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SMB

XXX CONGRESO NACIONAL DE BIOQUÍMICA

2-8 de noviembre de 2014 · Guadalajara, Jal.

PROGRAMA

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PROGRAMA

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XXX Congreso Nacional de Bioquímica

2 al 8 de noviembre, 2014 Guadalajara, Jal.

Mensaje de la Presidente

Estimados Colegas:

Bienvenidos a la Ciudad de Guadalajara.

Son muchas las razones que llevaron a la Mesa Directiva de la Sociedad Mexicana de Bioquímica a elegir a Guadalajara como sede de nuestro XXX Congreso. La ciudad de Guadalajara cuenta con una de las ofertas culturales más amplias en nuestro país, a lo cual se suma el interés del gobierno, de la Universidad de Guadalajara y de instituciones privadas por explotar los atributos culturales de la ciudad. La Universidad de Guadalajara es la tercera universidad más antigua en México y es reconocida por su prestigio académico internacional en las áreas de Medicina, Derecho, Administración de empresas, Biología, Arte, Arquitectura y Diseño. Asimismo, esta Universidad organiza la Feria Internacional del Libro de Guadalajara que se realiza cada año durante la última semana de noviembre y que constituye el evento de difusión literaria más importante de México, incluyendo una gran exposición de editoriales y la presentación de libros y conferencias, con una trayectoria y un gran prestigio de casi 30 años; así como el Festival Internacional de Cine de Guadalajara (conocido como Guadalajara Film Fest), con más de veinte años de trayectoria. El FICG es la actividad más importante que se lleva a cabo en México, en cuanto a cine se refiere.

El centro histórico de la ciudad de Guadalajara alberga edificios coloniales de carácter religioso y civil, los cuales destacan por su trascendencia arquitectónica e histórica. El Instituto Cultural Cabañas, mejor conocido como Hospicio Cabañas, es un edificio de estilo neoclásico, emblemático de la ciudad y que en 1997 fue declarado Patrimonio de la Humanidad por la UNESCO. En su interior se conservan algunos de los excepcionales murales de José Clemente Orozco, en particular "El Hombre de Fuego".

La ciudad de Guadalajara cuenta con científicos distinguidos que llevan a cabo investigación de excelencia reconocidos en los foros internacionales pertinentes. Destacan los grupos de investigación de la Universidad de Guadalajara, del Centro de Investigación Biomédica de Occidente del IMSS y del Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A. C. Algunos de ellos presentarán parte de su trabajo en dos de los simposia que tendrán lugar durante nuestro congreso.

En ocasión de la inauguración del XXX Congreso de la Sociedad Mexicana de Bioquímica, tendremos dos conferencias magistrales, la primera será presentada por el Dr. Fernando Serrano Migallón, Subsecretario de Educación Superior de la Secretaría de Educación Pública. El Dr. Serrano Migallón, es profesor de Ciencia política y Derecho constitucional en la Facultad de Derecho de la Universidad Nacional Autónoma de México, profesor en las licenciaturas de Administración pública y Relaciones internacionales de El Colegio de México, es miembro del Sistema Nacional de Investigadores. Su trabajo académico ha resultado en la publicación de un número importante de libros. Entre los temas que han interesado al Dr. Serrano Migallón se encuentra el papel de México como país de asilo. A este respecto ha publicado entre otros títulos: “El Asilo Político en México” (Editorial Porrúa) y “La Inteligencia Peregrina, Legado de los Intelectuales del Exilio Republicano Español en México” (El Colegio de México). Dice Serrano Migallón:

"La institución del asilo tiene su fundamento y origen en razones humanitarias de reconocimiento del primer derecho fundamental que tiene el hombre mismo, por el simple hecho de serlo, el derecho a que su vida, su integridad, le sean respetadas".

En su conferencia titulada: "México, País de Asilo", nuestro invitado analizará el papel fundamental que México ha jugado al ofrecer asilo y los beneficios y riqueza que ha recibido con la llegada de hombres y mujeres comprometidos y que tomaron a México como su patria y ejercieron una influencia importante en la educación y vida cultural de nuestro país.

La segunda conferencia será presentada por el Dr. José Bargas, Investigador titular del Instituto de Fisiología Celular de la Universidad Nacional Autónoma de México y miembro del Sistema Nacional de Investigadores. El Dr. Bargas ha desarrollado su investigación en el área de las neurociencias, particularmente en el estudio de la acción de la dopamina y acetilcolina en las neuronas y redes estriatales. El Dr. Bargas ha publicado más de 60 artículos científicos y su trabajo ha recibido un importante reconocimiento internacional.
Dicen Bargas y col:

"…….nos encontramos en el umbral de un nuevo paradigma que desplazará al celular-molecular. A partir del siglo XXI se trabaja en el diseño de herramientas experimentales y teóricas para empezar a ***entender*** *los entes biológicos a un nivel distinto".*

En su conferencia magistral, el Dr. Bargas atenderá, entre otras, las siguientes preguntas: ¿qué es lo que tienen de distinto las diversas células de los diferentes tejidos?, ¿cómo es que llevan a cabo funciones diferentes?, ¿cómo coordina sus acciones un grupo de 50 a 300 células?, ¿cómo trabajan en equipo? Asimismo, el Dr. Bargas nos propondrá una novedosa estrategia teórica y experimental que permita atender las preguntas planteadas, en cuanto a la función que desarrollan grupos de células.

La Mesa Directiva de la SMB ha preparado un nutrido programa que incluye, además de las inaugurales, cinco conferencias magistrales presentadas por importantes líderes académicos, tanto de México como del extranjero, 18 simposia en los que se presentarán trabajos de los miembros de cada una de las ocho ramas que constituyen nuestra Sociedad, así como 10 simposia con temas de gran importancia. Dos de ellos estarán dedicados a la labor realizada por los **Doctores ARMANDO GÓMEZ-PUYOU** y **EDGARDO ESCAMILLA MARVÁN**; ambos fueron investigadores del Instituto de Fisiología Celular. Uno más será dedicado al **Doctor JURE PISKUR**, de La Universidad de Lund en Suecia, uno de nuestros conferencistas invitados, quien falleciera el pasado mes de mayo.

Habrá 21 sesiones de presentaciones orales en las que participarán 144 estudiantes de diferentes instituciones de investigación de toda la República Mexicana. Tendremos cuatro sesiones de carteles en las que se presentarán alrededor de 220 carteles por día. Al igual que en el Congreso de Oaxaca, cada sesión combinará los 16 temas en los que se dividieron los trabajos, de suerte que todos los congresistas tengan la oportunidad de participar en las cuatro sesiones.

Tendremos cuatro sesiones tituladas *"Having coffee with ……",* en las que cada uno de ocho invitados se reunirá en una mesa de discusión con 10 alumnos, con el fin de que éstos tengan la oportunidad de acercarse a platicar con nuestros invitados. Al final del Congreso, el Dr. Claudio Scazzocchio del Deptartment of Microbiology, del Imperial College of London, impartirá el curso postcongreso: "Historia del Concepto de Gen", del 8 al 10 de noviembre, en el Hotel Riu. Para este propósito se han ofrecido 24 becas completas.

Nuestras actividades culturales incluirán: un brindis de bienvenida, una subasta de arte gráfico oaxaqueño, la presentación del ballet de la Universidad de Guadalajara y la cena de clausura.

Estoy segura de que ustedes disfrutarán nuestro programa, tanto científico como cultural, y que las interacciones que se den durante nuestro congreso servirán para estrechar los lazos de trabajo y amistad entre nuestra comunidad.

María Alicia González Manjarrez
Presidente SMB 2013-2015

Agradecimientos,

A la Dra. Marcia Hiriart, Directora del Instituto de Fisiología Celular,
Por su invaluable apoyo financiero, logístico y moral, para la realización de este congreso y al Instituto de Fisiología Celular, UNAM, por su permanente apoyo a la Sociedad Mexicana de Bioquímica.

Al Maestro Josué Valencia
Por su asesoría e incondicional apoyo en las diferentes actividades y etapas de organización del congreso.

A la Lic. Luz Jazmín Serrano Iñiguez,
De la Dirección de Relaciones Internacionales y Ciudades Hermanas, Municipio de Guadalajara
Por el apoyo prestado para diseñar la logística del congreso

A la Lic. María Teresa Castillo, Coordinadora de la organización total del congreso y del equipo de trabajo. Por su inteligente y profesional atención a cada detalle, a todos los aspectos que determinan el éxito de una reunión científica. Por su valiosa contribución en el llenado de todos los formatos que se prepararon para la solicitud de apoyos, establecimiento y mantenimiento de contacto con los invitados, edición del programa, contenido de los carteles diseñados para la difusión del congreso y múltiples sugerencias e indicaciones para mejorar y facilitar todas las actividades.
Un agradecimiento especial por su trato humano, gentil e incondicional, por su paciencia, creatividad y enorme solidaridad.

Al C. P. Francisco Arcos, coordinador de finanzas, por su cuidadoso, gentil y atento trabajo al frente de la contabilidad de la Sociedad.

A la Bióloga Andrea Ortiz Arcos, coordinadora de archivos e ingreso de socios. Por su valioso apoyo desde el inicio de la organización del congreso hasta la conclusión del mismo, particularmente en todos los aspectos referentes al ingreso de socios nuevos y mantenimiento y rescate de archivos.

A Rocío Romualdo Martínez, coordinadora de invitados. Por su impecable y muy valioso trabajo organizando la atención a los conferencistas invitados nacionales y extranjeros.

A la Bióloga Daniela Carmona León, por su trabajo en la preparación de archivos y material del congreso

A la Bióloga Belén Melo Munive, por su trabajo en la atención a los congresistas y organización del material pertinente.

Al Ingeniero Juan Barbosa Castillo, por la importante labor en la edición de la memoria electrónica.

A Ricardo Chávez Castillo, coordinador de difusión, por su muy valioso apoyo al frente de la distribución, y entrega oportuna de innumerables cartas, solicitudes y material de publicidad.

A Omar Chávez Castillo, coordinador de comunicación, por su siempre creativa aportación a la difusión de las actividades intra y extra congreso, por buscar la mejor manera de socializar las actividades, por haber propuesto y desarrollado nuestra presencia en Facebook.

A todos ellos nuestro reconocimiento y profundo agradecimiento.

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Hotel Riu Plaza Guadalajara, Guadalajara, Jal., November 2-8, 2014

SUNDAY 2		MONDAY 3	TUESDAY 4	THURSDAY 6	FRIDAY 7
REGISTRATION 11:00 – 17:30 **AND DURING ALL THE MEETING**	9:00 – 11:00	**Oral Presentations** **Technical Conferences**	**Oral Presentations** **Technical Conferences**	**Oral Presentations** **Research Programs in Germany**	**Plenary Sessions 12-13-14-15** - Molecular Epidemiology - Nanobiotechnology - Bioetica - Biochemistry and Plant Molecular Biology
	11:00 11:30	COFFEE BREAK			
	11:30 – 12:30	**Plenary Lecture** **Annie Pardo** **Fibrosis: Just A Scar Or An Aberrant Remodeling Process? The Paradigm of Idiopathic Pulmonary Fibrosis**	**Oral Presentations**	**Plenary Lecture** **Tomas Kirchhausen** **Dynamics of Endocytosis**	**Plenary Lecture** **Alexander D. Johnson** **How Transcription Circuits Evolve and Produce Biological Novelty**
	12:30 – 14:00	**LUNCH**			
	14:00 – 16:00	**Plenary Sessions 1-2-3-4** -Physicochemistry and Protein Design *IN MEMORIAM* DR. GOMEZ-PUYOU -Epigenetic Reg. Fungus Parasites and Plants -Human Genomics -Oxydative Stress and Health	**Plenary Sessions 5-6-7-8** -Evolutionary Genomics. Hisp/Mex -Physiological Relevance of Signalling Pathways Crosstalk -Genomics of Mendelian Diseases in Northwestern Mexico -Molecular Biology of Viruses	**Plenary Sessions 9-10-11** -Frontiers in Bioenergetics and Mitochondrial Function. -Genomics -Mechanisms of Neurodegeneration **Workshop PLoS-Open Access Publishing**	**Plenary Sessions 16-17-18** -Epigenetic Mechanisms. In Plants: Their Significance in Biotechnological Developments -Fungal Molecular Biology *IN MEMORIAM* DR. PISKUR -Biochemistry and Molecular Biology of Bacteria *IN MEMORIAM* DR. ESCAMILLA MARVÁN
	16:15 – 18:15	**Oral Presentations** **Having coffee with…** Annie Pardo / John P. Richard/ Philippe Silar / Gabaldon - Santiago E - DeLuna	**Oral Presentations** **16:15 – 17:15**	**Oral Presentations** **Having coffee with….** Tomas Kirchhausen / Michael Eisen Sandy Johnson / Peter Walter	**POSTER SESSION** **16:00 – 18:00**
OPENING CEREMONY 18:00 **OPENING LECTURE** **Fernando Serrano Migallón** **México, País de Asilo** **18:30 – 19:30**	18:15 – 20:15	**POSTER SESSION** **18:15 – 20:15**	**POSTER SESSION** **17:15 – 19:15**	**POSTER SESSION** **18:15 – 20:15**	**Plenary Lecture** **Peter Walter** **Unfolded Protein Response in Health and Disease** **18:15 – 19:15** **FINAL ANNOUNCEMENTS AND CLOSING CEREMONY**
PLENARY LECTURE **José Bargas** **Microcircuitos Cerebrales** **19:30 20:30**		Free time	**Plenary Lecture** **Michael Eisen** **The control of transcription during early development** **19:30 20:30**	Free time	**CLOSING DINNER** **21:00**
WELCOME COCKTAIL	21:00		**BUSINESS SESSIONS - SMB** **20:30**		
			Auction of Oaxaca Graphic Art **21.00HRS.**		

PLENARY LECTURES. Salón Guadalajara
PLENARY SESSIONS. Salón Guadalajara 1, 2, 3. Salón Vallarta
HAVING COFFEE WITH. Salón Tonalá
TECHNICAL CONFERENCES. Salón Vallarta
POSTER SESSIONS. Salón Jalisco
WELCOME COCKTAIL AND CLOSING DINNER. Salón Fiesta Guadalajara
ART AUCTION. Salón Orozco. Holiday Inn Select Hotel - Galeria de Noel Cayetano (Oaxaca)

Program of Events

Sunday November 2, 2014

11:00 – 17:30 Registration
Hotel RIU

Opening Ceremony, Plenary Lectures

Guadalajara Room

18:00 – 18:30 Opening Ceremony

18:30 – 19:30 **Opening Lecture**

México, País de Asilo
Fernando Serrano Migallón
Facultad de Derecho, UNAM

Chair: Alicia González Manjarrez
Instituto de Fisiología Celular, UNAM

19:30 – 20:30 **Plenary Lecture**

Cerebral Microcircuits
José Bargas
Instituto de Fisiología Celular, UNAM

Chair: Miguel Lara Flores
ENES - León, UNAM

20:30 – 22:00 Welcome Cocktail
Salón Fiesta Guadalajara

Oral presentations will be held in the Guadalajara Room and Vallarta Room, Hotel RIU

"Having coffee with" will be held in the Tonala Room, Hotel RIU

Poster presentations will be held in the Jalisco Room, Hotel RIU

Monday November 3, 2014

9:00 – 11:00 Oral Presentations 1
Guadalajara Room I

Systems Biology and Bioinformatics I

Chair: **Luis Delaye**
CINVESTAV – IPN – Unidad Irapuato

9:00 – 9:15	Dosage matters: Revealing the immediate fitness effects of experimental gene duplication **Diana Ascencio**, Adriana Espinosa & Alexander de Luna LANGEBIO, CINVESTAV-IPN
9:15 – 9:30	Transcriptomic analyses of the apterygotan insect *Thermobia domestica*: insights into the evolutionary origin of plant cell wall digestion in insects **Nancy Calderón Cortés**, Hirofumi Watanabe Escuela Nacional de Estudios Superiores Unidad Morelia, UNAM
9:30 – 9:45	The cellular crosstalks of metabolism by moonlighting activities **Adriana Espinosa** & Alexander de Luna LANGEBIO, CINVESTAV-IPN
9:45 – 10:00	Functional genomics characterization of Excretory/Secretory proteins from *T. solium* genome Sandra Gómez, Laura Adalid-Peralta, Hector Palafox-Fonseca, Xavier Soberón, Edda Sciutto, Gladis Fragoso, Raúl J Bobes, Juan P Laclette, Luis del Pozo Yauner and **Adrián Ochoa-Leyva** UGPAS, Facultad de Química, UNAM - INMEGEN
10:00 – 10:15	Characterization haem and hemoglobin binding membrane protein (*spbhp-37)* in *Streptococcus pneumoniae* **José de Jesús Olivares-Trejo**, María Elena Romero-Espejel, Mario A. Rodríguez and Sarita Montaño Posgrado Ciencias Genómicas, Universidad Autónoma de la Ciudad de México
10:15 – 10:30	Prediction of Internal Ribosome Entry Sites (IRES) in *Saccharomyces cerevisiae* using computational strategies **Esteban Peguero Sánchez** and Enrique Merino Pérez Instituto de Biotecnología, UNAM
10:30 – 10:45	Genome-wide open chromatin profiling reveal new components of *Anopheles gambiae* innate immunity system **Verónica Valverde Garduño**, Oscar Migueles, Zita Becerra and Manuel Castillo Méndez CISEI, Instituto Nacional de Salud Pública
10:45 – 11:00	Interaction of antimicrobial peptides with membrane models by molecular dynamics simulations **José Luis Velasco**, Ramón Garduño Juárez Instituto de Ciencias Físicas, UNAM

9:00 – 11:00 Oral Presentations 2
Guadalajara Room II

Biochemistry I

Chair: **Jaime García Mena**
CINVESTAV – IPN Zacatenco

9:00 – 9:15 Changes in *Arabidopsis thaliana* root proteome in response to capsaicin
Ana Laura Alonso Nieves, Argel Gastélum Arellánez, Jorge Molina Torres, Silvia Edith Valdés Rodríguez
CINVESTAV-IPN Unidad Irapuato

9:15 – 9:30 Identification of a R2R3-MYB transcription factor gene (*CaMyb31*) that regulates the capsaicinoid biosynthetic pathway in chili pepper fruits (*Capsicum annuum* L.)
Magda Lisette Arce Rodríguez and Neftalí Ochoa Alejo
CINVESTAV-IPN Unidad Irapuato

9:30 – 9:45 The biogenesis and control mechanisms of ribosomal activity in *Saccharomyces cerevisiae*.
Arnulfo Bautista-Santos, Reynaldo Tiburcio Félix and Samuel Zinker Ruzal.
Departamento de Genética y Biología Molecular. CINVESTAV-IPN Unidad Zacatenco

9:45 – 10:00 Altered Intracellular Ca^{2+} Signaling Exacerbates Mitochondrial Fragility in Ventricular Myocytes from Obese Rats
Judith Bernal-Ramírez, Noemí García, Julio Altamirano
School of Medicine and Health Sciences. Tecnológico de Monterrey

10:00 – 10:15 Crystallographic, biochemical studies and solution structure by the small-angle X-ray scattering evidencing the dimeric formation of thioredoxin 1 from white leg shrimp *Litopenaeus vannamei*
Adam Andrés Campos-Acevedo, Enrique Rudiño-Piñera
Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

10:15 – 10:30 Complex I activity decreases by low content of mitochondrial STAT3 obesity-induced
Ariel Charles Ponce, Josué Cantú Valdés and Noemí García
Department of Cardiology, School of Medicine, Tecnológico de Monterrey

10:30 – 10:45 Regulation of Deoxyxylulose phosphate synthase from *Arabidopsis thaliana*
Marel Chenge-Espinosa, Patricia León Mejía
Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

10:45 – 11:00 Discovery of the biosynthesis of arseno-organic molecules in *Actinobacteria*
Pablo Cruz Morales, Luis Yañez Guerra, Johannes Kopp, Jörg Feldmann and Francisco Barona Gómez
LANGEBIO, CINVESTAV – IPN Unidad Irapuato

9:00 – 11:00 Oral Presentations 3
Guadalajara Room III

Biotechnology I

Chair: **Gabriel Rincón Enríquez**
CIATEJ - Jalisco

9:00 – 9:15 Antitumor activity evaluation of the protein extracts from the biological states of the house fly *Musca domestica*.
Moyira Osny Aquino-Gil, Carlos Josué Solórzano-Mata, Víctor Manuel Aquino-Gómez, Itandehui Belem Gallegos Velasco, Socorro Pina-Canseco, Edgar Zenteno-Galindo y Yobana Pérez-Cervera.
Facultad de Medicina, UNAM-UABJO

9:15 – 9:30 Characterization of the transcription induced by the intoxication of Cry toxins in mosquitoes
Pablo Emiliano Cantón Ojeda, Mario Soberón Chávez, Alejandra Bravo de la Parra
Instituto de Biotecnología, UNAM

9:30 – 9:45 Impact and mechanisms of shock wave application on human cells
Karen Castaño-González, Luz María López-Marín, Blanca Edith Millán-Chiu, Francisco Fernández-Escobar, Carmen Yolanda Aceves-Velasco, Achim Max Loske-Mehling
Centro de Física Aplicada y Tecnología Avanzada, UNAM

9:45 – 10:00 Improvement of enzymatic saccharification yield in *Arabidopsis thaliana* by expression of rice *SUB1A* transcription factor
Lizeth Núñez-López, Blanca E. Barrera-Figueroa, Andrés Aguirre-Cruz, Julián M. Peña-Castro
Instituto de Biotecnología, Universidad del Papaloapan

10:00 – 10:15 Plant pathogen interaction of agave wilt: The response of the *Agave tequilana* Weber var. Azul to the infection of *Fusarium oxysporum*
Joaquín Alejandro Qui-Zapata, Gabriel Rincón-Enríquez, Emmanuel Bahena-Reyes, Patricia Dupré, José Manuel Rodríguez-Domínguez
Centro de Investigación y Asistencia en Tecnología y Diseño del Edo. de Jalisco A. C.

10:15 – 10:30 MicroRNA profiling of Arabidopsis *AtGRDP1* overexpression line reveals deregulation of mir159 and mir160 in response to ABA
Aída Araceli Rodríguez Hernández, Catalina Arenas Huertero and Juan Francisco Jiménez Bremont
División de Biología Molecular, IPICyT

10:30 – 10:45 Bioprospecting of yeast strains capable of producing microbial oils as feedstock for biodiesel
Luis Gerardo Treviño Quintanilla and Andrea Carina Martínez Burgos
Universidad Politécnica del Estado de Morelos

10:45 – 11:00 Don't be afraid of mass spectrometry (MS) – Strategies to generate and analyze MS data in a 'normal' biochemistry laboratory
Robert Winkler
CINVESTAV – IPN, Unidad Irapuato

9:30 – 11:00
Vallarta Room

Technical Conferences

Chair: **Deyamira Matuz Mares**
Facultad de Medicina, UNAM

9:30 – 10:00 Digital PCR: Overview and Applications
Maribel Acosta
LIFETECH TECHNOLOGIES

10:00 – 10:30 Microscopy as a tool for molecular and subcellular analysis - an overview
Christopher David Wood
Laboratorio Nacional de Microscopía Avanzada. Instituto de Biotecnología, UNAM
ACCESOLAB

10:30 – 11:00 SECUENCE CAPTURE: a simple single-step enrichment method.
Verenice Ramírez Rodríguez
ROCHE DIAGNOSTICS

11:00 – 11:30 Coffee Break
Foyer Guadalajara Room

11:30 – 12:30
Guadalajara Room

Plenary Lecture I

Fibrosis: Just a Scar or an Aberrant Remodeling Process?
The Paradigm of Idiopathic Pulmonary Fibrosis
Annie Pardo
Facultad de Ciencias, UNAM

Chair: **Alicia González Manjarrez**
Instituto de Fisiología Celular, UNAM

12:30 – 14:00 Lunch

14:00 – 16:00 Plenary Session 1
Guadalajara Room I

Physicochemistry and Protein Design

"*In Memoriam* Dr. Armando Gómez Puyou"

Chair: **Daniel Alejandro Fernández Velasco**
Facultad de Medicina, UNAM

14:00 – 14:30 "Brucei-fying" triosephosphate isomerase from *Trypanosoma cruzi,* or viceversa
Ruy Perez Montfort, Selma Díaz Mazariegos, Mónica Rodríguez Bolaños, Nallely Cabrera González, Marietta Tuena de Gómez Puyou and Armando Gómez Puyou
Instituto de Fisiología Celular, UNAM

14:30 – 15:00 Drug Discovery by Protein Structure Modeling. Proteins from Parasites as Target
Arturo Rojo Domínguez
Universidad Autónoma Metropolitana. Unidad Cuajimalpa

15:00 – 15:30 Reflections on the Catalytic Power of the TIM Barrel
John P. Richard
Department of Chemistry, University at Buffalo, USA

15:30 – 16:00 The unfolding story of a TIM barrel
D. Alejandro Fernández Velasco, Mariana Schulte-Sasse, Luis A. Becerril Sesín and Sergio Romero Romero

14:00 – 16:00 Plenary Session 2
Guadalajara Room II

Epigenetic Regulation in Fungus, Parasites and Plants

Chair: **Félix Recillas**
Instituto de Fisiología Celular, UNAM

14:00 – 14:30 A protosilencer with unique properties modulates subtelomeric gene expression in *Candida glabrata*
Irene Castaño, Eunice López Fuentes, Alejandro Juárez-Reyes, Leonardo Castanedo Ibarra and Alejandro De Las Peñas
Instituto Potosino de Investigación Científica y Tecnológica

14:30 – 15:00 The role of TrxG and PcG during Arabidopsis root development.
Sánchez MP, Petrone E, Vega R, Flores J, Aceves P, Steckenborn S, Garay-Arroyo A, García-Ponce B and Álvarez-Buylla RE.
Instituto de Ecología, UNAM

15:00 – 15:30 Paramutation: trangenerational epigenetic inheritance and development
Mario Arteaga-Vázquez, Omar Oltehua-López, Ana Dorantes-Acosta and Daniel Grimanelli
Laboratorio de Epigenética y biología del desarrollo, Universidad Veracruzana

15:30 – 16:00 Chromatin structure modulates differential gene expression of the chicken α-globin locus
Félix Recillas Targa, Christian Valdes Quezada, Cristian Arriaga Canon, Yael Fonseca Guzmán, Georgina Guerrero
Instituto de Fisiología Celular, UNAM

14:00 – 16:00 Plenary Session 3
Guadalajara Room III

Human Genomics

Chair: **Xavier Soberón**
Instituto Nacional de Medicina Genómica

14:00 – 14:30 The Human Genome, fourteen years latter
Francisco Xavier Soberon Mainero
Instituto Nacional de Medicina Genómica

14:30 – 15:00 Dynamics of the Human Genome
Rafael Palacios, Laura Gómez Romero, Kim Palacios-Flores, José Reyes, Delfino García, Margareta Boege, Guillermo Dávila, Margarita Flores
Universidad Nacional Autónoma de México

15:00 – 15:30 Patterns of Genetic Diversity in Latin America: Insights from Human Population Genomics
Andrés Moreno Estrada
School of Medicine, Stanford University

15:30 – 16:00 The impact of genomics in the study and clinical management of cancer
Alfredo Hidalgo Miranda
Laboratorio de Genómica del Cáncer, Instituto Nacional de Medicina Genómica

14:00 – 16:00 Plenary Session 4
Vallarta Room

Oxygen Reactive Species

Chair: **Luis Cárdenas**
Instituto de Biotecnología, UNAM

14:00 – 14:30 Energy/redox metabolism in gene-environment interactions, and dopaminergic cell death associated with Parkinson´s disease
Rodrigo Franco, Annadurai Anandhan, Pablo Hernández-Franco
Redox Biology Center, Universidad de Nebraska, USA

14:30 – 15:00 Nrf2 redox signaling confers long term protection in post-ischemic hearts
Ana Cecilia Zazueta Mendizabal
Instituto Nacional de Cardiología

15:00 – 15:30 Hepatocyte Growth Factor/c-Met as a master regulator of redox cellular status and oxidative stress
Luis E. Gómez-Quiroz, Denise Clavijo-Cornejo, Cristina Enríquez, Oscar Bello Monroy, Roxana U Miranda, Verónica Souza, Leticia Bucio, Ma. Concepción Gutiérrez-Ruiz
UAM Iztapalapa

15:30 – 16:00 REDOX state as an inductor and modulator of cellular senescence
Mina Konigsberg Fainstein, Mina Königsberg, Francisco Triana-Martínez, Luis A Maciel-Barón, Sandra L Morales-Rosales, Viridiana Y González-Puertos, Claudio Torres, Norma E López-Diazguerrero
UAM Iztapalapa

16:15 – 18:15 Oral Presentations 4
Guadalajara Room I

Oxygen Reactive Species

Chair: **David Manuel Diaz Pontones**
UAM - Iztapalapa

16:15 – 16:30 Colloidal Silver and cell viability association with markers of oxidative stress: heme oxygenase 1 Catalase 8 isoprostane and iron
Lucerito Esmeralda Avila Lagunes y **Elda Maria del Rocio Coutiño Rodríguez**
Instituto de Salud Pública, Universidad Veracruzana

16:30 – 16:45 The metallothionein gene from the white shrimp *Litopenaeus vannamei*: Characterization and expression in response to hypoxia
Monserrath Félix-Portillo, José Alfredo Martínez-Quintana, Alma B. Peregrino-Uriarte, Gloria Yepiz-Plascencia
Centro de Investigación en Alimentación y Desarrollo

16:45 – 17:00 A peptide that regulates the motility of sea urchin sperm, activates the mitochondrial metabolism mediated by the Carnitine Palmitoyl Transferase I (CPT-I)
Juan García-Rincón, Alberto Darszon & Carmen Beltrán
Instituto de Biotecnología, UNAM

17:00 – 17:15 Cyclophosphamide induces oxidative stress in brain, liver and kidney of normal rats treated
Laura Guerrero-Medrano, Calderón-Salinas José Víctor
Departamento de Bioquímica, CINVESTAV – IPN Zacatenco

17:15 – 17:30 C-Phycocyanin prevents renal mitochondrial dysfunction induced by cisplatin**.**
Berenice Fernández-Rojas, Daniela S. Rodríguez-Rangel, Fernando Granados-Castro, Negrete Mario, José Pedraza-Chaverri
Department of Biology, Facultad de Química, UNAM

17:30 – 17:45 Does Senescence Associated Secretory Phenotype (SASP) profile change when senescence is induced by different ways?
Luis Ángel Maciel Barón, Sandra Lizbeth Morales Rosales, Viridiana Yazmín González Puertos, Claudio Torres, Mina Königsberg Fainstein
Departamento de Ciencias de la Salud, UAM - Iztapalapa

17:45 – 18:00 Auranofin-induced oxidative stress causes redistribution of the glutathione pool in *Taenia crassiceps* cysticerci.
José de Jesús Martínez González, Alberto Guevara Flores, Luz Gisela Martínez García, Juan Luis Rendón Gómez e Irene Patricia del Arenal Mena
Departamento de Bioquímica, Facultad de Medicina, UNAM

18:00 – 18:15 The lipogenesis attenuation by hydrogen peroxide avoid the liver steatosis obesity-related but increase the mortality by starvation
José Raúl Pérez Estrada, David Hernández-García, Susana Castro Obregón, Luis Covarrubias
Instituto de Biotecnología, UNAM

16:15 – 18:15 Oral Presentations 5
Guadalajara Room II

Biochemistry II

Chair: **Martha Calahorra**
Instituto de Fisiología Celular, UNAM

16:15 – 16:30 Changes in water availability induce folding in intrinsically disordered stress proteins from plants.
Cesar L. Cuevas Velazquez, Gloria Saab Rincón, Carlos Amero, Maria Martinez Yamout, Gerard Kroon, Jane Dyson, Alejandra A. Covarrubias
Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

16:30 – 16:45 Protein-ligand interactions to understand the role of the mutations in the Shwachman-Diamond Syndrome
Eugenio de la Mora, Abril Gijsbers Alejandre, Adrián García Márquez, Alfonso Méndez Godoy, Nancy Marcial Bazaldúa, y Nuria Sánchez Puig
Instituto de Química, UNAM

16:45 – 17:00 Thrombin generation as parameter of response to therapeutic agents in patients with bleeding disorders
Hilda Luna-Záizar, Ana Rebeca Jaloma-Cruz, Sandra L. Ruiz-Quezada, Griselda G. Macías-López, María Amparo Esparza-Flores, Ana Cristina Ramírez-Anguiano, Fermín Paul Pacheco-Moisés.
CUCEI-Universidad de Guadalajara y Centro de Investigación Biomédica de Occidente, IMSS

17:00 – 17:15 Physicochemical study of the transcriptional repressor NagC from *Escherichia coli*
Dana Mariel Díaz Jiménez, Sergio Zonszein Strauss, Laura I. Álvarez Añorve, Mario L. Calcagno Montans.
Departamento de Bioquímica, Facultad de Medicina, UNAM

17:15 – 17:30 Effect of low-glucose cultivation on the *Entamoeba histolytica* energy metabolism
Rusely Encalada and Emma Saavedra
Departamento de Bioquímica. Instituto Nacional de Cardiología "Ignacio Chávez"

17:30 – 17:45 Analysis of differential compartmentalization between paralogous gene products: its role in functional diversification
Mirelle Citlali Flores-Villegas, Augusto Ortega-Granillo, Jure Piskur, Alicia González-Manjarrez
Institute of Cell Fiosiology, National Autonomous University of Mexico

17:45 – 18:00 Polymorphisms in mitochondrial genome affect the regulation of Cytochrome b synthesis in *Saccharomyces cerevisiae*
Aldo E. García Guerrero, Yolanda Camacho Villasana y Xochitl Pérez Martínez
Instituto de Fisiología Celular, UNAM

18:00 – 18:15 Diurnal restricted feeding schedules promotes changes in NF-KB presence in liver of WISTAR rats
Ana Cristina García-Gaytán, Mauricio Díaz-Muñoz, Isabel Méndez.
Instituto de Neurobiología, UNAM

16:15 – 18:15 Oral Presentations 6
Guadalajara Room III

Microbiology and Parasitology I

Chair: **Alejandro Carabin Lima**
Ciencias Microbiológicas, BUAP

16:15 – 16:30 Identification of genes involved in virulence of pUM505 plasmid from *Pseudomonas aeruginosa*
Ernesto Rodríguez Andrade, Víctor Meza Carmen, Carlos Cervantes and Martha Isela Ramírez Díaz
Instituto de Investigaciones Químico-Biológicas. UMSNH

16:30 – 16:45 Virulotyping of *Salmonella enterica* strains isolated from meat and dairy products from Michoacán
Lucia Paulina Maldonado Ruiz, Ma. Soledad Vázquez Garcidueñas, Gerardo Vázquez Marrufo
Ciencias Médicas y Biológicas "Dr. Ignacio Chávez". Universidad Michoacana

16:45 – 17:00 Effects of 17β-estradiol on the internalization of *Staphylococcus aureus* into bovine mammary epithelial cells
Ricardo Iván Medina Estrada, Joel Edmundo López Meza y Alejandra Ochoa Zarzosa
FMVZ, Universidad Michoacana de San Nicolás de Hidalgo

17:00 – 17:15 Metagenomic analysis of bacterial diversity in kefir and tibi grains
Verónica Lorena Cano García, Selvasankar Murugesan, Alberto Piña Escobedo, Héctor Mario Poggi Varaldo, Elvira Ríos Leal, Carlos Hoyo Vadillo, Bulmaro Cisneros Vega, Jaime García Mena
Genética y Biología Molecular, CINVESTAV – IPN Zacatenco

17:15 – 17:30 Identification of novel ciprofloxacin resistance genes of PUM505 plasmid from *Pseudomonas aeruginosa*
Víctor Manuel Chávez-Jacobo, Jesús Campos-García, Carlos Cervantes and Martha Isela Ramírez-Díaz
Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana

17:30 – 17:45 Cell wall caracterization and imnune sensig of no-albicans species
María de Jesús Navarro-Arias, Eine Estrada-Mata, María Fernanda Rodríguez-Preciado, Edgar Cordero-Roldan, Mercedes Guadalupe López-Pérez and Héctor Manuel Mora-Montes.
División de Ciencias Naturales y Exactas. Universidad de Guanajuato

17:45 – 18:00 Inhibitory activity of *Flammulina velutipes* extracts against the *S. schenckii*: cell - host interaction
Svetlana Kashina, Lérida L. Flores Villavicencio, Myrna Sabanero López
Departamento de Biología, Universidad de Guanajuato

18:00 – 18:15 *Entamoeba histolytica* Infection Modulates the Expression of microRNA in Human Colon Cancer Cells
Itzel López-Rosas, Olga Hernández de la Cruz, José Ali Flores-Pérez, César López-Camarillo, Laurence A. Marchat
Escuela Nacional de Medicina y Homeopatía, IPN

16:15 – 18:15 Having coffee with.....
Tonala Room

Four Round Tables for students
chosen from oral presentations

Annie Pardo	Alejandro Bustos Cortés	Nadia Aglae Rangel-Gauna
	Minerva Mata Rocha	Nadia Jacobo Herrera
	Jaquelina Julia Guzmán Rodríguez	Mónica Alejandra Anaya Segura
	Rebeca Pérez Cabeza de Vaca	Selvasankar Murugesan
	Ma. De Lourdes Delgado Aceves	Yuriana Oropeza Almazan
	Chair: Luisa Alba Lois	
John P. Richard	Judith Bernal Ramírez	Erika Gómez Chang
	Sergio Romero Romero	Estefanía Herrera Herrera
	Sandra Martínez Jarquín	Diana Fabiola Díaz Jiménez
	Magda Lisette Arce Rodríguez	Pablo Cruz Morales
	Geovani López Ortíz	Jorge Marcos Víquez
	Chair: Daniel Alejandro Fernández	
Philippe Silar	Oscar Rodríguez Lima	Laura García Segura
	Ángel Andrade Torres	Carlos Vladimir Muro Medina
	Jazmín Espinosa Rivero	Lucero Romero Aguilar
	Ramón Alberto Batista García	
	Emma Beltrán Hernández	
	Chair: Jesús Aguirre Linares	
Santiago Elena and Tony Gabaldon	José Luis Aguilar	Diana Ascencio
	Eréndira Rojas Ortega	Adrian Ochoa Leyva
	Dariel Márquez Gutiérrez	Esteban Peguero Sánchez
	Mariana Schulte Sasse	José Luis Velasco
	Alejandro Juárez Reyes	José de Jesús Olivares Trejo
	Chair: Alexander De Luna Fors	

18:15 – 20:15 Poster Session 1

B	BASIC BIOCHEMISTRY	1 – 37
SB	SYSTEMS BIOLOGY & BIOINFORMATICS	1 – 16
BT	BIOTECHNOLOGY	1 – 40
CD	CELL DIFFERENTIATION	1 - 29
G	GENETICS	1 – 23
GR	GENETIC REGULATION & EPIGENETIC	1 – 22
T	TOXICOLOGY	1 – 17
ST	SIGNAL TRANSDUCTION	1 – 32

Jalisco Room

9:00 – 11:00 Oral Presentations 7
Guadalajara Room I

Medicine, Health and Nutrition I

Chair: **Edith Araceli Cano Estrada**
Universidad Autónoma del Estado de Hidalgo

9:00 – 9:15 Potential Biomarkers for Duchenne Muscular Dystrophy and Carrier Detection
Mónica Alejandra Anaya Segura, Ikuri Álvarez Maya, Guillermina Ávila Ramírez, Froylan Arturo García Martínez, Luis Ángel Montes Almanza, Benjamín Gómez Díaz, Luz Berenice López Hernández
CIATEJ

9:15 – 9:30 Diffuse reflectance spectroscopy as a possible tool to complement liver biopsy for grading hepatic fibrosis in paraffin-preserved human liver specimens
Ursula Dinorah Arroyo Camarena, Diego Adrián Fábila Bustos, María Dolores López Vancell, Marco Antonio Durán Padilla, Guadalupe Itzel Azuceno García, Israel Torres Castro, Suren Stolik, José Manuel de la Rosa, Gabriela Gutiérrez Reyes, Galileo Escobedo González
Unidad de Medicina Experimental, Hospital General de México

9:30 – 9:45 Physicochemical characterization and antimicrobial activity of propolis collected in the apiary "Las agujas" from the "La primavera" forest
María de Lourdes Delgado Aceves, Georgina Ivette López Cortés, Angelina Carolina Rodríguez Aranda, Cintia Gutiérrez Villegas, Patricia Paola Ochoa Guzmán, Jesús Ángel Andrade Ortega, Rafael Ordaz Briseño, Alfonso Enrique Islas Rodríguez
Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara

9:45 – 10:00 Cytotoxicity of defensins γ-thionin (*Capsicum chinense*) and PaDef (*Persea americana* var. *drymifolia*) against cancer cell lines
Jaquelina Julia Guzmán-Rodríguez, Rodolfo López-Gómez, Alejandra Ochoa-Zarzosa and Joel E. López-Meza
Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, UMSNH

10:00 – 10:15 New treatment for breast and colorectal cancers inhibiting mTOR pathway and glycolysis by promoting apoptosis and autophagy
Nadia J. Jacobo-Herrera, Verónica García-Castillo, Gabriela Figueroa-González, Carlos Pérez-Plasencia, Luis Ignacio Terrazas-Valdés, Alejandro Zentella-Dehesa
Instituto Nacional de Ciencias Médicas y Nutrición/ FESI- UNAM

10:15 – 10:30 Age-related reduction of muscle mass and strength in fast but not slow skeletal muscle. The role of costamer-nuclei proteins interactions
Joel Medina Monares, Rocío Álvarez Medina and Alicia Ortega Aguilar
Departamento de Bioquímica, Facultad de Medicina, UNAM

10:30 – 10:45 Distal colon microbiota and Short chain Fatty Acids in association with Mexican Childhood Obesity
Selvasankar Murugesan, Maria Luisa Pizano Zárate, Hugo Martínez-Rojano, Flor María Galván Rodríguez, Alberto Piña Escobedo, Carlos Hoyo Vadillo, Jaime García Mena
Departamento de Genética y Biología Molecular, CINVESTAV – IPN Zacatenco

Biochemistry III

Chair: **Lina Raquel Riego**
Instituto Potosino de Investigación Científica y Tecnológica

9:00 – 9:15 Study of the rearrangement of the canonical RNAD canonical enzymes during mRNA decay
Jaime García-Mena, Alberto Piña-Escobedo, Ben F Luisi, Lilianha Domínguez Malfavón
Genética y Biología Molecular, CINVESTAV-IPN Zacatenco

9:15 – 9:30 Dynamics and chaotropic agents effects on the amyloidogenic lambda 3r light chain studied by NMR spectroscopy
Paloma Gil Rodríguez, Carlos Amero Tello
Centro de Investigaciones Químicas, UAEM

9:30 – 9:45 An intramitochondrial PKA modulates placental steroidogenesis in a cAMP independent manner
Erika Gómez-Chang, Ma. Teresa Espinosa-García, Sofía Olvera-Sánchez, Oscar Flores-Herrera and Federico Martínez
Bioquímica, Facultad de Medicina, UNAM

9:45 – 10:00 Role of p53 on tumor energy metabolism
Ileana Hernández-Reséndiz and Sara Rodríguez Enríquez
Bioquímica. Instituto Nacional de Cardiología

10:00 – 10:15 Characterization of a hyaluronidase from the venom of the spider *Brachypelma verdezi*
Estefanía Herrera Herrera, Herlinda Clement and Gerardo Corzo
Medicina Molecular y Bioprocesos, Instituto de Biotecnología,UNAM

10:15 – 10:30 Complex IV heterodimeric COX2 in *Polytomella* sp: Import and protein-protein interactions
Alejandra Jiménez Suárez, Héctor Vicente Miranda Astudillo, Martha Lilia Colina Tenorio, Miriam Vázquez Acevedo, Diego González Halphen
Instituto de Fisiología Celular, UNAM

10:30 – 10:45 Analyses of the oligomeric organization of paralogous proteins in *S. cerevisiae*: Leu4 and Leu9 a specific case
Geovani Lopez, Mijail Lezama, James González, Ximena Martínez, Maritrini Colón and González Manjarrez Alicia
Bioquímica y Biología Estructural. Instituto de Fisiología Celular, UNAM

10:45 – 11:00 DEAD-box RNA-helicases from *Bacillus subtilis* display differences in RNA-unwinding activity
Diana Fabiola Díaz-Jiménez, Gabriela Olmedo-Álvarez and Luis Gabriel Brieba de Castro
CINVESTAV – IPN Unidad Irapuato

9:00 – 11:00 Oral Presentations 9
Guadalajara Room III

Epigenetic, Genetic Regulation I

Chair: **Cristina Aranda Fraustro**
Instituto de Fisiología Celular, UNAM

9:00 – 9:15 Heterologous expression of *Debaryomyces hansenii* catalase genes in *Saccharomyces cerevisiae*
Claudia Segal-Kischinevzky, Diego Noriega, Román Castillo, Ángel García, Viviana Escobar, Víctor Valdés-López, Luisa Alba-Lois
Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM

9:15 – 9:30 Mediator as a link between development and abiotic stress response in plants
Martínez-Camacho Carol, Núñez-Ríos Tania, Sawers Ruairidh, Gillmor Stewart
Laboratorio Nacional de Genómica para la Biodiversidad. CINVESTAV – IPN

9:30 – 9:45 Small RNAs regulate the response to injury of the filamentous fungus *Trichoderma atroviride*
José Manuel Villalobos-Escobedo, Nohemí Carreras-Villaseñor, Joel Rodríguez-Medina, Cei Abreu-Goodger and Alfredo Herrera-Estrella
LANGEBIO – CINVESTAV – IPN Unidad Irapuato

9:45 – 10:00 *BAT1* & *BAT2* paralogous subfunctionalization is determined by expression divergence and chromatin reorganization in the yeast *Saccharomyces cerevisiae*
James González, Joseph Strauss, Geovani López, and Alicia González
Depto. de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

10:00 – 10:15 In search for *cis*-regulatory elements activated by Dengue virus infection in *Aedes aegypti* midgut cells
Manuel Castillo Méndez, Graciela H. Gleasson, Krystal Maya Maldonado, José Ramos Castañeda and Verónica Valverde Garduño
CISEI, Instituto Nacional de Salud Pública. CINVESTAV – IPN, Irapuato

10:15 – 10:30 Polyamines and Abscisic Acid: a new link between two pathways involved in stress responses in *Arabidopsis thaliana*
Diana Sánchez Rangel, Ana Isabel Chávez Martínez and Juan Francisco Jiménez Bremont
Department of Molecular Biology, IPICYT

10:30 – 10:45 Tumor-soluble factors derived from the breast cancer cell line ZR75.30 mimic a TNF pro-inflammatory transcriptome and phenotype in primary human endothelial cells.
Alejandro Zentella-Dehesa, Janini Mejía-Rangel, Emilio J Córdova, Lorena Orozco, Ventura-Gallegos JL, Felipe Vadillo-Ortega, Patricio Gariglio
Facultad de Medicina, UNAM/INMEGEN. IIB-UNAM. INCMNSZ

10:45 – 11:00 *Caenorhabditis elegans* as a model to study the involvement of microRNAs in lipotoxicity
Jonathan Alcántar Fernández, Ana M Salazar Martínez, Rosa E Navarro González, Martha Elva Pérez Andrade, Juan Miranda Ríos
Unidad de Genética de la Nutrición, IIB, UNAM e Instituto Nacional de Pediatría

9:00 – 11:00
Vallarta Room

Technical Conferences II

Chair: **Adriana Julián Sánchez**
Facultad de Medicina, UNAM

9:00 – 9:30 Cell Mutiparameter Analysis using Fluorescence Microscopy. High-content analysis – Automated imaging and analysis of cellular assays
Sandra Rosa da Silva
Product Group Manager - Latin America
GE Healthcare Life Sciences

9:30 – 10:00 Reporter Bioassays to Assess Therapeutic Antibodies in ADCC and Immunotherapy Programs
Mei Cong
Director of Customer Assay Services. Department of Research, Promega Corporation
UNIPARTS

10:00 – 10:30 Optimizing protein purification using new Chromatography generation (NGC)
Mariana Perez
Especialista en Aplicaciones México
BIORAD

10:30 – 11:00 Fluorescence-based Detection of Proteins: Quantification in Western Blots and in Cells
Michael Van Waes
QUIMICA VALANER

11:00 – 11:30 Coffee Break
Foyer Guadalajara Room

11:30 – 12:30 Oral Presentations 10
Guadalajara Room I

Cell Differentiation and Immunology

Chair: **Omar Arroyo Helguera**
Universidad Veracruzana

11:30 – 11:45 Cortactin deficiency causes increased ROCK1-mediated actin-contractility and decreased adrenomedullin secretion leading to enhanced endothelial permeability
Alexander García-Ponce, Alí Francisco Citalán-Madrid, Hilda Vargas-Robles, Abigail Betanzos, Klemens Rottner, Dietmar Vestweber, Michael Schnoor
Department of Molecular Biomedicine, CINVESTAV – IPN Zacatenco

11:45 – 12:00 Asymmetric Cell Division of Corneal Epithelial Cells
Eber Gómez-Flores, Erika Sánchez-Guzmán, Federico Castro-Muñozledo
Departamento de Biologia Celular, CINVESTAV – IPN Zacatenco

12:00 – 12:15 Does IFC-305 compound could be modulating the Kupffer cell phenotype?
Rebeca Pérez Cabeza de Vaca, Nuria Guerrero Celis and Victoria E Chagoya de Sánchez
Biologia Celular y Desarrollo, IFC, UNAM

12:15 – 12:30 HS1 regulates the neutrophil extravasation cascade during inflammation by converging PKA signaling into integrin activation
Michael Schnoor, Dietmar Vestweber
Molecular Biomedicine, CINVESTAV – IPN Zacatenco

11:30 – 12:30 Oral Presentations 11
Guadalajara Room II

Biochemistry IV

Chair: **Vicente Castrejón Téllez**
Instituto Nacional de Cardiología "Ignacio Chávez"

11:30 – 11:45 Allostery and cooperativity, together forever?
Jorge Marcos Víquez and Mario L. Calcagno
LFQIP, Bioquímica, Facultad de Medicina, UNAM

11:45 – 12:00 Studies on functional divergence between *Saccharomyces cerevisiae ALT1* and *ALT2* using *Kluyveromyces lactis KlALT1* and *Lachancea Kluyveri LkALT1* as "ancestral type yeast
Ximena Martínez de la Escalera Fanjul, Maritrini Colón González, Lina Riego-Ruiz & Alicia González
Bioquímica y Biología Estructural. Instituto de Fisiología Celular UNAM

12:00 – 12:15 Physicochemical properties correlate with evolutionary categories of bacterial expansins
Claudia Martínez-Anaya, Sonia Dávila, Ernesto Pérez-Rueda, Nina Pastor, Lorenzo Segovia
Instituto de Biotecnología, UNAM

12:15 – 12:30 *In vivo* detection of compounds from organisms by mass spectrometry using low-temperature plasma (LTP) ionization
Sandra Martínez-Jarquín, Robert Winkler
Biotecnología y Bioquímica. CINVESTAV Unidad Irapuato

11:30 – 12:30 Oral Presentations 12
Guadalajara Room III

Genetics

Chair: **Fernando Fernández Ramírez**
Hospital General de México

11:30 – 11:45 Study of the expression of gene *Ccs* (*Capsanthin-capsorubin synthase)* AT differente maturation stages in two different morphotypes of Habanero Pepper (*Capsicum chinense Jacq.)* by RT-PCR Real-Time Technique
Margarita Aguilar-Espinosa, Fátima Alejandra Borges-García, Renata Rivera- Madrid
Centro de Investigación Científica de Yucatán

11:45 – 12:00 Detection of mutations related to Multidrug-Resistencia in clinical *Mycobacterium tuberculosis* from Mexico by next generation sequencing technology
Minerva Mata Rocha, Vanessa M. González Covarrubias, Omar F. Cruz Correa, Francisco X. Soberón Mainero
Instituto Nacional de Medicina Genómica

12:00 – 12:15 Analysis of the effects of HPV16 E2 expression on the cell cycle in HaCaT cells
Janet Sánchez Ramos, Victoria Domínguez Catzín, Alfredo García Venzor, Erick Gustavo Ramírez Salazar and José Efraín Garrido Guerrero
Genética y Biología Molecular. CINVESTAV – IPN Zacatenco

12:15 – 12:30 The nuclear higher-order structure (NHOS) defined by the topological relations DNA-nuclear matrix is species-specific
Evangelina Silva-Santiago and Armando Aranda-Anzaldo
Laboratorio de Biología Molecular, Facultad de Medicina, UAEM

12:30 – 14:00 Lunch

14:00 – 16:00 Plenary Session 5
Guadalajara Room I

Evolutionary Genomics Hispanic-Mexican

Chair: **Alexander De Luna Fors**
LANGEBIO CINVESTAV Irapuato

14:00 – 14:30 Evolution of genomes by copy and paste: phylogenomics reveals non-vertical evolutionary relationships in fungi
Toni Gabaldón
ICREA Research Professor, Comparative Genomics Group, Centre for Genomic Regulation (CRG), Barcelona, Spain

14:30 – 15:00 Ecological metagenomics of cycads and their bacterial symbionts: metabolic insights into a million-year old association
Angélica Cibrian-Jaramillo, Pablo Cruz, Antonio Hernández, José Antonio Corona, Luis Delaye, Francisco Barona-Gómez.
Laboratorio Nacional de Genómica de la Biodiversidad, CINVESTAV

15:00 – 15:30 Experimental evolution of genome architecture and complexity in RNA viruses
Santiago F. Elena, Anouk Willemsen, José L. Carrasco, Mark P. Zwart
Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain

15:30 – 16:00 Evolution of the transcription circuit underlying biofilm formation in *Candida* species
Eugenio Mancera, Alexander D. Johnson
Microbiology & Immunology, University of California, San Francisco, USA

14:00 – 16:00 Plenary Session 6
Guadalajara Room II

Physiological Relevance of Signiling Pathways Crosstalk

Chair: **Marina Macias Silva**
Instituto de Fisiología Celular, UNAM

14:00 – 14:30 TGF-beta and GPCR Signals Converge to Regulate the Stability of Ski and SnoN Transcriptional Cofactors in Hepatocytes
Marina Macías Silva
Departamento de Biología Celular y Desarrollo, IFC - UNAM

14:30 – 15:00 RhoGEFs: integral components of signaling modules
José Vázquez-Prado
Department of Pharmacology, CINVESTAV-IPN Zacatenco

15:00 – 15:30 Modulation of Toll-like receptor 4 (TLR4) signaling in mast cells
Claudia González Espinosa
Departamento de Farmacobiología, CINVESTAV-IPN Sede Sur

15:30 – 16:00 Modulation of G protein-coupled receptors by receptor tyrosine kinases
J. Adolfo García-Sáinz
Instituto de Fisiología Celular, UNAM

14:00 – 16:00 Plenary Session 7
Guadalajara Room III

Genomics of Mendelian Diseases in NorthWestern Mexico

Simposio Universidad Anfitriona

Chair: **Bertha Ibarra Cortés**
Universidad de Guadalajara

14:00 – 14:30 Genomic approach of a monogenic disease: hemophilia as a model
Ana Rebeca Jaloma Cruz
Centro de Investigación Biomédica de Occidente, IMSS

14:30 – 15:00 Hemoglobinopathies
Bertha Ibarra Cortés and F. Javier Perea
Centro Universitario en Ciencias de la Salud, Universidad de Guadalajara

15:00 – 15:30 Fragile X Syndrome and Cancer
J. Patricio Barros Núñez, Mónica Rosales Reynoso, Berenice Ochoa Hernández
Centro de Investigación Biomédica de Occidente, IMSS

15:30 – 16:00 Application of Genomic Medicine to Chronic-Degenerative disorders
Juan S. Armendáriz Borunda
Centro Universitario en Ciencias de la Salud, Universidad de Guadalajara

14:00 – 16:00 Plenary Session 8
Vallarta Room

Molecular Biology of Viruses

Chair: **Ramón González García Conde**
Facultad de Ciencias, UAEM

14:00 – 14:30 Protective immune response against rotavirus infection
Fernando Esquivel-Guadarrama
Facultad de Medicina, UAEM

14:30 – 15:00 Epidemiology and evolution of Influenza A virus, application of next-generation sequencing
Pavel Isa Haspra
Instituto de Biotecnología. UNAM

15:00 – 15:30 Molecular pathogenesis of human papillomavirus oncoproteins
Lourdes Gutiérrez Xicoténcatl
CISEI, Instituto Nacional de Salud Pública

15:30 – 16:00 The adenoviral oncoproteins: Multiple roles in virus-cell interactions
Ramón A. González
Facultad de Ciencias, UAEM

16:15 – 17:15 Oral Presentations 13
Guadalajara Room I

Neuroscience and Neurobiology

Chair: **Patricia Ferrera Boza**
Instituto de Investigaciones Biomédicas, UNAM

16:15 – 16:30 Systemic activation of innate immunity induced by LPS inhibits long- and short-term plasticity and increases phosphorylation of TrkB receptor in the MF-CA3 synapse of the hippocampus
Martin Avila and Emilio J. Galvan
Department of Pharmacobiology. CINVESTAV-IPN South Campus

16:30 – 16:45 Sirtuin modulation protects against spinal motor neuron loss induced by chronic excitotoxicity *in vivo*
Rafael Lazo-Gómez, Ricardo Tapia
División de Neurociencias, Instituto de Fisiología Celular, UNAM

16:45 – 17:00 TRPV1 channel regulation by steroidal molecules and its analgesic effect
Sara L. Morales-Lázaro, Itzel Llorente, Félix Sierra-Ramírez, Barbara Serrano-Flores, Carlos Díaz-García, Marcia Hiriart, Sidney A. Simon, Tamara Rosenbaum.
División de Neurociencias, Instituto de Fisiología Celular, UNAM

17:00 – 17:15 Striato-nigral circuits control sugar-induced inflexible intake
Luis A Tellez, Tatiana L Ferreira, Sara Medina, Wenfei Han and Ivan E de Araujo.
The J.B. Pierce Laboratory & Department of Psychiatry. Yale University.

16:15 – 17:15 Oral Presentations 14
Guadalajara Room II

Toxicology

Chair: **Federico Centeno Cruz**
Instituto Nacional de Medicina Genómica

16:15 – 16:30 Anti -tumor and Immuno modulatory Effect of 6-Pentadecyl Salicylic Acid in an *in Vivo* Model
Josephin Nerling Rashida Gnanaprakasam, Estrada Muñiz E, Vega L
Departamento de Toxicología.CINVESTAV – IPN Zacatenco

16:30 – 16:45 Las nanopartículas de dióxido de titanio son internalizadas mediante fagocitosis en células gliales
Elizabeth Huerta-García, Sandra Gissela Márquez-Ramírez, María del Pilar Ramos-Godinez, Ernesto Alfaro-Moreno, Alejandro López-Saavedra, Rebeca López-Marure.
Instituto Nacional de Cardiología "Ignacio Chávez"

16:45 – 17:00 Diethyldithiophosphate (DEDTP) Induces Changes in Tumor Infiltrating Lymphocytes, Macrophages and Natural Killer Cells in 4T1 Murine Breast Cancer Model
Dunia Margarita Medina Buelvas, Elizabeth Estrada Muñiz, Libia Vega Loyo
Departamento de Toxicología.CINVESTAV – IPN Zacatenco

17:00 – 17:15 CYP2E1 induction leads to oxidative stress and cytotoxicity in cerebellar granule neurons
Valencia-Olvera Ana Carolina, Morán Julio, Camacho-Carranza Rafael, and Espinosa-Aguirre Jesús Javier
Instituto de Investigaciones Biomédicas, UNAM

16:15 – 17:15 Oral Presentations 15
Guadalajara Room III

Signal Transduction I

Chair: **Teresa Hernández Sotomayor**
Centro de Investigación Científica de Yucatán

16:15 – 16:30 Analysis of profibrosing molecules in cells stimulated with cigarette smoke extract
Semiramis Stephania García Trejo, Marco Antonio Checa Caratachea, Moisés Eduardo Selman Lama, Annie Pardo Cemo, Víctor Manuel Ruiz López.
Instituto Nacional de Enfermedades "Respiratorias. Ismael Cosio Villegas"

16:30 – 16:45 Smad transcriptional co-factors Ski and SnoN exhibit a highly dynamic turnover in normal *versus* transformed hepatocytes
Cassandre Caligaris, Genaro Vázquez-Victorio, Marcela Sosa-Garrocho, Diana G. Ríos-López, Alvaro Marín-Hernández, Rafael Moreno-Sánchez, Marina Macías-Silva
Instituto de Fisiología Celular, UNAM

16:45 – 17:00 GPI/AMF inhibition blocks the development of the metastatic phenotype of mature multi-cellular tumor spheroids
Juan Carlos Gallardo-Pérez and Sara Rodríguez-Enríquez
Departamento de Bioquímica. Instituto Nacional de Cardiología

17:00 – 17:15 The flavonoid epicatechin actives the PI3K/Akt pathway in healthy mouse's hearts
Sergio De los Santos Enriquez, Viridiana García Pérez, Israel Ramírez Sánchez, Ileana Patricia Canto Cetina, Ramón Mauricio Coral Vázquez
Escuela Superior de Medicina-IPN. CMN 20 de Noviembre, ISSSTE.

17:15 – 19:15 Poster Session 2

B	BASIC BIOCHEMISTRY	38 – 75
BT	BIOTECHNOLOGY	41 – 83
E	BIOCHEMISTRY EDUCATION	1 – 5
OR	OXYGEN REACTIVE SPECIES	1 – 18
I	IMMUNOLOGY	1 – 16
M	MEDICINE, HEALTH &NUTRITION	1 – 23
MP	MICROBIOLOGY & PARASITOLOGY	1 – 22
RG	GENETIC REGULATION & EPIGENETIC	23 – 45
ST	SIGNAL TRANSDUCTION	33 – 63

Jalisco Room

19:30 – 20:30
Guadalajara Room

Plenary Lecture II

The control of transcription during early development
Michael Eisen
Howard Hughes Medical Institute
University of California, Berkeley

Chair: **Guadalupe Espin**
Instituto de Biotecnología, UNAM

9:00 – 11:00 Oral Presentations 16
Guadalajara Room I

Signal Transduction II

Chair: **Roberto Sánchez Olea**
Universidad Autónoma de San Luis Potosí

9:00 – 9:15 Exploring the role of Rho GTPases on leptin-induced migration, of breast cancer cells in culture
Eduardo Castañeda-Saucedo, Napoleón Navarro-Tito, Miguel A. Mendoza-Catalán, Mercedes Calixto-Gálvez, Itzel Carranza-Mendoza
Universidad Autónoma de Guerrero

9:15 – 9:30 Compromised Pak1 activity sensitizes FA/BRCA-proficient breast cancer cells to PARP inhibition
Luis Enrique Arias Romero, Olga Villamar Cruz y Jonathan Chernoff
Cancer Biology Program, Fox Chase Cancer Center, USA. FESI, UNAM

9:30 – 9:45 Akt inhibits β-catenin signaling
Itzel Zenidel Gutiérrez Martínez, José Antonio Hernández Trejo, Aurora Candelario Martínez and Porfirio Nava Domínguez
Departamento de Fisiología, Biofísica y Neurociencias. CINVESTAV, IPN Zacatenco

9:45 – 10:00 Akt-induced apoptosis during inflammation is regulated by 14.3.3 proteins
José Antonio Hernández Trejo, Itzel Zenidel Gutiérrez Martínez, Mauricio Efrén Gómez Suarez, Marcela Hernández Ruiz, Dimelza Lisett Suarez Pérez, Aurora Antonia Candelario Martínez, Jose Luna Muñoz, Vianney Ortiz Navarrete and Porfirio Nava Domínguez
Fisiología, Biofísica y Neurociencias. CINVESTAV – IPN Zacatenco

10:00 – 10:15 Novel RACK1 ligands from the Dinoflagellate *Symbiodinium microadriaticum* identified by the yeast two-hybrid system
Tania Islas Flores and Marco A. Villanueva Méndez
Instituto de Ciencias del Mar y Limnología, Unid. Acad. de Sist. Arrecifales, UNAM

10:15 – 10:30 Real time monitoring of the acrosomal reaction in human sperm
Esperanza Mata Martínez, Claudia Sánchez-Cárdenas, Alberto Darszon y Claudia L Treviño
Instituto de Biotecnología-UNAM

10:30 – 10:45 Functional characterization of *AtGRDP1* gene during the growth and development of *Arabidopsis thaliana*
Carlos Vladimir Muro-Medina, Aída Araceli Rodríguez-Hernández, Adriana Leticia Salazar Retana, Jocelin Itzel Ramírez Alonso and Juan Francisco Jímenez-Bremont
División de Biología Molecular. IPICyT

10:45 – 11:00 Futile Hog1p phosphorylation in response to hiperosmotic stress in *Saccharomyces cerevisiae*
Aracely Vázquez–Ibarra, Miriam Rodríguez-González and Roberto Coria.
Genética Molecular, Instituto de Fisiología Celular, UNAM

9:00 – 11:00 Oral Presentations 17
Guadalajara Room II

Biochemistry V

Chair: **Nuria Sánchez Puig**
Instituto de Química, UNAM

9:00 – 9:15 Dynamic characterization of the native state of amiloidogenic protein 6aJL2 and the mutant R24G
Roberto Carlos Maya Martinez, Paloma C. Gil Rodríguez, Carlos Amero Tello
Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos

9:15 – 9:30 Molecular cloning, expression and transport analysis of the first SWEET transporter from *Zea mays*
Sobeida Sánchez-Nieto, Roberto D.Carvente-García, Sara L. Morales-Lázaro, Tamara Rosenbaum, Paulina Aguilera-Alvarado
Departamento de Bioquímica, Facultad de Química, UNAM

9:30 – 9:45 Variable and new electron transport pathways in *Candida albicans*, and other surprises.
Antonio Peña, Norma Silvia Sánchez, and Martha Calahorra
Department of Molecular Genetics. Instituto de Fisiología Celular. UNAM

9:45 – 10:00 Free amino acids profile and consumption during early development of the Pacific red snapper *Lutjanusperu* larvae
Renato Peña, Silvie Dumas, Ivette Moguel-Hernández, Noemí García-Aguilar
Unidad Piloto de Maricultivos. CICIMAR-IPN

10:00 – 10:15 The folding mechanism of the lysine, arginine, ornitine binding protein (LAO)
Jesús Renan Vergara Gutiérrez, Haven A. López Sánchez, Nancy O. Pulido-Mayoral, Alejandro Sosa Peinado, Rogelio Rodríguez-Sotres, D. Alejandro Fernández Velasco
Departamento de Bioquímica. Facultad de Medicina.UNAM

10:15 – 10:30 Kinetic characterization of glycogen synthesis and degradation pathways in tumor cells
Marco Antonio Reyes-García and Álvaro Marín-Hernández
Departamento de Bioquímica, Instituto Nacional de Cardiología, Ignacio Chávez

10:30 – 10:45 Purification and characterization of Alt1 and Alt2 of *Saccharomyces cerevisiae*: Functional divergence of the enzymes involved in alanine metabolism
Rojas- Ortega Eréndira, Diaz -Guerrero Miguel Ángel, Gonzalez- Manjarrez Alicia
Instituto de Fisiología Celular, UNAM

10:45 – 11:00 Pseudohysteresis in an enzyme following a ping pong bi bi kinetic mechanism. The case of Thioredoxin-glutathionereductase (TGR)
Juan Luis Rendón, Alberto Guevara Flores, I. Patricia del Arenal Mena, and Juan Pablo Pardo
Departamento de Bioquímica, Facultad de Medicina, UNAM

9:00 – 11:00 Oral Presentations 18
Guadalajara Room III

Microbiology and Parasitology II

Chair: **Irma Romero Álvarez**
Facultad de Medicina, UNAM

9:00 – 9:15 A *Burkholderia cenocepacia* gene encoding a non-functional tyrosine phosphatase is required for the delayed maturation of the bacteria-containing vacuoles in macrophages
Angel Andrade and Miguel A. Valvano
Microbiology and Immunology, University of Western Ontario

9:15 – 9:30 Characterization of lignocellulolytic activities from a halophile strain of Aspergillus caesiellus isolated from a sugarcane bagasse fermentation
Ramón Alberto Batista-García, Edgar Balcázar-López, Estefan Miranda-Miranda, Ayixón Sánchez-Reyes, Laura Cuervo-Soto, Denise Aceves-Zamudio, Karina Atriztán-Hernández, Catalina Morales-Herrera, Rocío Rodríguez-Hernández and Jorge Folch-Mallol
Centro de Investigación en Biotecnología, UAEM

9:30 – 9:45 The nonphosphorylated IIA^{Ntr} protein induces RpoS degradation by ClpAP protease in *Azotobacter vinelandii*
Luis Felipe Muriel Millan, María Soledad Moreno León, Guadalupe Espín Ocampo
Microbiología Molecular, Instituto de Biotecnología, UAM

9:45 – 10:00 Inhibitory effect of *Parthenium hysterophorus* on *Helicobacter pylori* adherence *in vitro*
Jazmín P. Espinosa Rivero, Suleima Ríos Tovar, Erika P. Rendón Huerta, Martha Lydia Macías Rubalcava and Irma Romero
Bioquímica. Facultad de Medicina, UNAM

10:00 – 10:15 The recombinant enzyme 3-hydroxy-3-methyl glutaryl coenzyme A reductase from *Candida glabrata* (rec-HMGRCg) as a model for studying synthetic inhibitors
Dulce María-Andrade Pavón, José Antonio-Ibarra, César Hugo-Hernández Rodríguez, Lourdes-Villa Tanaca
Microbiología, Escuela Nacional de Ciencias Biológicas – IPN

10:15 – 10:30 Isolation and partial characterization of TATA binding protein (TBP) gene of *Taenia solium*
Oscar Rodríguez-Lima, Lucia Jiménez, Ángel Zarain-Herzberg, Ponciano García-Gutiérrez and Abraham Landa
Departamento de Microbiología y Parasitología, Facultad de Medicina, UNAM

10:30 – 10:45 Membrane topology and identification of functional amino acids in the ArnT protein from *Burkholderia cenocepacia*
Faviola Tavares-Carreon, Kinnari B. Patel and Miguel A. Valvano
Microbiology and Immunology, Western University, London, Ontario, Canada

10:45 – 11:00 Synthesis of acetate in *Entamoeba histolytica*
Citlali Vázquez Martínez, Erika Pineda, Emma Saavedra
Departamento de Bioquímica. Instituto Nacional de Cardiología Ignacio Chávez

9:00 – 11:00
Vallarta Room

DAAD CONFERENCE

Research in Germany

9:00 – 9:40 Investigación en Alemania: Consejos sobre la organización y el financiamiento de proyectos conjuntos de investigación
Susanne Faber
Coordinadora de Marketing, DAAD México

9:40 – 10:20 Los programas de beca de la Fundación Alexander von Humboldt (AvH)
Claudia L. Treviño Santa Cruz
Representante Alexander von Humboldt Stiftung

10:20 – 11:00 Los programas de la Fundación Alemana para la Investigación Científica (DFG)
Christina Siebe
Representante Deutsche Forschungsgemeinschaft (DFG)

11:00 – 11:30 Coffee Break
Foyer Guadalajara Room

11:30 – 12:30
Guadalajara Room

Plenary Lecture III

Dynamics of Endocytosis
Tomas Kirchhausen
Boston Children´s Hospital

Chair: **Maria Eugenia Gonsebatt**
Instituto de Investigaciones Biomédicas, UNAM

12:30 – 14:00 Lunch

14:00 – 16:00 Plenary Session 9
Guadalajara Room I

Frontiers in Bioenergetics and Mitochondrial Function

Chair: **Marietta Tuena Sangri**
Instituto de Fisiología Celular, UNAM

14:00 – 14:30 Protein synthesis inside mitochondria: the story of subunit 1 of the cytochrome *c* oxidase
Xochitl Pérez Martínez, Yolanda Camacho Villasana, Angélica Zamudio Ochoa, Juan Pablo Mayorga Juárez, Rodolfo García Villegas, Miguel Shingú Vázquez, Aldo E. García Guerrero, Emmanuel Frías Jiménez
Genética Molecular, Instituto de Fisiología Celular, UNAM

14:30 – 15:00 Dissecting the peripheral arm of the mitocondrial ATP synthase of chlorophycean algae.
Diego González-Halphen, Miriam Vázquez-Acevedo, Héctor Miranda-Astudillo, Martha Lilia Colina-Tenorio, Araceli Cano-Estrada, Lorenzo Sánchez-Vásquez y Alexa Villavicencio-Queijeiro
Instituto de Fisiología Celular, UNAM

15:00 – 15:30 Unveiling the δsubunit of the α-proteobacterial F_1F_o-ATPase nanomotor: a novel natural inhibitor of rotation different to bacterial ε and mitochondrial IF_1.
Mariel Zarco-Zavala, Edgar Morales-Ríos, Guillermo Mendoza-Hernández, Gerardo Pérez-Hernández, Francisco Mendoza-Hoffmann, Raquel Ortega, and José J García-Trejo
Facultad de Química and Facultad de Medicina, UNAM

15:30 – 16:00 Energy associated with the binding of nucleotides in the isolated α and β subunits and their role in $(\alpha\beta)_3$ and $\alpha_3\beta_3\gamma$ subcomplexes.
Guillermo Salcedo Barrientos, Marietta Tuena de Gómez-Puyou, Enrique García Hernández
Bioquimica y Biología Estructural. Instituto de Fisiología Celular, UNAM

14:00 – 16:00 Plenary Session 10
Guadalajara Room II

Genomics

Chair: **Luis Herrera Estrella**
LANGEBIO – CINVESTAV – IPN Irapuato

14:00 – 14:30 Genomic history of domestication of common bean
Alfredo Herrera-Estrella, Anna Vlasova, Martha Rendón-Anaya, Salvador Capella-Gutiérrez, Miguel Hernández-Oñate, André Minoche, IonasErb, Francisco Câmara-Ferreira, Pablo Prieto-Barja, André Corvelo, Walter Sanseverino, Gastón Westergaard, Juliane C. Dohm, Georgios JoanisPappas Jr, Soledad Saburido-Alvarez, DarekKedra, Irene Gonzalez, Luca Cozzuto, María A. Aguilar-Morón, Nuria Andreu, O. Mario Aguilar, Jordi García-Mas, Maik Zehnsdorf, Martín P. Vázquez, Alfonso Delgado-Salinas, Luis Delaye, Ernesto Lowy, Alejandro Mentaberry, Rosana P. Vianello-Brondani, José Luís García, Tyler Alioto, Federico Sánchez, Heinz Himmelbauer, Marta Santalla, CedricNotredame, Toni Gabaldón, and Roderic Guigó
LANGEBIO – CINVESTAV – IPN Irapuato

14:30 – 15:00 Architecture and evolution of a minute plant genome
Enrique Ibarra-Laclette, Eric Lyons, Victor Albert and **Luis Herrera-Estrella**
LANGEBIO – CINVESTAV – IPN Irapuato

15:00 – 15:30 Common bean transcriptomics: analysis of global regulators for the rhizobia symbiosis and the abiotic stress responses
Georgina Hernández, Jamie O'Rourke, Luis P. Íñiguez, Damien Formey, Bárbara Nova-Franco, Ana B Mendoza-Soto, Alfonso Leija, Sara I. Fuentes, Pablo Peláez, Mario Ramírez, Federico Sánchez, Lourdes Girard, José L. Reyes, Carroll P. Vance
Centro de Ciencias Genómicas, UNAM

15:30 – 16:00 Genome-wide perspectives on evolution and molecular adaptation in polar bear
Charlotte Lindqvist
Department of Biological Sciences, University at Buffalo (SUNY), Buffalo

14:00 – 16:00 Plenary Session 11
Guadalajara Room III

Mechanisms of Neurodegeneration

Chair: **Carlos Beas**
GENIAR, Universidad de Guadalajara

14:00 – 14:30 Neuronal damage and epileptogenesis induced by monosodium glutamate neonatal treatment
Monica E Ureña-Guerrero y Carlos Beas-Zárate
Departamento de Biología Celular y Molecular, CUCBA, Universidad de Guadalajara

14:30 – 15:00 Mast cells and their involvement in brain damage and epileptogenesis processes
Luisa Rocha
México

15:00 – 15:30 Ferritin a stress oxidative marker in the neurodegenerative processes
Alberto Lazarowski
Facultad de Farmacia y Bioquímica- Universidad de Buenos Aires, Argentina

15:30 – 16:00 Are early metabolic alterations in the hippocampus of the mouse model APPswe/PS1d9 alzheimer's disease the cause of disease?
Antoni Camins Espuny
Centro de Investigación Biomédica en Red. Facultad de Farmacia. España

14:00 – 16:00 Workshop
Vallarta Room

Workshop PLoS-Open Acces Publishing

Chair: **Michael Eisen**
Howard Hughes Medical Institute
University of California, Berkeley

16:15 – 18:15 Oral Presentations 19
Guadalajara Room I

Medicine, Health &Nutrition

Chair: **Alfredo Tellez Valencia**
Universidad Juárez del Estado de Durango

16:15 – 16:30 Silencing of the mitochondrial calcium uniporter improves post-ischemic cardiac dysfunction and attenuates mitochondrial Ca^{2+} overload and apoptosis in rat myoblast and cardiomyocytes
Yuriana Oropeza-Almazán Alberto Marbán, Eduardo Reyes, Jesús R. Garza y Gerardo García-Rivas
Escuela de Medicina y Ciencias de la Salud. Tecnológico de Monterrey

16:30 – 16:45 Energy metabolism in human triple-negative breast carcinoma
Silvia Cecilia Pacheco Velázquez and Sara Rodríguez Enríquez.
Departamento de Bioquímica, Instituto Nacional de Cardiología "Ignacio Chávez"

16:45 – 17:00 Membrane fluidity of kidney microsomes during development of diabetes
Ismael Herminio Pérez Hernández, Ricardo Mejía Zepeda
Facultad de Estudios Superiores Iztacala, UNAM

17:00 – 17:15 Genetic determinants associated with antibiotics resistance in *Mycobacterium tuberculosis* strains from Michoacán
Liliana Pérez Reyes, Gerardo Vázquez Marrufo, Christian Serrato Morales, Miriam Espinoza-de Anda y Ma. Soledad Vázquez-Garcidueñas
Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez". Universidad Michoacana

17:15 – 17:30 Expression and clinical relevance of heat shock proteins of 90 kDa, Hsp90α and Hsp90β, in patients with renal carcinoma
Nadia Aglae Rangel-Gauna, Ma. Delia Pérez-Montiel, Miguel A. Jiménez-Ríos, David Cantú de León, Luis Alonso Herrera-Montalvo, Carlo César Cortés-González
Instituto Nacional de Cancerología, IIB, UNAM

17:30 – 17:45 MicroRNA amplification from neonatal screening samples
Sandra Patricia Rodil García, Angélica Montoya Contreras, Luis Antonio Salazar Olivo
Instituto Potosino de Investigación Científica y Tecnológica

17:45 – 18:00 Combination of cell therapy and gene therapy reduces experimental liver fibrosis
Alejandra Meza-Ríos, Juan Armendáriz-Borunda, Leonel García-Benavides, Jesús García-Bañuelos, Adriana Salazar-Montes, Ana Sandoval-Rodríguez
Institute for Molecular Biology and Gene Therapy, Department of Molecular Biology and Genomics, Health Sciences University Center, University of Guadalajara

18:00 – 18:15 Drug resistance promoted by hypoxia in human medulloblastoma cells involves modulation of extracellular pH control proteins, cytochrome P450 enzymes and cell cycle arrest
Jesús Valencia Cervantes, Daniel Martínez Fong, Sara Rodríguez Enríquez, José Antonio Arias Montaño, Víctor Manuel Dávila Borja
Laboratorio de Oncología Experimental, Instituto Nacional de Pediatría

16:15 – 18:15 Oral Presentations 20
Guadalajara Room II

Biochemistry VI

Chair: **Juan Pablo Pardo**
Facultad de Medicina, UNAM

16:15 – 16:30 Lipid droplet accumulation in *Ustilago maydis* depends on the nitrogen source
Lucero Romero Aguilar, Guadalupe Guerra Sánchez, Ariana Zavala Moreno, Juan Pablo Pardo Vázquez, Mónica Montero Lomelí, Christian A Cárdenas Monroy
Microbiología. Escuela Nacional de Ciencias Biológicas, IPN

16:30 – 16:45 Kinetic and thermodynamic control in the thermal unfolding of bacterial Triosephosphate Isomerases
Sergio Romero Romero, Miguel A. Costas Basín, Adela Rodríguez Romero, D. Alejandro Fernández Velasco
Fisicoquímica e Ingeniería de Proteínas, Facultad de Medicina, UNAM

16:45 – 17:00 Using X-rays to describe electronic fluxes in REDOX enzymes
Enrique Rudiño-Piñera, Eugenio De la Mora, Hugo Serrano-Posada, Sonia Patricia Rojas-Trejo, César S. Cardona-Félix and Claudia Rodríguez-Almazán
Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

17:00 – 17:15 Different expression of the inhibitory δsubunit of the F_1F_O-ATPase nanomotor among free-living, symbiotic, and parasiticα-proteobacteria
Francisco Mendoza-Hoffmann, Mariel Zarco-Zavala, Raquel Ortega, Edgar Morales-Ríos & José J García-Trejo
Biología, Facultad de Química, UNAM

17:15 – 17:30 Evolutive and structural correlation of reconstructed eukaryotic ancestors of the enzyme *triosephosphate isomerase*.
Mariana Schulte-Sasse Jiménez, Nancy O Pulido Mayoral, Miguel Costas Basín, Enrique García Hernández, Adela Rodríguez Romero and D. Alejandro Fernández-Velasco
Facultad de Medicina, UNAM

17:30 – 17:45 Functionalization of Multi-Walled Carbon Nanotubes with *Amaranthus Leucocarpus* Lectin
Carlos Alejandro Vásquez Martínez, Pedro Hernández Cruz, Itandehui Gallegos Velasco, Yadira Gochi Ponce, Alma Dolores Pérez Santiago
Instituto Tecnológico de Oaxaca

17:45 – 18:00 Disentangling the tertiary-quaternary coupling mechanism of allosteric transition of Glucosamine-6-phosphate deaminase of *Escherichia coli*.
Roberto Jareth Vazquez-Nuñez, Sergio Zonszein Strauss, Laura I Álvarez-Añorve, Mario L Calcagno
Departamento de Bioquímica, Facultad de Medicina, UNAM

18:00 – 18:15 Structural and thermodynamic study of the LAO protein of S. typhimurium and the binding of different ligands
Rubén Priego Jiménez, Nancy O Pulido Mayoral, Alejandro Sosa Peinado, Enrique Rudiño Piñera and D Alejandro Fernández Velasco
Departamanto de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

16:15 – 18:15 Oral Presentations 21
Guadalajara Room III

Genetic Regulation

Chair: **Alma Rosa Villalobos**
Universidad de Guadalajara

16:15 – 16:30 Global changes in expression of microRNAs in *Caenorhabditis elegans* worms subjected to fasting
Laura García Segura, Armando Hernandez Mendoza, Cei Abreu Goodger, Martha Elva Perez Andrade, Jonathan Alcantar Férnandez, Juan Miranda Rios
Unidad de Genética de la Nutrición. IIB, UNAM e Instituto Nacional de Pediatría

16:30 – 16:45 Systematic identification of subtelomeric silencing pathways in *Saccharomyces cerevisiae*
Alejandro Juárez-Reyes, Jhonatan Hernández-Valdés & Alexander de Luna
Laboratorio Nacional de Genómica para la Biodiversidad. CINVESTAV – IPN

16:45 – 17:00 Epigenetic deregulation of BORIS and CTCF in breast cancer
Iliana Alcalá-Moreno, Ernesto Soto-Reyes, Daniela Morales-Espinosa, Lissania Guerra-Calderas, Rodrigo González-Barrios, Héctor Aquiles Maldonado-Martínez, David Cantú de León, Clementina Castro, Luis A. Herrera
Unidad de Investigación Biomédica en Cáncer – INCAN / IIB, UNAM

17:00 – 17:15 The role of ADX proteins in chromatin organization
Silvia Meyer Nava, Mario Zurita and Viviana Valadez Graham
Instituto de Biotecnología, UNAM

17:15 – 17:30 Impact of Random Mutagenesis of the Transcriptional Regulator IscR of *Dickeya dadantii* Over Genetics Regulation of Iron-Sulfur Clusters Biogenesis
Diego Eloyr Navarro-López, Evangelina Esmeralda Quiñones-Aguilar, Joaquín Alejandro Qui-Zapata, Gabriel Rincón-Enríquez
Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco AC

17:30 – 17:45 Aim25 and its role during aging and oxidative stress in the yeast *Saccharomyces cerevisiae*
José Luis Aguilar, Raymond Laboy, Fabiola Jaimes, Alexander de Luna y Soledad Funes
Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV – Irapuato

17:45 – 18:00 Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichoderma atroviride*
Beltrán-Hernández Emma, Molina-Torres Jorge, Herrera-Estrella Alfredo
Laboratorio Nacional de Genómica para la Biodiversidad CINVESTAV- Irapuato

18:00 – 18:15 Global Methylation Profiling in Mexican Patients with Locally Advanced Cervical Cancer
Marcela Angélica De La Fuente Hernández, Jorge Fernández Retana, David Francisco Cantú de León, Jaime Alberto Coronel Martínez, Carlos Guadalupe Pérez Plasencia
Instituto Nacional de Cancerología

16:15 – 18:15 Having coffee with.....
Tonala Room

Four Round Tables for students chosen from oral presentations

Tomas Kirchhausen	José Carlos Campero	José Manuel Villalobos Escobedo
	Diana Sánchez Rangel	Ana Cristina García Gaytán
	Jonathan Alcántar Fernández	Sobeida Sánchez Nieto
	José Alfredo Villanueva	Jesús Renán Vergara
	Carol Martínez Camacho	Araceli Vázquez Ibarra
	Chair: *Rubén López Revilla*	
Michael Eisen	James González	Eber Gómez Flores
	Brisa Aranzazú Campos Oliver	Luis Ángel Maciel Barón
	Manuel Castillo Méndez	Dana Mariel Diaz Jiménez
	Luis Enrique Arias Romero	Faviola Tavares Carreón
	Arnulfo Bautista Santos	Luis Felipe Muriel Millán
	Chair: *Lina Riego*	
Sandy Johnson	Ximena Martínez de la Escalera	Itzel López Rosas
	Mijail Lezama	Claudia Martínez Anaya
	Claudia Segal	Evangelina Silva Santiago
	Elda Maria del Rocio Coutiño	Janeth Sánchez Ramos
	Jonathan Lozano Salgado	Semiramis Stephania García Trejo
	Chair: *Gloria Soberón*	
Peter Walter		Erika Garay Garduño
	Mirelle Citlali Flores Villegas	Esperanza Mata Martínez
	Alejandro Zentella	Elizabeth Huerta García
	Juan García Rincón	Sara Morales Lázaro
	Rusely Encalada	Luis A. Téllez
	Verónica Lorena Cano García	Cassandre Caligaris
		Ana Carolina Valencia Olvera
	Chair: *Leonardo Peraza*	

18:15 – 20:15 Poster Session 3

B	BASIC BIOCHEMISTRY	76 – 113
SB	SYSTEMS BIOLOGY & BIOINFORMATICS	17 – 33
PH	PHARMACOLOGY	1 – 24
I	IMMUNOLOGY	17 – 31
M	MEDICINE, HEALTH & NUTRITION	24 – 47
MP	MICROBIOLOGY & PARASITOLOGY	23 – 45
N	NEUROSCIENCE & NEUROLOGY	1 – 16
GR	GENETIC REGULATION & EPIGENETIC	46 – 65
T	TOXICOLOGY	18 – 34
ST	SIGNAL TRANSDUCTION	64 – 92

Jalisco Room

9:00 – 11:00 Plenary Session 12
Guadalajara Room I

Molecular Epidemiology

Chair: **Rubén López Revilla**
Instituto Potosino de Investigación Científica y Tecnológica

9:00 – 9:30	Molecular epidemiology of infectious diseases **Rubén López-Revilla** Biología Molecular. Instituto Potosino de Investigación Científica y Tecnológica
9:30 – 10:00	Genetic diversity and transmission of the *Mycobacterium tuberculosis* Complex in San Luis Potosí **Julio Juárez-Álvarez** Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica
10:00 – 10:30	Experimental pulmonary tuberculosis, epidemiological contributions **Rogelio Hernández-Pando** Patología. Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"
10:30 – 11:00	High prevalence and anomalous association with neoplastic lesions suggests an outbreak of human papiloma virus type 33 cervical infection in San Luis Potosí, Mexico **Raúl De la Rosa-Martínez** Biología Molecular. Instituto Potosino de Investigación Científica y Tecnológica

9:00 – 11:00 Plenary Session 13
Guadalajara Room II

Nanobiotechnology

Chair: **Rafael Vázquez Duhalt**
Centro de Nanociencias y Nanotecnología, UNAM

9:00 – 9:30	Nanobiotechnology: challenges and opportunities in an emerging discipline **Victor Manuel Castaño Meneses** Centro de Física Aplicada y Tecnología Avanzada, UNAM
9:30 – 10:00	Biological systems as targets for study of new properties induced by nanomaterials **María del Carmen González Castillo** Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí
10:00 – 10:30	Heterogeneous biocatalysis. Laccase as an example **Sergio A. Águila** Departamento de Bionanotecnología, Centro de Nanociencias y Nanotecnología, UNAM

10:30 – 11:00 Virus-like nanoparticles as potential carriers of cytochrome P450 for chemo therapy pro-drug activation
Rafael Vazquez-Duhalt
Centro de Nanociencias y Nanotecnología, UNAM

9:00 – 11:00 Plenary Session 14
Guadalajara Room III

Bioética

Chair: **Ricardo Tapia Ibargüengoytia**
Instituto de Fisiología Celular, UNAM

9:00 – 9:30 Por qué es importante la laicidad en bioética
Roberto Blancarte
El Colegio de México/Colegio de Bioética

9:30 – 10:00 De las Células Troncales a la Dignidad Humana pasando por el Cigoto
Rubén Lisker
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

10:00 – 10:30 Muerte encefálica y trasplante de órganos: ¿estamos bien?
Patricio Santillán Doherty
Instituto Nacional de Enfermedades Respiratorias, Colegio de Bioética

10:30 – 11:00 Ética científica: la responsabilidad de los científicos y los límites de la ciencia
Ricardo Tapia
División de Neurociencias, Instituto de Fisiología Celular, UNAM.

9:00 – 11:00 Plenary Session 15
Vallarta Room

Biochemistry and Plant Molecular Biology

Chair: **Miguel Lara Flores**
ENES -León, UNAM

9:00 – 9:30 MEDIATOR 25 regulates root architecture and intraspecific plant competition Via auxin signaling
José López-Bucio, Javier Raya-González, Randy Ortiz-Castro, León Francisco Ruíz-Herrera, Edith Muñoz-Parra, Ramón Pelagio-Flores
Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana

9:30 – 10:00 *Phaseolus vulgaris* TOR gene is required for rhizobial infection, nodule development and symbiotic nitrogen fixation
Kalpana Nanjareddy

10:00 – 10:30 Zygotic Genome Activation in inbred and hybrid embryos of *Arabidopsis thaliana*
Stewart Gillmor, Del Toro De León, Gerardo, García Aguilar, Marcelina
LANGEBIO, CINVESTAV – IPN Unidad Irapuato

10:30 – 11:00 Tracking the genetic pathway of pollen rejection in *Nicotiana*
Felipe Cruz-García, Liliana García Valencia , Lilia Gracida Bernal y Yuridia Cruz-González Zamora
Departamento de Bioquímica, Facultad de Química, UNAM

11:00 – 11:30 Coffee Break
Foyer Guadalajara Room

11:30 – 12:30
Guadalajara Room

Plenary Lecture IV

How Transcription Circuits Evolve and Produce Biological Novelty
Alexander D. Johnson
University of California, San Francisco

Chair: **Miguel Lara Flores**
Escuela Nacional de Educación Superior, UNAM. León

12:30 – 14:00 Lunch

14:00 – 16:00 Plenary Session 16
Guadalajara Room I

Epigenetic Mechanisms in Plants:
Their significance in Biotechnological Developments

Chair: **Clelia De la Peña Seaman**
Centro de Investigación Científica de Yucatán

14:00 – 14:30 Epigenetic regulation in the common bean (*Phaseolus vulgaris* L.) and its potential use for crop improvement
Raúl Álvarez-Venegas, Aarón Barraza, José Luis Cabrera-Ponce, and Keren Martínez-Aguilar
Department of Genetic Engineering. CINVESTAV – IPN Unidad-Irapuato

14:30 – 15:00 Epigenetic Control by Small RNAs during Maize Somatic Embryogenesis
Tzvetanka D. Dinkova, Vasti T. Juárez-González, Carolina Chávez-Hernández, José L. Contreras-Guerra, Naholi D. Alejandri-Ramírez
Departamento de Bioquímica. Facultad de Química, UNAM

15:00 – 15:30 miRNAs and fruit development
Stefan de Folter, Ricardo A. Chávez-Montes, Yolanda Ruiz-Suárez, Valentín Luna-García, Karla L. González-Aguilera, Andrés Cruz-Hernandez, Nayelli Marsch-Martínez, Flor de Fátima Rosas-Cárdenas
LANGEBIO CINVESTAV IPN Irapuato

15:30 – 16:00 Epigenetic changes during in vitro culture of important plants
Clelia De-la-Peña
Centro de Investigación Científica de Yucatán

14:00 – 16:00 Plenary Session 17
Guadalajara Room II

Fungal Molecular Biology
"In Memoriam" Dr. Jure Piskur

Chair: **Jesús Aguirre Linares**
Instituto de Fisiología Celular, UNAM

14:00 – 14:30 Order vs chaos: the regulation of vesicle traffic in *Neurospora crassa*
Meritxell Riquelme and Eddy Sánchez-León
Center for Scientific Research and Higher Education of Ensenada (CICESE)

14:30 – 15:00 How do fungi transform dead plants into fruiting bodies?
Philippe Silar
Laboratoire Interdisciplinaire des Energies de Demain
Univ Paris Diderot, Sorbonne Paris

15:00 – 15:30 A non-canonical function of the yeast high osmolarity glycerol (HOG) pathway
Roberto Coria
Genética Molecular, Instituto de Fisiología Celular, UNAM

15:30 – 16:00 ROS signaling and fungal development
Jesús Aguirre
Biología Celular y Desarrollo, Instituto de Fisiología Celular, UNAM

14:00 – 16:00 Plenary Session 18
Guadalajara Room III

Biochemistry and Molecular Biology of Bacteria
"*In Memoriam* Dr. José Edgardo Escamilla Marván"

Chair: **Guadalupe Espín Ocampo**
Instituto de Biotecnología, UNAM

14:00 – 14:30 A new proposal for bacterial evolution: What we have learned from the analysis of *Azotobacter vinelandii* genome
Gloria Soberón Chávez, Adrián González-Casanova, Eneas Aguirre-von-Wobeser; Guadalupe Espín, Luis Servín-González, Noemi Kurt, Dario Spanò and Jochen Blath
Instituto de Investigaciones Biomédicas, UNAM

14:30 – 15:00 Post-transcriptional and post-translational alginates regulation synthesis in *Azotobacter vinelandii.*
Miguel Castañeda, Liliana López Pliego, Itzel Paulina Morales, Ana Laura Rodríguez.
Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla

15:00 – 15:30 Isolation and Characterization of Cr(VI)-Reducing Bacteria with Potential Application in Soil Bioremediation Strategies
Katy Juárez, Paloma Lara, Italia Moreno, Bianca Flores, Fernando Mckay, Enrique Morett
Instituto de Biotecnología, UNAM

15:30 – 16:00 Biosynthesis of arseno-organic molecules in Actinobacteria
Francisco Barona-Gómez, Pablo Cruz-Morales, Luis Yañez-Guerra, Hilda E. Ramos-Aboites, Fernanda Iruegas-Bocardo, Johannes Kopp, Jörg Feldmann
Evolution of Metabolic Diversity Laboratory, LANGEBIO, CINVESTAV – IPN Irapuato

16:00 – 18:00 Poster Session 4

B	BASIC BIOCHEMISTRY	113 – 151
BT	BIOTECHNOLOGY	84 – 125
OR	OXYGEN REACTIVE SPECIES	18 – 33
G	GENETICS	24 – 43
M	MEDICINE, HEALTH & NUTRITION	48 – 71
MP	MICROBIOLOGY & PARASITOLOGY	46 – 68
N	NEUROSCIENCE & NEUROLOGY	17 – 32
GR	GENETIC REGULATION & EPIGENETIC	67 – 86
V	VIROLOGY	1 – 18

Jalisco Room

18:15 – 19:15
Guadalajara Room

Plenary Lecture V

Unfolded Protein Response in Health and Disease
Peter Walter
Howard Hughes Medical Institute/
University of California, San Francisco

Chair: **Alicia González Manjarrez**
Instituto de Fisiología Celular,UNAM

19:15 – 19:45 Final Announcements

Closing Ceremony

21:00 Closing Dinner

Poster Sessions

SYSTEMS BIOLOGY & BIOINFORMATICS

SB-1
Inferring microRNA functions: regulation beyond direct targets. Cesaré Ovando-Vázquez, **Roberto Álvarez-Martínez** and Cei Abreu-Goodger. LANGEBIO – CINVESTAV – IPN. Irapuato

SB-2
Evolution of Codon Usage Bias in Bacteria. **Guadalupe Arellano** and Luis Delaye. CINVESTAV – IPN Unidad Irapuato

SB-3
Adaptive mutations typically increase the performance of metabolism independently from one another. **J Abraham Avelar Rivas** & Alexander de Luna. LANGEBIO – CINVESTAV – IPN. Irapuato

SB-4
Evolutionary codependence as crucial information during LAO protein redesign. **Jesús Agustín Banda Vázquez**, Rogelio Rodríguez Sotres, Karina Marisol Maya Ramírez y Alejandro Sosa Peinado. Facultad de Medicina. UNAM

SB-5
Core and pan-genome analysis of the genus *Streptococcus*. **Hugo Rafael Barajas de la Torre** and Luis David Alcaraz. Departamento de Ecología de la Biodiversidad. Instituto de Ecología, UNAM

SB-6
Molecular flexibility of a family of periplamic binding proteins for basic aminoacids: LAO-binding protein. **Tania Raquel Berrocal Gama**, Andrés Escandón Flores, Alejandro Sosa Peinado. Facultad de Medicina. UNAM

SB-7
Evaluation of parameters and adequate sampling to measure sea urchin sperm motility. **Cecile Bustamante-Gómez,** Juan E Sosa-Hernández, Aarón Vazquez Jiménez, Jesús Santana-Solano, Jesús Rodríguez-González and Blanca Estela Galindo**.** CINVESTAV – IPN. Monterrey

SB-8
High-resolution genome-wide aging screens reveal novel molecular mechanisms of lifespan extension by dietary restriction. **Sergio E. Campos-Rodríguez** & Alexander de Luna. LANGEBIO – CINVESTAV - IPN. Irapuato

SB-9
Evolutionary and physiological correlation of redundant gene dosage within the *Debaryomyces hansenii* genome, a preliminary approach. **Angeles Cancino-Rodezno**, Luis Lozano, Luisa Alba-Lois, Viviana Escobar-Sánchez, Claudia Segal-Kischinezky, Víctor Valdés-López, Alfonso Vilchis-Peluyera. Departamento de Biología Celular, Facultad de Ciencias, UNAM

SB-10
Microbial evolution: a systems biology approach. **Luis Delaye**. CINVESTAV – IPN. Irapuato

SB-11
Dendroctonus rhizophagus Thomas Bright cytochrome *CYP6DG1V1* structure prediction and interaction analysis with α-pinene. **María Fernanda López Gómez**, Gema Ramírez Salinas, José Correa Basurto, Gerardo Zúñiga y Arnulfo Albores Medina. CINVESTAV – IPN Zacatenco

SB-12
Improving sequence capture design for genetically modified organisms identification by next generation sequencing. **Roberto Galindo Ramírez**, Salvador Ángel Romero Martínez y Abraham Itzcóatl Acatzi Silva. Subdirección de Secuenciación y Bioinformática. SENASICA

SB-13
BAC-end sequencing of a BAC-based genomic library of *Saccharomyces pastorianus* for genome sequence assembly improvement. **Gómez-Muñoz Cintia**, Riego-Ruiz Lina, Damas-Buenrostro Luis. Instituto Potosino de Investigación Científica y Tecnológica, A.C.

SB-14
Evolution of global gene expression from fermentative to glycerol-based respiratory growth in *Escherichia coli* and *Schizosaccharomyces pombe*: A comparative transcriptome analysis using RNA-seq. Joivier Vichi Lozada, Leticia Olvera Rodríguez, Enrique Morett Sánchez Verónica Jiménez Jacinto y **Armando Hernández Mendoza**. Facultad de Ciencias, UAEM

SB-15
Study *In silico* of protein-protein interaction of T-type voltage-gated Ca^{2+} channels and auxiliary subunits.**Teresa Hernández-Segura**, Nidia-Beltrán, Jacaranda Rosendo-Pineda & Heriberto Manuel Rivera., Facultad de Medicina, UAEM

SB-16
Phylogenetic analysis of Zinc-dependent alcohol dehydrogenases and aldehyde dehydrogenases in animals, **Adriana Julián-Sánchez**, Paola Vara-Cisneros, Juan José Alvarado-Leaño, Carlos Gaona-López, Aída Hernández-Tobías, and Héctor Riveros-Rosas. Facultad de Medicina, UNAM

BASIC BIOCHEMISTRY

B-1
Cleavage of recombinant Proenkephalin A overexpressed in Chinese hamster ovary cells. Inés Velázquez-Quesada, **Pilar Sarah Acevo-Rodríguez**, Silvia Duran, Marie-Hélène Metz-Boutigue, Osvaldo Vindrola, María-Rosa Padrós. Instituto de Fisiología, BUAP

B-2
Factors regulating the expression of heterologous proteins in *E.coli*; using seven homologous triosephosphate isomerases (TPI) from different sources. **Beatriz Aguirre López**, Nallely Cabrera González, Concepción José Núñez, Marietta Tuena Sangri, Ruy Pérez Montfort and Armando Gómez Puyou. Instituto de Fisiología Celular UNAM

B-3
Insights in the structure and association of the capsomer of Parvovirus B19. **Mayra Patricia Alcántara-Sol**, Ismael Bustos-Jaimes. Facultad de Medicina, UNAM

B-4
Characterization of two cysteine-less wild-type triosephosphate isomerases and a cysteine containing mutant enzyme from lactic acid bacteria. **Ruy Perez-Montfort**, Nallely Cabrera Gonzalez, Alfredo Torres Larios, Andres Hernandez Arana and Valeria Guzman Luna. Instituto de Fisiología Celular, UNAM

B-5
Identification of lectin from teosinte coleoptile (*Zea diploperennis, Zea mexicana* y *Zea parviglumis)* by RT-PCR. **Jaquelina Alvarado Gil**, María del Socorro Pina Canseco, Eduardo Pérez Campos, Gabriel Mayoral Andrade, Flavio Aragón Cuevas, Alejandro Cisneros Solano, Nora Hilda Rosas Murrieta, Margarito Martínez Cruz. Instituto Tecnológico de Oaxaca

B-6
Analysis of Enzyme Kinetic Aspects of Phenanthrene Dihydrodiol Dehydrogenase in *Mucor circinelloides* YR-1. **Jazmin Areli Alvarez Copado**, Roberto Zazueta Sandoval. Universidad de Guanajuato

B-7
PepGMV infection benefit the interaction of the host plant with the withefly *T. vaporariorum* by changing the defense responses. **Yesenia Ithaí Ángeles López**, Martin Heil. CINVESTAV – IPN Unidad Irapuato

B-8
Development and evaluation of a recombinant sperm-activating peptide tagged with fluorescent proteins. **César Arcos Hernández**, Francisco Romero Corpus, Yoloxochitl Sánchez Guevara, Alberto Darszon, Takuya Nishigaki and Carmen Beltrán. Instituto de Biotecnología, UNAM

B-9
Identification of structural determinants involved in the difference of conformational change in EF-hand motifs. **Emma L. Arévalo Salina**, Joel Osuna Quintero, Humberto Flores Soto y Gloria Saab Rincón. Instituto de Biotecnología, UNAM

B-10
Engineering the reaction media to enhance the enzymatic synthesis of a sugar-based surfactant. **Rodrigo A Arreola Barroso**, Gloria Saab Rincón. Instituto de Biotecnología, UNAM

B-11
Electrophoretic analysis of maize stressed with *A. parasiticus.* **Adriana M. Arrioja López**, Marco A. Sánchez Medina, Pérez Cervera Yobana, Alma D. Pérez Santiago. Instituto Tecnológico de Oaxaca

B-12
Capsaicinoid accumulation in *in vitro* cultured *Capsicum chinense* placental tissue depends on *in situ* synthesis of valine and phenylalanine. **Fray M. Baas-Espinola**, Lizbeth A. Castro-Concha and María de Lourdes Miranda-Ham. Centro de Investigación Científica de Yucatán

B-13
Effect of the R3W, C27G, F211I, and Q279 somatic mutations present in human cancer cells on Gpn3 function. **Angel Adán Barbosa Camacho**, Selene C Acosta Morales, Olga L Ramírez Ramírez, Sonia G Peña Gómez, Angélica Y Robledo Rivera, Roberto Sánchez Olea, and Mónica R Calera. Instituto de Física, Universidad Autónoma de San Luis Potosí

B-14
Establishment of a Silver Staining System on Polyacrylamide Gels for Electrophoretic Analysis of Protein Patterns from Human Urine. **Mónica Janett Muñoz Contreras**, Magda Hernández Hernández, Jaime Morales Romero, Hilda Montero L de Guevara, Arturo Rodríguez Hernández, Clara Luz Sampieri Ramírez. Facultad de Química, Universidad Veracruzana

B-15
Evaluation of the cytotoxic activity of organic extracts of *Juniperus monticola* form *monticola* Martínez on breast human cancer cell lines. **Dalia Barrios-Palacios**, Nadia Judith Jacobo-Herrera, Claudia Tzasná Hernández-Delgado, Manuel Jiménez-Estrada. Instituto de Química, UNAM

B-16
Relation between simvastatin and mitochondrial uncoupling protein 2 in PAE cells. **Adán Barrios Rivera**, Andrea Ximena Girón Ceballos, Vicente Castrejón-Téllez. Instituto Nacional de Cardiología "Ignacio Chávez"

B-17
Thermodynamic characterization of three Triosephosphate isomerases from representative, yet unexplored species from the *Eukarya* domain. **Luis Arturo Becerril Sesín**, Mariana Schulte Sasse Jiménez, Sergio Romero Romero and D Alejandro Fernández Velasco. Depto. Bioquímica. Facultad de Medicina. UNAM

B-18
Influence of Microbial Inoculation on Phenolic Content and the Therapeutic Properties of Common Bean. **Vanessa Dianne Bravo García**, Huberto González-Rayo, Alejandra Ochoa Zarzosa, Josué Altamirano-Hernández. IIQB, Universidad Michoacana

B-19
Evaluation of melatonin as an adjuvant therapy in a model of experimental autoimmune encephalomyelitis. **Ana L Briones Torres**, Janeeth Bañuelos León, Margarita Cid Hernández, Celso Cortés Romero, Genaro Gabriel Ortiz y Fermín Pacheco Moisés. Departamento de Química. CUCEI. Universidad de Guadalajara

B-20
Increased lung apoptosis in *Autophagin-1* deficient mice after bleomycin-induced injury. **Sandra Cabrera Benítez**, Mariana Maciel Herrerías, Teresa Nava Ramírez, Fabián Vergara Ovalle, Miguel Gaxiola Gaxiola, Moisés Selman Lama, Annie Pardo Cemo FC, UNAM

B-21
Metabolic effect of constant high pH on *S. cerevisiae*. **Martha Calahorra**, Antonio Peña, Norma Silvia Sánchez, Helber Álvarez, Jorge Ramírez and Laura Ongay. Instituto de Fisiología Celular. UNAM

B-22
Oligomerization of nitrilases in catalytic filaments. **Alejandro Evaristo Cáliz Rodríguez**, Georgina Garza-Ramos Martínez. Departamento de Bioquímica, Facultad de Medicina, UNAM

B-23
Protection of human ALDH2 of the inactivation by lipid peroxidation products by site directed mutagenesis. **Luis F Calleja-Castañeda** and José S Rodríguez-Zavala. Instituto Nacional de Cardiología

B-24
Matrix metalloproteinase (MMP)-19 deficient fibroblasts display a profibrotic phenotype. **María del Jazmín Calyeca Gómez**, Daniel Paul Jara Pelaez, Yair Romero López, Luis Antonio Plácido Méndez, Vilma Maldonado Lagunas, Moisés Eduardo Selman Lama, Annie Pardo Cemo. Facultad de Ciencias, UNAM

B-25
Functional analysis of the NADP-Dependent glutamate dehydrogenase (NADP-*Kl*Gdh1) of *Kluyveromyces lactis* and (NADP-*Kl*Gdh1) of *Lacchancea kluyvery*. **José Carlos Campero-Basaldua**, James E. González Flores, Dariel Márquez Gutiérrez and M Alicia González Manjarrez. Department of Biochemistry and Structural Biology. Instituto de Fisiología Celular, UNAM

B-26
Structural Determinants of Amyloid Fibril Formation in Triosephosphate Isomerase. **Edson Norberto Cárcamo Noriega**, Gloria Saab Rincón, Filiberto Sánchez López. Instituto de Biotecnología, UNAM

B-27
Expression and activity of the alternative oxidase in *Ustilago maydis* under different carbon and nitrogen sources. **Christian Adrián Cárdenas Monroy**, Lucero Romero Aguilar, Héctor Vázquez Meza, Gabriela Piñón Zárate and Juan Pablo Pardo Vázquez. Facultad de Medicina, UNAM

B-28
Structural studies of glutamyl tRNA reductase. **Daniel Eduardo Rodríguez Chamorro**, Alfredo Torres Larios. Instituto de Fisiología Celular, UNAM

B-29
Localization of enzymes involved in nitrogen metabolism in *Capsicum chinense* Jacq. using *in situ* hybridization techniques. **Lizbeth A. Castro-Concha**, Raúl A. Manzanilla Rivas and María de Lourdes Miranda-Ham. Unidad de Bioquímica y Biología Molecular de Plantas, CICY

B-30
Study of the interactions of atypical subunit ASA1 in the ATP synthase of *Polytomella* sp. **Lilia Colina Tenorio**, Héctor Vicente Miranda Astudillo, Araceli Cano Estrada, Miriam Vázquez Acevedo, Diego González Halphen. Instituto de Fisiología Celular. UNAM

B-31
The Role of the Erythrocytes in the Serum Enzyme Levels that Regulates Nitrogen Metabolism in Humans: the OTC. **Martha L Contreras Zentella**, Pablo Rangel Silva, Rolando Hernández Muñoz. Instituto de Fisiología Celular, UNAM

B-32
cDNA cloning of fructose bisphosphatase and tissue expression in the shrimp *Litopenaeus vannamei*. **Abdiel Keni Cota Ruiz**, Alma Beatriz Peregrino Uriarte, Gloria Yepiz Plascencia. Centro de Investigación en Alimentación y Desarrollo, A. C

B-33
Proteolytic activity in pollen and pollen tube by 2D zymographic analysis. **Yuridia Cruz González Zamora**, Lilia Angélica Bernal Gracida, Felipe Cruz García. Facultad de Química, UNAM

B-34
Physicochemical characterization of the folding and binding mechanisms of ArtJ, a substrate-binding protein from *Geobacillus stearothermophilus*. **Francisco Aarón Cruz Navarrete**, Haven López, Nancy O Pulido Mayoral, Jesús Renan Vergara Gutiérrez, Alejandro Sosa Peinado and D Alejandro Fernández Velasco. Departamento de Bioquímica, Facultad de Medicina, UNAM

B-35
Rewriting the terminar marking mechanism by deamidation in human triosephosphate isomerase. **Ignacio de la Mora-de la Mora**, Alfredo Torres-Larios, Sergio Enríquez Flores, Gabriel López-Velázquez, Sara Teresa Méndez-Cruz, Itzhel García-Torres, Saúl Gómez-Manzo, Jaime Marcial-Quino, América Vanoye Carlo, Horacio Reyes-Vivas, Adriana Castillo-Villanueva, Angélica Torres Arroyo, Jesús Oria-Hernández. Laboratorio de Bioquímica Genética, Instituto Nacional de Pediatría

B-36
Kinetic and metabolic analysis of tumor Krebs cycle. **Isis Del Mazo-Monsalvo**, Ricardo Jasso-Chávez and Álvaro Marín-Hernández. Instituto Nacional de Cardiología, Ignacio Chávez

B-37
Molecular characterization and tissue expression of betaine aldehyde dehydrogenase from white shrimp *Litopenaeus vannamei*. **Delgado-Gaytan F**, Hernández-Palomares MLE, Soñanez-Organis JG, Stephens-Camacho NA, Sánchez-Paz JA, Muhlia-Almazán A, Valenzuela-Soto EM, Rosas-Rodríguez JA. Centro de Investigación en Alimentación y Desarrollo, A.C.

BIOTECHNOLOGY

BT-1
Isolation and identification of thermophilic bacteria degrading hydrocarbons obtained from hot springs. **Ramiro Aldama Hernández**, Gladis Yazmín Escobar Puebla, Jeiry Toribio Jiménez, Miguel Ángel Rodríguez Barrera and Yanet Romero Ramírez. Research Laboratory in Biotechnology and Microbial Genetics, Autonomous University of Guerrero

BT-2
Curtobacterium sp. strain MR2 exhibits two lead-resistance mechanisms dependent of cell density. **Carlos Juan Alvarado López**, Luis Gerardo Treviño Quintanilla, Laura Stephania Colín Rosette. Universidad Politécnica del Estado de Morelos

BT-3
The role of Zn in the growth and development of *Phaseolus vulgaris* L.: anatomical and morphological effects. **Julio César Amezcua-Romero**, Miguel Lara Flores. ENES, Unidad León– UNAM

BT-4
Alkaline phosphatase is a putative receptor of Cry1Ab and Cry1Ac toxins from *Bacillus thuringiensis* in stem borer *Diatraea magnifactella* Dyar. **Iván Arenas Sosa,** Fernando Zúñiga Navarrete, Ángel Flores Alcantar, Laura Patricia Lina García, Mary Carmen Torres Quintero, Guadalupe Peña Chora and Víctor Hernández Velázquez. Centro de Investigación en Biotecnología, UAEM

BT-5
Role of the rhizosphere microbiome in the phytoremediation of the organochlorine pesticide endosulfan by Basil *(Ocimum basilicum)* **Jackeline Lizzeta Arvizu Gómez**, Alejandro Hernández Morales, Aideé Cristina Ibarra Parra, Guadalupe Yaraceth Ochoa De Luna, Gabriel Palma Ayala, Verónica Alejandra Mondragón Jaimes, Jesús Bernardino Velázquez Fernández. Posgrado en Ciencias Biológico Agropecuarias y Pesqueras. Universidad Autónoma de Nayarit

BT-6
Insight of the active site of a fungal laccase: A molecular dynamic simulation. **Mayra Guadalupe Avelar Frausto**, Carmen Nina Pastor Colón, Sergio Andrés Águila Puentes, Marcela Ayala Aceves. Instituto de Biotecnología, UNAM

BT-7
Study of Agave tequilana leaves an alternative biomass of sugars and cellulosic material for the production of second generation bioethanol. **María Elisa Evangelina Avila Gaxiola**, Jorge Carlos Avila Gaxiola, Gelacio Atondo Rubio, Oscar Jesús Velarde Escobar, Francisco Ramos Brito, Josefa Adriana Sañudo Barajas, Cristo Manuel Yee Rendon. Facultad de Ciencias Físico – Matemáticas, Universidad Autónoma de Sinaloa.

BT-8
Study of acid-enzymatic hydrolysis of Agave tequilana Weber azul leaf for the production of second generation bioethanol. **Maria Elisa Evangelina Avila-Gaxiola**, Jorge Carlos Avila Gaxiola, Lorena Amaya-Delgado Oscar Jesús Velarde Escobar, Francisco Ramos Brito, Gelacio Atondo-Rubio, Cristo Manuel Yee-Rendon. Facultad de Ciencias Físico-Matemáticas, Universidad Autónoma de Sinaloa

BT-9
Effect of fatty acids and derivativesfrom avocado on the regulation of innate immunity in bovine mammary epithelial cells infected with *Staphylococcus aureus.* **Marisol Báez-Magaña**, Rafael Salgado-Garciglia, Rodolfo López-Gómez, Lourdes Macías Rodríguez, Alejandra Ochoa-Zarzosa, Joel Edmundo López-Meza. FMVZ, UMSNH

BT-10
Molecular characterization of the acidic dehydrin Ops DHN1 from *Opuntia streptacantha*. **IE Hernández-Sánchez**, DM Martynowicz, AA Rodríguez-Hernández, AL Salazar-Retana, A Becerra-Flora, MB Pérez-Morales, SP Graether and JF Jiménez-Bremont. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica AC

BT-11
Characterization of cold-adapted digestive cathepsin D aspartic peptidase in American lobster (*Homarus americanus*). **Betsaida Bibo**, Anthony O'Donoghue, Liliana Rojo, Charles Craik, Fernando García-Carreño. Centro de Investigaciones Biológicas del Noroeste

BT-12
Isolation of first genes from carotenoid biosynthesis pathway phytoene synthase, phytoenedesaturase and ζ-carotene isomerase from *Bixaorellana*. **Oralia Caamal-Hau**, Yair Cárdenas-Conejo, Margarita Aguilar Espinosa, Nayeli Romero-López, and Renata Rivera-Madrid. Centro de Investigación Científica de Yucatán

BT-13
Production of xylanases from *Serratia sp.* isolated of the gut of bark beetle of *Dendroctonus* genera(Curculionidae: Scolytinae). **Claudia Cano Ramírez**, Alejandro Santiago Hernández, Gerardo Zúñiga Bermúdez, Flor Nohemí Rivera Orduña and Ma. Eugenia Hidalgo Lara. CINVESTAV – IPN Zacatenco

BT-14
Degradation of antibiotics by basidiomicetes fungi (*Trametes versicolor, Lentinulae dodes, Lentinula boryana, Pleurotus djamor, Pleurotus djamor var roseus*). **Víctor Carpanta**, Alexis Joavany Rodríguez, Ma. Lourdes Acosta- Urdapilleta, Elba Villegas. Facultad de Ciencias Biológicas, UAEM

BT-15
Biomimetic coats for improving the interaction and stability of DNA-delivering nanoparticles. **DG Carrasco-González**, LM López-Marín, B Millán-Chiu, G Hernández-Padrón, P Salas-Castillo and AM Loske-Mehling. Centro de Física Aplicada y Tecnología Avanzada, UNAM

BT-16
Functionalization of iron nanoparticles (FeNP´s) with proteins secreted by *Streptococcus pneumonia,*which bind haem and haemoglobin. **Mariana Carrillo Morales**, Edgar Augusto Ortiz Benítez, Alejandra Stephany Rodríguez Leviz, Norma Velázquez Guadarrama, Jesús Fandiño Armas y José de Jesús Olivares Trejo. Posgrado en Ciencias Genómicas. Universidad Autónoma de la Ciudad de México

BT-17
Regulation of morricinsynthesis. **Luz Edith Casados-Vázquez** and Eleazar Barboza Corona. División de Ciencias de la Vida. Departamento de Alimentos. Universidad de Guanajuato

BT-18

Preliminary analysis of protein content in huitlacoche. **Ariana Zavala Moreno**, Juan Pablo Carrillo Montes, Lucero Aguilar Romero y Roberto Arreguín Espinosa. Instituto de Química. UNAM

BT-19

Pycnoporus sanguineus acetonic extract insecticidal effect on *Spodoptera frugiperda* larvae. Silvia Hernández, Laura Lina, Ma. Lourdes Acosta-Urdapilleta, Alexis Rodríguez, Laura Lina, **Elba Villegas,** Centro de Investigación en Biotecnología, UAEM

BT-20

Expression of the hemagglutinin-neuraminidase´s ectodomain from *Rubulavirus porcine* in the yeast *Pichia pastoris*. **José Luis Cerriteño-Sánchez**, , Gerardo Santos-López, Nora Hilda Rosas-Murrieta, Julio Reyes-Leyva, Irma Herrera-Camacho. Laboratorio de Bioquímica y Biología Molecular. ICUAP-BUAP

BT-21

Partial characterization of a lectin-protease inhibit or fraction from Tepary bean (*Phaseolus acutifolius*). **Cervantes Jiménez Ricardo**, Zamora Huerta Andrés, Herrera García Andrea, Sosa Márquez Iván, López Martínez FJ, Blanco Labra Alejandro, Castro Guillen José Luis, Mendiola Olaya Elizabeth, Moreno Celis Ulisses, Ferriz Martínez Roberto, García Gasca Teresa. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro

BT-22

Transcriptional profile of leaves in *Solanum lycopersicum* mycorrhizal plants using RNA-seq. **Rocío Guadalupe Cervantes-Gámez**, Abraham Cruz-Mendívil, Claudia María Ramírez-Douriet, Mario Alonso Bueno-Ibarra, Carlos Ligné Calderón-Vázquez, Melina López-Meyer. CIIDIR-Sinaloa. IPN

BT-23

Physiological and molecular characterization of the response to drought in four *Brachypodium distachyon* natural variants. **Mariana E Cesario-Solis**, Amado Ortiz-Yescas, Julián M Peña-Castro, Blanca E Barrera-Figueroa Biotecnología Vegetal. Instituto de Biotecnología, Universidad del Papaloapan

BT-24

Diazotrophic potential among bacterial communities associated with wild and cultivated agaves. **Damaris Desgarennes**, Etzel Garrido, Miryam J Torres-Gómez, Juan José Peña-Cabriales and Laila P Partida-Martínez. Ingeniería Genética, CINVESTAV – IPN. Irapuato

BT-25

Expressed genes in *Capsicum annum* transformed roots in interaction with *Rhizoctonia sp.* Binucleate. **César Díaz-Pérez**, María de Jesús González Ramos, Lenin Sánchez Calderón, Saúl Fraire Velázquez. Laboratorio de Biología Integrativa de Plantas y Microorganismos. Universidad Autónoma de Zacatecas

BT-26

Isolation and Characterization of Denitrifying rhizobacteria from *Portulacaoleracea* L. in Chinampa: an Anthropogenic Crop Soil and Wetland in the Valley of Mexico. **Salvador Embarcadero Jiménez**, Brenda Román Ponce, Flor Nohemí Rivera Orduña, En Tao Wang-Hu. Escuela Nacional de Ciencias Biológicas. IPN

BT-27

Intra and extracellular metabolite analyses on the accumulation of aromatic intermediates as an effect of carbon flux increase due to the presence of the Plasmid pJLB*aroGfbrtktA* in a ΔaroC *E. coli* strain. **Magda Karina Espíndola Martínez**, Luz María Martínez-Mejía, Guillermo Gosset Lagarda, and Georgina Teresa Hernández-Chávez. Instituto de Biotecnología, UNAM

BT-28

Detection of anti-cancer proteins in the atypical crystalline inclusions of two *Bacillus thuringiensis* isolates. **Astrid Nalleli Espino Vázquez**, Luis Jesús Galán Wong y Benito Pereyra Alférez. Instituto de Biotecnología. Facultad de Ciencias Biológicas. UANL

BT-29

Evaluation of soil *Streptomyces* strains with antagonist activity againstdiverse fungal plant pathogens. **Zahaed Evangelista-Martínez**, Evangelina Quiñones-Aguilar, Gabriel Rincón-Enríquez, Luis Valera-Montero. CIATEJ

BT-30
Study of the hydrodynamic effects and dissolved oxygen on the growth, morphology and laccase production by *Pleurotusostreatus* CP50 in submerged cultures. **Karen Ibeth Fernández Alejandre,** Raunel Ticoco Valencia, Mario A Caro, Enrique Galindo, Leobardo Serrano Carréon. Instituto de Biotecnología, UNAM

BT-31
Immobilization of *Burkolderia* sp. on different supports for biodegradation of methyl parathion. **Maikel Gilberto Fernández-López,** Elida Carolina Popoca-Ursino, Ma. Laura Ortiz-Hernández. Environmental Research Laboratory, Biotechnology Research Center, UAEM

BT-32
Isolation and identification of entomopathogenic bacteria from *Diatraea magnifactella* in sugarcane soils from Morelos state. **Christian Jesús Flores Bahena**, Verónica Obregón, Laura Lina, Francisco Medrano, Elba Villegas. Centro de Investigación en Biotecnología, UAEM

BT-33
Analysis of the endophytic bacterial communities associated with two wild sympatric species of Cacti. **Citlali Fonseca-García**, Etzel Garrido and Laila P Partida-Martínez. Departamento de Ingeniería Genética. CINVESTAV – IPN Unidad Irapuato

BT-34
Determination of carbon flow to the common aromatic pathway in *Escherichia coli* mutants lacking glucose phosphoenol pyruvate: phosphotranspherase system (PTS) and non-PTS transporters. **Juan Carlos Fragoso Jiménez**, Luz María Martínez Mejía, Georgina Hernández Chávez, Noemí Flores Mejía, Alfredo Martínez Jiménez, Guillermo Gosset Lagarda. Departamento de Biocatálisis, Instituto de Biotecnología, UNAM

BT-35
Same virus, new targets: Parvovirus B19 meets hepatocytes. **Brenda Franco-Marcelo**, Ismael Bustos-Jaimes. Departamento de Bioquímica, Facultad de Medicina, UNAM

BT-36
Un-targeted metabolic profiling for the discovery of highly heritable metabolites linked to coffee (*C. canephora*) cup quality. **Roberto Gamboa Becerra**, Robert Winkler. CINVESTAV- IPN, Unidad Irapuato

BT-37
Study of RsmA over-expression effects on the expression of the *alg*D gene in *A. vinelandii.* **María Eugenia Valentina García Aguilar**, Miguel Castañeda Lucio, Liliana López Pliego. Centro de Investigaciones Microbiológicas, Instituto de Ciencias, BUAP

BT-38
Redesign of the Shikimate Dehydrogenaese Enzyme from *E. coli*. Inversion on the cofactor specificity. **José Fernando García Guevara,** Lorenzo Segovia Forcella. Instituto de Biotecnología, UNAM

BT-39
Kinetic and structural characterization of mutants of the interactions surface of the C-terminal tail of nitrilase from *R. pyridinovorans***. Agustín Gómez Aguilar**, Georgina Garza-Ramos Martínez. Departamento de Bioquímica, Facultad de Medicina, UNAM

BT-40
Lipid bilayer disruption originated by a parasporal protein of *Bacillus thuringiensis subspecies neoleonensis,* GM18. **Erika González Álvarez**, Abelardo Chávez Montes, Hugo Luna Olvera, Luis Galan Wong, Azucena González-Horta. Laboratorio de Ciencias Genómicas, UANL

CELL DIFFERENTIATION

CD-1
Neural induction of bone marrow mesenchymal stromal cells and olfactory neuro-epithelial cells. **Alejandro Aguilera-Castrejón**, Herminia Pasantes, Juan José Montesinos Montesinos, Gloria Benitez-King, Marta E.

Castro-Manrreza and Gerardo Ramos-Mandujano. División de Neurociencias, Instituto de Fisiología Celular, UNAM

CD-2

Functional analysis of the *WIP* gene as a developmental regulator in the liverwort *Marchantia polymorpha.* **Mariana Andrade Medina**, Daniel Ramos Patlan, Eduardo Flores, John Bowman', and Nayelli Marsch Martínez. Biotecnología y Bioquímica, CINVESTAV – IPN Irapuato

CD-3

Effect of iodine deficiency on the migration and invasion of 3AsubE human trophoblasts cells. Zendy Evelyn Olivo Vidal, Erika Alarcòn Cruz, José Oscar Garcìa Carmona, Juan Carlos Conde Alarcòn, **Omar Arroyo-Helguera.** Instituto de Salud Pùblica, Universidad Veracruzana

CD-4

Effect of L-carnitine and a phosphorus analog of L-carnitine on *in vitro* insulin-like response. **Anaguiven Avalos Soriano**, Francisco Josué López Martínez, Ricardo de la Cruz Cordero, Jorge Luis Rosado Loría , Teresa Garcia Gasca. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro

CD-5

Characterization of cyclins B1;2 and B2;1 duringmaize germination. **María Fernanda Ayub-Miranda**, Brendy B García-Ayala, Jorge M Vázquez-Ramos and Aurora Lara-Núñez. Facultad de Química. UNAM

CD-6

IL-6 role during proliferation induction in MCF-7 cells treated with elderly serum. **Bertha Alicia Barajas–Gómez**, Viridiana Yazmin González Puertos, Pedro Posadas Rodríguez, Oscar Rosas–Carrasco, Teresa Juárez, Jorge Antonio García–Álvarez, Mina Konigsberg–Fainstein, Pablo Damián–Matzumura, Armando Luna–López. Postgrado in Experimental Biology, UAMI

CD-7

Expression of the Dystrophin-Associated Protein Complex in Human Adipose Tissue. **Byanka S Espinoza-López**, Angélica Montoya-Contreras, José Romo-Yáñez, Cecilia Montañez, Jaime Parés, José Refugio Medina-León, Luis A Salazar-Olivo. Div. Biología Molecular, IPICyT

CD-8

Papillomavirus oncogenes E6/E7 and estradiol promote mouse ear regeneration by different mechanisms. **Celina García**, David Hernández-García, Concepción Valencia, Mariana Werner, José Raúl Pérez and Luis Covarrubias Instituto de Biotecnología, UNAM

CD-9

ERK1/2 are involved in acrosome reaction through its activation by FAK. **Enrique O Hernández González**, Mónica L Salgado Lucio y Ana L Roa Espitia. Biología Celular, CINVESTAV – IPN Zacatenco

CD-10

Glial cell-derived Neurotrophic Factor favors the differentiation of mouse embryonic stem cells to motor neurons. **Daniel E Cortés P**, Iván Velasco Velázquez. Instituto de Fisiología Celular, UNAM

CD-11

Glucose and sucrose have a differential impact on embryo axes during maize germination. **Aurora Lara-Núñez**, Brendy B García-Ayala and Jorge M Vázquez Ramos. Facultad de Química. UNAM

CD-12

Ustilago maydis: A comparative microarray analyses at different stages of basidiocarps development. **Claudia Geraldine León-Ramírez**, José Luis Cabrera-Ponce, Domingo Martínez-Soto, José Alejandro Sánchez-Arreguín and José Ruiz Herrera. CINVESTAV - IPN. Departamento de Ingeniería Genética. Unidad Irapuato

CD-13

The *Podospora anserina* endoplasmic reticulum-shaping proteins and their role in sexual development. **Antonio de Jesús López-Fuentes**, Karime Naid Nachón-Garduño, Fernando Suaste-Olmos, Leonardo Peraza-Reyes. Instituto de Fisiología Celular, UNAM

CD-14
Regulation of seed structure and root development by the glutamate-carboxipeptidase *altered meristem program 1(AMP1)*. **Marina López-García**, J Raya-González, JS López-Bucio, AA Guevara-García and J López-Bucio. IIQB, Universidad Michoacana de San Nicolás de Hidalgo

CD-15
Molecular characterization of auxin transport inhibitors and their role in root morphogenesis. **Viridiana Magaña-Dueñas**, Ramón Pelagio-Flores and José López-Bucio. IIQB, Universidad Michoacana de San Nicolás de Hidalgo

CD-16
Regulation of root hair development in *Arabidopsis thaliana* by *Pseudomonas aeruginosa* and related quorum-sensing signals. **César Nahúm Maldonado Cortés**, Lourdes Iveth Macías Rodríguez and Jose López Bucio. IIQB, Universidad Michoacana de Sán Nicolás de Hidalgo

CD-17
TRPV4 channel is necessary for appropriated establishment of the tight junctions in corneal epithelium and regulates its barrier function in combination with EGF. **Jacqueline Martínez Rendón**, Erika Sánchez Guzmán, Angélica Rueda, Federico Castro- MuñozLedo and Refugio García-Villegas. CINVESTAV – IPN Zacatenco

CD-18
UTP induces migration and EMT in ovarian cancer cells. **Martínez-Ramírez AS**, Vázquez-Cuevas FG, Garay E, Arellano RO. Neurobiología Celular y Molecular, Instituto de Neurobiología, UNAM

CD-19
Dystroglycan depletion inhibits the functions of differentiated HL-60 cells. **Ivette Astrid Martínez-Vieyra**, Alma Delia Martínez-Zárate, Lea Alonso-Rangel, Bulmaro Cisneros, Steve J. Winder, Doris Cerecedo. Escuela Nacional de Medicina y Homeopatía, IPN

CD-20
Notch-1 and Notch-2 show differential activity on cell proliferation and differentiation of rabbit corneal epithelial cells. **José Carlos Alfonso Mata Lozano**, Erika Sánchez Guzmán and Federico Castro Muñozledo. Biología Celular, CINVESTAV – IPN Zacatenco

CD-21
Unusual nuclear localization of TRPV4 ionic channel in renal epithelial Cells (MDCK). José Arturo Matamoros-Volante, Jacqueline Martínez-Rendón and María del Refugio García-Villegas. Fisiología, Biofísica y Neurociencias, CINVESTAV-IPN. Zacatenco

CD-22
Assessing the nitric oxide production in the basal land plants: the moss Physcomitrella patens as model system. **Rigoberto Medina-Andrés**, Alejandro Solano-Peralta, Juan Pablo Saucedo-Vázquez, Selene Napsucialy-Mendivil, Jaime Arturo Pimentel Cabrera, Martha Elena Sosa-Torres, Joseph G. Dubrovsky and Verónica Lira-Ruan. Laboratorio de Fisiología y Desarrollo Vegetal, Facultad de Ciencias, UAEM

CD-23
Analysis of the Biological activity of the Paired Domain-lacking isoform of the transcription factor PAX6 (PAX6ΔPD) in mammalian corneal epithelium differentiation. **Rosa Guadalupe Meza Aguilar**, Diana Reséndez-Pérez, Refugio García Villegas, Erika Sánchez Guzmán and Federico Castro Muñozledo. Departamento de Biología Celular, CINVESTAV - IPN

CD-24
α6 integrin as a marker of rabbit corneal epithelial cells with migratory ability: Study in RCE1(5T5) cell line. **Diana Guadalupe Meza-Aguilar**, Erika Sánchez-Guzmán, Federico Castro-Muñozledo. Departamento de Biología Celular, CINVESTAV-IPN

CD-25
The estructure and function of *Ustilago maydis* proteinase A. **Cinthia Valentina Soberanes-Gutiérrez**, José Ruiz-Herrera and Lourdes Villa-Tanaca. Genética Microbiana. Microbiología ENCB-IPN.

CD-26
Cellular localization of the RE-1 Silencing Transcription factor in Lung Cancer cell lines. **Carlos Ortuño Pineda**, Jesús Valdés Flores, Catalina Flores Maldonado, Adán Arizmendi Izazaga, Ricardo Martinez Baltazar, Napoleón Navarro Tito, Alan G. Yáñez Olvera, Oscar del Moral Hernández, Miguel Ángel Mendoza Catalán. Unidad Académica de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

CD-27
Effect of PPAR agonist compounds on adipocite and myocite differentiation, and changes in energy metabolism of mesenchymal stem cells from pigs'bone marrow. **Rosa Martha Pérez-Serrano,** María Laura González-Dávalos, Armando Shimada Miyasaka, Anaid Antaramian Salas, Alfredo Varela Echavarría, María Ofelia Mora Izaguirre. RuMeN, Facultad de Estudios Superiores Cuautitlán, UNAM

CD-28
Sperm survival mediated by integrins. **Ana Lilia Roa-Espitia**, Monica L Salgado Lucio and Enrique Othón Hernández-González. Departamento de Biología Celular. CINVESTAV-IPN

CD-29 AfeA, TmpA and TmpB are membrane enzymes that regulate asexual development in *Aspergillus nidulans.* **Olivia Sánchez,** Gabriela Soid-Raggi, Rosa Fajardo-Somera, and Jesús Aguirre. IFC-UNAM

GENETIC

G-1
Transcription-associated adaptive mutagénesis in DNA-repair deficient *Bacillus subtilis* cells. **Verónica Ambriz Aviña** and Mario Pedraza Reyes. Biología, Universidad de Guanajuato

G-2
Analysis of Type I-like and Type II-like telomeres of telomerase-negative survivors of *Ustilago maydis* by PCR amplification and Bal31 digestion kinetics. **Estela Anastacio Marcelino**, Reynaldo Galicia Sarmiento, Candelario Vázquez Cruz y Ma. Patricia Sánchez Alonso. Instituto de Ciencias, BUAP

G-3
Expression and subcellular localization of Dp71 isoforms in PC12 cells: colocalization with β-dystroglycan and α-syntrophin. **Jorge Aragón**, Alejandro Martínez-Herrera, José Romo-Yáñez, Víctor Ceja, Coztli Ocelotl Azotla Vilchis, Lourdes Montserrat Siqueiros Márquez, Alma Herrera-Salazar and Cecilia Montañez. Genética y Biología Molecular, CINVESTAV – IPN Zacatenco

G-4
Detection of DNA of preys in the diet of a generalist predator: threshold in the number of detectable preys and time consumption. **Lourdes Bao Fundora**, Carla Sánchez-Hernández, Ricardo Ramírez-Romero, José Sánchez Martínez and Nicolas Desneux. CUCBA. Universidad de Guadalajara

G-5
Analysis of expression of the genes ζ-carotene desaturase (ZDS) and carotene isomerase (CRTISO) in different plant tissues of *Bixa orellana* L. by RT-PCR Real-time technique. **Vincent Cerbantez-Bueno**, Margarita Aguilar-Espinosa, Rivera-Madrid Renata. CICY

G-6
Role of ribosomal protein S1 in the translation of adenine- or uracil-rich mRNAs. **Juan Carlos Cifuentes Goches**, Manuel Alberto Castillo Méndez, María del Rosario Salinas Tobón, Gabriel Guarneros Peña, Javier Hernández Sánchez. Genética y Biología Molecular. CINVESTAV-IPN Zacatenco

G-7
Expression of the green fluorescent protein in members of the *Sporothrix schenckii* complex. **Diana Marcela Clavijo-Giraldo**, José Ascensión Martínez-Álvarez, Nancy Edith Lozoya-Pérez, Sergio Casas-Flores, and Héctor Manuel Mora-Montes. Departamento de Biología, Universidad de Guanajuato.

G-8
RFLP-PCR's standardization to identify *CYP2C9's* allelic variants (*2, 3* and *SNP4)* in the Mexican population. **Patricia Cuautle-Rodríguez**, Nidia Samara Rodriguez-Rivera, Fernando Castillo-Nájera, Oscar Eduardo Campos-Ramos, Isaac González Romero, Juan Arcadio Molina-Guarneros. FM - UNAM

G-9
Genome-wide copy number analysis in Müllerian aplasia. **Fernando Fernández**, Eva Martínez-Peñafiel, Carlos Venegas-Vega, Saraí Valerio, Adriana del Castillo, Alicia Cervantes, Jaime Berumen, Susana Kofman. Unidad de Genética, Hospital General de México

G-10
Genome-wide identification of the genes mediating antagonistic pleiotropy of aging in the budding yeast. **Erika Garay**, Abraham Avelar & Alexander de Luna. LANGEBIO. CINVESTAV – IPN

G-11
Characterization of dystrophin mutant Dp40c-L170Pexpression in PC12 cells. **César Pastor García Cruz**, Jorge Aragón Medrano, Alejandro Martínez-Herrera, Víctor Ceja Orozco, Ma. Luisa Bazán Tejeda, Rosa Ma. Bermúdez Cruz y Cecilia Montañez Ojeda. Genética y Biología Molecular, CINVESTAV – IPN Zacatenco

G-12
Frequency of polymorphisms of five genes involved in athletic performance in an Mexican population**. Froylan Arturo García-Martínez**, Luis Ángel Montes-Almanza, Luz Berenice López-Hernández, Guillermina Ávila-Ramírez, Cecilia de los Ángeles Nieto-Gómez, José Gilberto Franco-Sánchez, Andrea Pegueros-Pérez, Benjamín Gómez-Díaz. UNAM

G-13
Comparison of mutation profiles in the Duchenne Muscular Dystrophy gene among populations: implications for exon skipping and stop codon read-trough therapies. **Benjamín Gómez-Díaz**, Alexandra Berenice Luna-Angulo, Carolina Zúñiga-Guzmán, Mónica Anaya-Segura, Rosa Elena Escobar-Cedillo, Héctor Rangel-Villalobos, Francisco Javier Estrada-Mena, Ramón Mauricio Coral-Vázquez and Luz Berenice López-Hernández. Instituto Nacional de Rehabilitación.

G-14
Functional divergence of duplicate genes coding DEAD RNA helicases in *Bacillus subtilis*. **José Antonio González Gutiérrez**, Itzel Aidé Vargas-Pérez, Gabriela Olmedo Álvarez. CINVESTAV – IPN Irapuato

G-15
Describing the epistatic interactions among genes that extend yeast lifespan. **Jorge González de la Cruz**, Erika Garay& Alexander de Luna. LANGEBIO. CINVESTAV – IPN Irapuato

G-16
Wnt signaling pathway alterations induced by *Trichinella spiralis* muscle larvae excretory-secretory products in primary myoblast cultures. **Lizbeth Hernández Ancheyta**, María del Rosario Salinas Tobón, Javier Hernández Sánchez. Genética y Biología Molecular. CINVESTAV – IPN

G-17
Functional characterization of *Candida tropicalis MNN4* and *OCH1*. **Hernández-Chávez Marco J**, Mora-Montes Héctor M. Departamento de Biología, Universidad de Guanajuato

G-18
Resveratrol increases expression of the sarco/endoplasmic reticulum Ca^{2+}-ATPase in mice hearts. **María Dolores Hernández-Huerta**, Abigail Guzmán-Bárcenas, Octavio Villanueva-Sánchez, Gabriela Rodríguez, Ángel Alfonso Zarain-Herzberg. Facultad de Medicina, UNAM

G-19
Extra chromosomal complementation of *trt1*-disrupted mutant of *Ustilago maydis*. **Guillermo M Horta Valerdi**, Estela Anastacio Marcelino, Candelario Vázquez Cruz y Patricia Sánchez Alonso. Instituto de Ciencias, BUAP

G-20
Increased expression of Hedgehog (Hh) molecules and EMT-related Hh pathway downstream target genes in transgenic mice K14E7**. Eloísa Ibarra Sierra,** Enoc M. Cortés Malagón, Victor H Garzón Barrientos, Rodolfo Ocadiz Delgado, José A Munguía Moreno, José Bonilla Delgado, José Díaz Chávez, Alejandro García Carrancá, Patricio Gariglio. Genetics and Molecular Biology, CINVESTAV – IPN Zacatenco

G-21
Nuclear export of β-Dystroglycan. **Guadalupe Elizabeth Jiménez Gutiérrez**, Griselda Vélez Aguilera, Bulmaro Cisneros Vega. CINVESTAV – IPN Zacatenco

G-22
Getting a mutant gene in *Gallibacterium anatis* 12656-12*qseC*. **Ana Jaqueline López Ochoa**, Patricia Sánchez Alonso, Erasmo Negrete Abascal, Candelario Vázquez Cruz. Centro de Investigaciones en Ciencias Microbiológicas. ICUAP, BUAP

G-23
Characterization of cis-elements that negatively regulate transcription of *EPA* genes through silencing proteins of *Candida glabrata.* **Eunice López-Fuentes and Irene Castaño**. IPICYT

GENETIC REGULATION & EPIGENETIC

GR-1
Insights into the evolution and domain structure of Ataxin-2 proteina cross eukaryotes. **Domingo Jiménez López**, Laura Aguilar-Henonin and Plinio Guzmán. CINVESTAV Unidad Irapuato

GR-2
Changes in internalization and regulators of virulence gene expression after *S. aureus* pre-treatment with TNFα and IL-10. **Daniela Angel-Andrés**, Octavio Silva-García, Víctor Manuel Baizabal-Aguirre, Javier Oviedo-Boyso, Alejandro Bravo-Patiño, Marcos Cajero-Juárez, Martha Patricia Chávez-Moctezuma and Juan José Valdez-Alarcón. FMVZ, Universidad Michoacana de San Nicolás de Hidalgo

GR-3
Role of PARP/PARG [(Poly-ADP-ribosyl) polymerase/glycohydrolase] in thepathogenic fungus *Fusarium oxysporumf.sp.lycopersici.* **Carlos A. Araiza-Cervantes**, Nancy E. Lozoya-Pérez, María Isabel González Roncero, Guadalupe Martínez Cadena, Georgina E. Reyna López. Depto. de Biología. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

GR-4
Chromatin Immunoprecipitation Analysis by RT-PCR in the yeast *Saccharomyces cerevisiae.* **Cristina Aranda**, Alan Anuart González Rangel, Lourdes Valenzuela and Alicia González. Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

GR-5
Spatio-temporal analysis of the *Pv*KNOLLE promoter activity in *Phaseolus vulgaris* transgenic roots inoculated with *Rhizobium tropici*. **Emmanuel Ayala Guzmán**, Elizabeth Monroy Morales, Carmen Quinto and Rosana Sánchez-López. Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-6
Comparison of the effect of HPV 18 and 11 (high and low risk types) E6 oncoproteins on the proliferation and migration of C33A cells. **Osvaldo Bautista-Isidro**, Verónica García-Castillo, Eduardo López-Urrutia and Carlos Pérez-Plasencia.Unidad de Biomedicina. FES Iztacala-UNAM

GR-7
The role of the CDK8 module of Mediator in vegetative development of *Arabidopsis thaliana.* **Manuel Buendía-Monreal** and Stewart Gillmor. LANGEBIO – CINVESTAV, IPN Irapuato

GR-8
Construction of the recombinant proteins for the NMD putative factors Upf1, Upf2 and Upf3 of *Ustilago maydis.* **Xadeni Burgos Gamez**, Nancy Martínez Montiel, Rebeca D Martínez Contreras. Instituto de Ciencias. BUAP

GR-9
Interaction of NFAT, AP-1, NF-kB, Sp1 and STAT3 protein and its involvement in the expression of IL-10 in U937 monocytes stimulated with LPS/PGE2. **Jorge A Calzada Martínez**, Sandra R. Reyes Carmona,

Nora H Rosas Murrieta, Irma P Herrera Camacho, Julio R Reyes Leyva, Lourdes Millán Pérez Peña. Instituto de Ciencias, BUAP

GR-10

Methylation analysis of genes for the T-type voltage-dependent calcium channels in cervical cancer derived cell lines. **Katherine Lilibeth Campaña Valencia**, Pedro Chávez Olmos, Francisco Javier Camacho Arroyo y José Efraín Garrido Guerrero. Genética y Biología Molecular. CINVESTAV - IPN

GR-11

Transcriptional regulation of *GDH3* in *Saccharomyces cerevisiae*. **Brisa Aranzazú Campos-Oliver**, Maritrini Colón-González, José Carlos Campero-Basaldúa, James González-Flores, Cristina Aranda-Fraustro and Alicia González-Manjarrez. Instituto de Fisiología Celular. UNAM

GR-12

Phenotype analysis of *Phaseolus vulgaris* transgenic roots expressing *Pv*CLC2-RNAi OR *Pv*CLC3-RNAi. **Alfredo Capistrán López**, Elizabeth Monroy Morales, Carmen Quinto and Rosana Sánchez-López. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-13

Unraveling the function of *Phaseolus vulgaris RbohD* during nodulation. **Gabriela Carmona Pulido**, Manoj Kumar Arthikala, Noreide Nava, Jesús Montiel and Carmen Quinto. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-14

Analysis of the expression patterns of the Mitogen Activated Protein Kinase Kinases (MAPKKs) encoded on genome the *Arabidopsis thaliana*. **Yessica Casales-Tlatilpa**, Jesús Salvador López-Bucio, Maricela Ramos-Vega, Patricia Leónand Ángel Arturo Guevara-García. Facultad de Biología, UAEM

GR-15

Analysis of the interaction between the yKu, Abf1 and Rap1 proteins of *Candida glabrata* and the*cis*-acting regulatory elements in the E-R telomere. **Leonardo Castanedo-Ibarra**, Alejandro De Las Peñas and Irene Castaño. Laboratorio de Microbiología Molecular, IPICYT

GR-16

Site specific DNA methylation analysis of the RXRA promoter region from umbilical cord blood and its relationship to maternal nutritional status during pregnancy. **Diana Chávez Lizárraga**, Felipe Vadillo Ortega, Carmen Canchola Sotelo, Jorge Beltran Montoya Erika Chavira Suárez
Escuela Superior de Medicina, IPN

GR-17

Methylation-sensitive high resolution melting analyses tosite specific LEPR gene promoter methylation from umbilical cord related with the maternal BMI and weight gain during pregnancy. **Erika Chavira Suárez**, Jesús Jorge Beltrán Montoya, Carmen Canchola Sotelo, Felipe Vadillo-Ortega. Facultad de Medicina, UNAM

GR-18

mir-26: A Putative Epigenetic Regulator in Iflammation-Associated Colorrectal Cancer Development. **Coronel-Hernández Jossimar**, García-Castillo Verónica, López-Urrutia Eduardo and Pérez-Plasencia Carlos.Unidad de Biomedicina. FES Iztacala-UNAM

GR-19

Silencing the *PvKEULE*GENES in *Phaseolus vulgaris* transgenicroots: effect on the nodulation. **Jonathan Gilberto Cuéllar Vega**, Marco Adán Juárez Verdayes, Raúl Dávila Delgado, Elizabeth Monroy Morales, Carmen Quinto y Rosana Sánchez-López. Instituto de Biotecnología, UNAM

GR-20

Analysis of genes involved in polar transport, signaling and metabolism of auxin during primary root development of *Pachycereus pringlei* (*Cactaceae*)**. Andrés Cuevas-Moreira**, Marta Matvienko, Joseph G Dubrovsky and Svetlana Shishkova. Instituto de Biotecnología, UNAM

GR-21

Genetic and transcriptional analysis of Zygotic Genome Activation in early embryogenesis of *Arabidopsis thaliana.* **Gerardo Del Toro**, Marcelina García-Aguilar and Stewart Gillmor. LANGEBIO–CINVESTAV.

GR-22
Ca^{2+}-dependent pathways control calsequestrin-2 expression in cardiomyocytes. **Rafael Estrada-Avilés**, Gabriela Rodríguez, José Manuel Medina-Cervantes, Ángel Zarain-Herzberg. Departamento de Bioquímica, Facultad de Medicina, UNAM

TOXICOLOGY

T-1
Modulation of nerve growth factor expression (NGF) by ionizing radiation in different mouse tissues. **Albarrán-Ponce LÁ**, Fajardo-Miranda RM, Gamboa de Buen MI, Valdovinos-Flores C and Gonsebatt ME. Instituto de Investigaciones Biomédicas, UNAM

T-2
Effect of airborne fine particle exposure on $5HT_{1A}$- and D_2-receptor density and signaling in rat striatum, prefrontal cerebral cortex and olfactory bulb**. María de los Angeles Andrade Oliva**, Octavio Gamaliel Aztatzi Aguilar, José Antonio Arias-Montaño, Andrea De Vizcaya-Ruiz. Toxicología y Fisiología, Biofísica y Neurociencias, CINVESTAV – IPN. Zacatenco

T-3
Fluoride induced tubular injury and apoptosis in male Wistar rat subchronically exposed through drinking water and challenged with gentamicin. **Olivier C Barbier**, Mariana C Cárdenas González, Tania L Jacobo Estrada, Luz M Del Razo. Toxicología, CINVESTAV – IPN. Zacatenco

T-4
Genotoxic effect of temephos in human lymphocytes**. Alma Betsaida Benitez Trinidad**, Guillermina Vázquez-Estrada, José Francisco Herrera Moreno, Monserrat Sordo Cedeño, Patricia Ostrosky Wegman, Irma Martha Medina Díaz, María de Lourdes Robledo Marenco, Briscia Socorro Barrón Vivanco, Yael Yvette Bernal Hernández, Romero Bañuelos Carlos Alberto, Aurora Elizabeth Rojas García. Universidad Autónoma de Nayarit

T-5
Chronic low level arsenic exposure induces progressive aberrant DNA methylation that correlates with cell transformation in HaCaT cell line. **Federico Centeno Cruz**, Francisco Barajas Olmos, Elizabeth Ortiz Sánchez, José Luis Cruz Colín, Ivan Imaz Rosshandler, Claudia Rangel Escareño, Lorena Orozco. Instituto Nacional de Medicina Genómica

T-6
Proteomic characterization of venom from male and female scorpions of the species *Centruroides limpiduslimpidus*. **Jimena Isaias Cid Uribe**, Georgina Gurrola Briones, Fredy Coronas Valderrama and Lourival Domingos Possani. Instituto de Biotecnología-UNAM

T-7
Cloning and expression of a serinoprotease from the venom of *Bothrops ammodytoides*. **Herlinda Clement**, Vianey Flores, Alejandro Alagón, Adolfo de Roodt and Gerardo Corzo. Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

T-8
Isolation and characterization of a phospholipasein *Palythoacaribaeorum*. **Miguel Cuevas Cruz** and Roberto Arreguin-Espinosa. Graduate in Marine Science sand Limnology, UNAM

T-9
Coadministration of High Doses of Rosuvastatin and a Cholesterol-Rich Diet Produces Premature Death in CD-1 Male Mice. **Juan Cuauhtémoc Díaz-Zagoya**, Isela Esther Juárez-Rojop, Andrés Eliud Castell-Rodríguez, Jorge Luis Blé-Castillo, Rodrigo Miranda-Zamora. Facultad de Medicina, UNAM

T-10
Evaluation of lipid profile and association to cardiac risk factors in rural communities of state of Chihuahua exposed to fluoride in drinking water. **Iván Alejandro Domínguez Guerrero**, Luz María Del Razo, Olivier

Barbier, Mónica I Jiménez Cordova, Blanca Sánchez-Ramírez, María de Lourdes Ballinas-Casarrubias, Rocío Infante-Ramírez, Efraín Villareal, María del Carmen González-Horta. Facultad de Ciencias Químicas. Universidad Autónoma de Chihuahua

T-11

Genotoxic Safety of Jacareubein in Bone Marrow Cells of Female Balb/c Mice. **Elizabet Estrada Muñiz**, Lucero G Mireles López, Ricardo Reyes-Chilpa, Libia Vega. CINVESTAV-IPN

T-12

Expression and folding of cysteine rich neurotoxins from the venoms of spiders and coral snakes. **Vianey Flores-Lara**, Herlinda Clement, Alejandro Alagon, Gerardo Corzo. Instituto de Biotecnología, UNAM

T-13

Phage-displayed variants of the Css2 toxin from *Centruroides suffuses suffusus*. **García-Montelongo M**, Restano-Cassulini R, Becerril B, Ortiz E. Instituto de Biotecnología, UNAM

T-14

Participation of mTOR in the transport of aminoacids involved in glutathione (GSH) synthesis in mouse striatum. **Carla Garza Lombó**, Lourdes Massieu Trigo, Mauricio Díaz Muñoz, Pavel Petrosyan, María E Gonsebatt. Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, UNAM

T-15

Genotoxicity of Dialkilophosphates in Lymphocytes and Human Hepatic Cells. **José Arturo Godínez Izquierdo**, Elizabeth Estrada Muñoz, Libia Vega Loyo. Toxicología. CINVESTAV-IPN

T-16

Effects of temephos on DNA damage in human liver carcinoma (HepG2) cells. **José Francisco Herrera Moreno**, Francisco Alberto Verdín-Betancourt, Alma Betsaida Benitez Trinidad, Irma Martha Medina Díaz, Monserrat Sordo Cedeño, Patricia Ostrosky Wegman, María de Lourdes Robledo Marenco, Yael Yvette Bernal Hernández, Briscia Socorro Barrón Vivanco, Aurora Elizabeth Rojas García. Universidad Autónoma de Nayarit

T-17

Evaluation of glomerular filtration rate, cystatin-C, β-2-microglobulin, KIM-1 and clusterin on adult population environmentally exposed to fluoride. **Mónica I Jiménez Córdova**, Mariana C Cárdenas González, Carmen González Horta, Guadalupe Aguilar Madrid, Ángel Barrera Hernández, Efraín E Villarreal Vega, Iván A Domínguez Guerrero, Rocío Infante Ramírez, Olivier C Barbier, Luz M Del Razo. Departamento de Toxicología, CINVESTAV-IPN

SIGNAL TRANSDUCTION

ST-1

Signal integration by P-Rex1, a multidomain RacGEF, linked to its interaction with cAMP-dependent Protein Kinase. **Sendi Rafael Adame-García**, Lydia Chávez-Vargas, R Daniel Cervantes-Villagrana, Guadalupe Reyes-Cruz and José Vázquez-Prado. CINVESTAV IPN Zacatenco

ST-2

Subchronic treatment with ferric nitrilotriacetate (FeNTA) induces AP-1 activation and cyclin D1 overexpression: possible mechanisms of renal carcinogenicity. **Francisco Antonio Aguilar Alonso**, José Dolores Solano Becerra, Chabetty Vargas Olvera , Claudia Martinez Martinez, Telma Olivia Pariente Pérez, María Elena Ibarra Rubio. Facultad de Química, UNAM

ST-3

Study of the role played by the protein p32 (gC1qR) in maintaining the malignant phenotype of colon cancer. **Sara Ruth Albarrán-Gutiérrez**, Cristina Castañeda-Patlán y Martha Robles-Flores. UNAM

ST-4
The phosphoinositide-dependent protein kinase 1 inhibitor , UCN-01, induces fragmentation. Possible role of metallo proteinases. **Rocío Alcántara-Hernández**, Aurelio Hernández-Méndez and J Adolfo García-Sáinz. Instituto de Fisiología Celular. UNAM

ST-5
Function/structure of the human alpha 1d adrenoceptor. **Marco Antonio Alfonzo Méndez**, María Teresa Romero Ávila, Jean Alberto Castillo Badillo and J Adolfo García-Sáinz. Instituto de Fisiología Celular, UNAM

ST-6
Regulation of the regulatory subunit of Protein Kinase A subcellular localization in *Saccharomyces cerevisiae.* **Francisco Manuel Amezola-Chávez**, Mariana Hernández-Elvira, Francisco Torres-Quiroz. Instituto de Fisiología Celular, UNAM

ST-7
The effects of Hydrogen Sulfide on Protein Kinase A activation inthe yeast *Saccharomyces cerevisiae*. **Juan de Dios Ayala-González**, Francisco Torres-Quiroz. Bioquímica y Biología Estructural. Instituto de Fisiología Celular, UNAM

ST-8
Akt degradation by direct interaction with Bag5. **Ismael Bracho-Valdés**, Paola Moreno-Alvarez, Jorge Carretero-Ortega, Estanislao Escobar Islas, Adán Olguín-Olguín, J. Alberto Olivares Reyes, J. Silvio Gutkind, Guadalupe Reyes-Cruz, José Vázquez-Prado. Departments of Pharmacology, Cell Biology and Biochemistry, CINVESTAV-IPN

ST-9
Molecular features and expression analysis of RACK1 from the symbiotic dinoflagellate *Symbiodinium microadriaticum.* **Marco A Villanueva Méndez**, Esmeralda Pérez Cervantes, Tania Islas Flores, Montserrat Loredo Guillén and Gabriel Guillén Solís. Instituto de Ciencias del Mar y Limnología, UNAM

ST-10
Effect of KPNA2 down regulation on the nuclear localization of small GTPase Rac1 in HaCaT cells. **Calixto-Gálvez Mercedes**, De la Cruz-López Karen G, García-Hernández Alejandra P, Navarro-Tito Napoleón, Castañeda-Saucedo Eduardo. Ciencias Químico Biológicas, Universidad Autónoma de Guerrero

ST-11
The global regulator CsrA is required for proper BarA activation and *UvrY* translation. **Martha I Camacho**, Adrián F Álvarez y Dimitris Georgellis. Instituto de Fisiología Celular. UNAM

ST-12
The sustained activation of AT1R prevents the Akt/PKB desensitization in hepatic C-9 cells. **Jesús Castillo Hernández**, Rafael Rubio, Jesús Alberto Olivares Reyes. CINVESTAV – IPN Zacatenco

ST-13
Biochemical and molecular features of a HSP90-like protein from *Symbiodinium microadriaticum* that undergoes phosphorylation changes in response to light. **Raúl E Castillo Medina** and Marco A Villanueva Méndez. Instituto de Ciencias del Mar y Limnología, UNAM

ST-14
Constitutively active RhoGEFs, representing the most abundantly expressed branch of endothelial RhoGEFs, including RGS-RhoGEFs, Intersectins, PLEKHG5 and NGEF, reveal a strong angiogenic signal transduction potential. **Alejandro Castillo-Kauil**, Ricardo Hernández-García, Guadalupe Reyes-Cruz and José Vázquez-Prado. CINVESTAV-IPN Zacatenco

ST-15
Detecting phosphorylations in Efl1 with Mass Spectometry. **Nina del Carmen Castro Moreno**, Eugenio de la Mora Lugo, Nuria Sánchez Puig. Instituto de Química, UNAM

ST-16
Prostaglandin E_2 differentially alters protein expression in female *Anopheles albimanus* mosquito midgets. **Febe E Cázares-Raga**, Alejandra del Pilar Ochoa Franco, Fernando García- Gil de Muñóz, Mario Henry Rodríguez, Fidel de la Cruz Hernández-Hernández. CINVESTAV-IPN Zacatenco

ST-17
Contribution of bone marrow-derived cells to tumor growth: Potential role of RhoGEFs. Rodolfo **Daniel Cervantes-Villagrana**, Ricardo Hernández-García, Lydia Chávez Vargas, Alejandro Castillo Kauil, Guadalupe Reyes-Cruz and José Vázquez-Prado. CINVESTAV-IPN Zacatenco

ST-18
LPS of *Helicobacter pylori* regulates claudins expression via ERK1/2. **Christian O. Chavarría-Velázquez**, Luis F. Montaño-Estrada, Erika P. Rendón-Huerta. Facultad de Medicina, UNAM

ST-19
Regulating of the activity of NF-κB by action of glycine dare channel Ca^{+2} in adipocytes. **Erika Contreras Nuñez**, Gerardo Blancas Flores, Julio Almanza Pérez, Miguel Cruz Lopéz, Rubén Román Ramos, Francisco Javier Alarcón Aguilar. DCBS, UAM-Iztapalapa

ST-20
The effect of multiple carboxylase deficiency in decrease the mitochondrial mass is mediated via STAT3-HIF1a-BNIP3 in brain. **Jose Ahmed Corella Vazquez**, Estefanía Ochoa Ruiz, Rodrigo Díaz Ruiz, Antonio Velázquez Arellano. Instituto de Investigaciones Biomédicas. UNAM

ST-21
Mechanism for the specific targeting of methyltransferases to chemoreceptors in *Pseudmonas aeruginosa* PAO1. **Andrés Corral-Lugo**, Cristina García-Fontana and Tino Krell. Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain

ST-22
The transcriptional regulator Atf1 has key roles in cellular stress and asexual reproduction in *Trichoderma atroviride*. **Víctor Alejandro Correa Pérez**, Fidel Landeros Jaime, José Antonio Cervantes Chávez, Edgardo Ulises Esquivel Naranjo. Universidad Autónoma de Querétaro

ST-23
Identification of DNA binding proteins to the regulatory region implicated in the *STP1* (*SUGAR TRANSPORTER PROTEIN 1*) gene sugar regulation in *Arabidopsis thaliana*. **María-Isabel Cruz-López** and Elizabeth Cordoba. Instituto de Biotecnología, UNAM

ST-24
Role of the redundant proteins Ssk2p and Ssk22p in the endoplasmic reticulum stress response in the yeast *Saccharomyces cerevisiae*. **Uriel Cruz-Martínez**, José Francisco Torres-Quiroz, Laura Kawasaki-Watanabe, Roberto Coria. Instituto de Fisiología Celular, UNAM

ST-25
Regulation of voltage-dependent calcium channels by G proteins in rat β-pancreatic cells. **Lizbeth de la Cruz**, Silvia Valdés-Rives, Isabel Arenas, David García. Facultad de Medicina, UNAM

ST-26
Effects of endotoxin tolerance on the cytokine production and the secretory machinery of mas cells activated through the high affinity IgE receptor (FcεRI). **Zyanya Patricia Espinosa Riquer** and Claudia González Espinosa. Departamento de Farmacobiología, CINVESTAV-IPN

ST-27
The SAPK signaling pathway regulates cellular stress and responses to light in *Trichoderma atroviride.* **Edgardo Ulises Esquivel Naranjo**, Mónica García Esquivel, Víctor Alejandro Correa Pérez, Jorge Luis Parra Arriaga, Fidel Landeros Jaime, José Antonio Cervantes Chávez, Herrera Estrella A. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro

ST-28
Significant suppression of colitis-related colorectal cancer in a murine model. **Gabriela Figueroa-González**, Verónica García-Castillo, Víctor Montesinos-Fuentes, Nadia Jacobo-Herrera, Lizbeth Palma-

Berré, Eduardo López-Urrutia, Sonia León-Cabrera, Luis Ignacio Terrazas-Valdés and Carlos Pérez-Plasencia. Unidad de Biomedicina. FES Iztacala-UNAM

ST-29

Extracellular ATP Induces Apoptosis through P2X7 Receptor activation in MDA-MB 231 cells. **Flores-Flores Marycruz**, Monjaraz-Eduardo, Avelino Cruz José Everardo. Instituto de Fisiología BUAP

ST-30

Cancer metabolic hallmarks blockade induces death by autophagy in triple negative breast cancer cell line. **Verónica García-Castillo,** Nadia J Jacobo-Herrera, Eduardo López-Urrutia, Gabriela Figueroa-González, Víctor M Lara-Camacho, Mallely C Ávila-García, y Carlos Pérez-Plasencia. Unidad de Biomedicina. FES Iztacala-UNAM

ST-31

ZmPDK1, a new component of the *Zea mays* TOR pathway. **Verónica Garrocho-Villegas**, Maria Julissa Ek-Ramos, Raúl Aguilar, Lourdes Segura, Luis Felipe Jiménez and Estela Sánchez de Jiménez. Facultad de Química, UNAM

ST-32

Endogenous Ribosomal Heterogeneity in Maize Tissues: Possible Biological Relevance. **Ricardo Hernández Hermenegildo**, Cruz Jesus Ginez Ramos and Estela Sánchez de Jiménez. Facultad de Química, UNAM

BASIC BIOCHEMISTRY

B-38
Evaluation of Plant Lectins Interaction with *Aspergillus parasiticus*. **Edmar de Jesús Díaz García**, Marco Antonio Sánchez Medina, María del Socorro Pina Canseco, Alma Dolores Pérez-Santiago. Instituto Tecnológico de Oaxaca

B-39
Identification of the amino acids responsible for the differences in susceptibility to inactivation by methylmethane thiosulfonate of the triosephosphate isomerases from *Trypanosoma brucei* and *T. cruzi*. **Selma Díaz Mazariegos**, Itzhel García Torres, Nallely Cabrera González and Ruy Perez Montfort. Depto. Bioquímica y Biología Estructural. IFC - UNAM.

B-40
Analysis of glycerol dehydrogenases in *Mucor circinelloides* YR-1. **Arelí Durón Castellanos**, Roberto Zazueta-Sandoval. Div. de Ciencias Naturales y Exactas. Universidad de Guanajuato

B-41
Amino acids as modulators of root development in habanero pepper (*Capsicum chinense*Jacq.). Mildred Rubi Carrillo-Pech, Angélica Anahí. Serralta-Interian, Ernesto Palacios-Medel, Ángel Virgilio Domínguez-May, Candy Susana Fano-Silva, **Ileana Echevarría-Machado.** Unidad de Bioquímica y Biología Molecular de Plantas. CICY

B-42
Structural studies of the human triosephosphate isomerase. Artificial deamidation in the amino acid residues 15 and 71 and his effect over structure. **Sergio Enríquez Flores**, Alfredo Torres Larios, Ignacio De La Mora De La Mora, Sara T. Méndez, Adriana Castillo Villanueva, Saúl Gómez Manzo, Gabriel López Velázquez, Jaime Marcial Quino, Angélica Torres Arroyo, Itzhel García Torres, Horacio Reyes Vivas, Jesús Oria Hernández. Laboratorio de Bioquímica Genética, Instituto Nacional de Pediatría

B-43
Changes in affinity and molecular basis of multiple specificity of a periplasmic binding protein for basic amino acids; The LAO-protein. **Andrés Escandón Flores**, Nancy O Pulido Mayoral, Alejandro Fernández-Velasco, and Alejandro Sosa Peinado, Depto. Bioquímica, Facultad de Medicina, UNAM

B-44
Characterization of Dystroglycan on Kasumi 1 cell line. **Marco Antonio Escarcega-Tame**, Ivette Astrid Martínez-Vieyra, Lea Alonso-Rangel, Doris Cerecedo. Escuela Nacional de Medicina y Homeopatía, IPN

B-45
Assembly and distribution of the OXPHOS complexes and supercomplexes reflect energy metabolism in two human glioblastoma cell lines. **Diana Lashidua Fernández Coto**, Juan Fernando Minauro Sanmiguel, Karina Salvador Severo, Álvaro Marin Hernández. Unidad de Investigación Médica en Genética Humana, Hospital de Pediatría, CMN Siglo XXI, IMSS

B-46
Structural and functional characterization of Whiteleg shrimp thioredoxin (*Litopenaeus vannamei*). **Arline Fernández Silva**, Hugo A Gómez Uribe, Carlos Amero Tello. Centro de Investigaciones Químicas, UAEM

B-47
Effect of chronic exposition to extreme low frequency electromagnetic fields on fatty acid contents and lipoperoxidation in different regions of rat's brain. **Alan Emmanuel Flores Poblano**, Jesús Martínez Sámano, Leticia Verdugo Díaz, Oscar Iván Luqueño Bocardo, Marco Antonio Juárez Oropeza, Patricia Victoria Torres Durán. Facultad de Medicina, UNAM

B-48
NMR assignment and dynamics of LAO protein. **Leidys French Pacheco**, Renan Vergara, Isabel Velazquez, Alejandro Sosa and Carlos Amero. Centro de Investigaciones Químicas, UAEM

B-49
Study of the Pet309 function by site directed mutagenesis in *Saccharomyces cerevisiae* mitochondria. **Jesús Emmanuel Frías Jiménez**, Yolanda Camacho Villasana, Xochitl Pérez Martínez. Instituto de Fisiología Celular, UNAM

B-50
Identification of a lectin from blue mussel (*Mytilus edulis)* belonging to an unclassified family of lectins. **Efrén García Maldonado** and Alejandra Hernández Santoyo. Depto. de Química de Biomacromoléculas, Instituto de Química, UNAM

B-51
New target of proton pump inhibitors: inactivation of triosephosphate isomerase from *Giardia lamblia* by Omeprazole derivatives. **Itzhel García-Torres**, Ignacio De la Mora De la Mora, Saúl Gómez-Manzo, Jaime Marcial Quino, América Vanoye Carlo, Jesús Oria Hernández, Horacio-Reyes-Vivas, Gabriel Navarrete-Vázquez, Gabriel López-Velázquez, Sergio Enríquez-Flores. Instituto Nacional de Pediatría

B-52
Is the loss of protein interaction in Ribosome Biogenesis the cause of Shwachman-Bodian-Diamond Syndrome? **Abril Gijsbers Alejandre**, Nancy Marcial , Takuya Nishigaki y Nuria Sánchez Puig. Department of Chemistry of Biomacromolecules, Institute of Chemistry, UNAM

B-53
Structural and functional characterization of four pathologic variants causing Glucose-6-phosphate dehydrogenase deficiency in humans. **Saúl Gómez Manzo**, Jessica Terrón Hernández, Ignacio De la Mora-De la Mora, Alejandra Abigail González Valdez, Itzhel García-Torres, Jaime Marcial-Quino, América Vanoye-Carlo, Jesús Oria-Hernández, Gabriel López-Velázquez, Sergio Enríquez-Flores. Lab. Bioquímica Genética, Instituto Nacional de Pediatría

B-54
Reduction of nitrate and ammonium assimilation during induced capsaicinoid accumulation in *in vitro* cultured placentas of habanero pepper (*Capsicum chinense* Jacq.). **David J Góngora-Espínola**, Guadalupe G Martín-Morales, Aurora J Trujillo-Bernat, Lizbeth A Castro-Concha and María de Lourdes Miranda-Ham. U. de Bioquímica y Biología Molecular de Plantas, CICY

B-55
Computational Models of Pores Made of Light Chain Variable Domains: Another Cytotoxic Species? **Diego S Granados**, César Millán-Pacheco, Nina Pastor. Unidad Profesional Interdisciplinaria de Ingeniería, IPN Guanajuato

B-56
Tracheal cryopreservation: CASPASE-3 expression in tracheal epithelium and in mixed glands. **Isabel Guadarrama-Sánchez,** Avelina Sotres-Vega, J.Alfredo Santibañez-Salgado, Jaime Villalba-Caloca, José Luis Torre-Jaime, Jazmín Azaarel García-Montes, Miguel Gaxiola-Gaxiola. Lung Transplantation Research Unit. National Institute of Respiratory Diseases

B-57
Is glycerol employed as osmolyte in the symbiotic dinoflagellate *Symbiodinium?*. **Luis Parmenio Suescún Bolívar** , Patricia E Thomé Ortíz. Instituto de Ciencias del Mar y Limnología. UNAM

B-58
Unexpected redox homeostasis in the free-living platyhelminth *Dugesia* sp. **Alberto Guevara Flores**, Álvaro Miguel Herrera Juárez, José de Jesús Martínez González, I Patricia del Arenal Mena, and Juan Luis Rendón. Departamento de Bioquímica, Facultad de Medicina, UNAM

B-59
Intracellular localization of the six *Arabidopsis thaliana* isoforms of soluble inorganic pyrophosphatase. **Francisca Morayna Gutiérrez-Luna**, Lilian G Valencia-Turcotte and Rogelio Rodríguez-Sotres. Depto. de Bioquímica, Facultad de Química, UNAM

B-60
Purificación y caracterización parcial de una enzima tipo quimotripsina de *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). **María del Carmen Hernández Hernández**, Elizabeth Mendiola Olaya, José Luis Castro Guillen, Alejandro Blanco Labra. CINVESTAV – IPN Unidad Irapuato

B-61
Synthesis of omeprazole analogs with potential capacity to inactivate the triosephosphate isomerase of Giardia lamblia (GlTIM). **Beatriz Hernández Ochoa**, Gabriel-Navarrete Vázquez, Angélica Torres-Arroyo, Adriana Castillo-Villanueva, Sara Méndez, Jesús Oria-Hernández y Horacio Reyes Vivas. Laboratorio de Bioquímica Genética, Instituto Nacional de Pediatría. SS.

B-62
Analysis of the O-GlcNAcylation state of the vitamin D receptor (VDR) in human monocytes cultured under hyperglycemic conditions. **Fernando Hernández-Sánchez**, Manuel Salgado-Cantú, Madeline Balderas- Anaya, Ricardo Vega-Hernández and Martha Torres-Rojas. Instituto Nacional de Enfermedades Respiratorias.

B-63
Comparative study of *Synechococcus* PCC 7335 and CCC9, two different strategies for cyanobacterial nitrogen fixation and the harvesting of light. **Priscila Herrera Salgado**, Lourdes E Leyva Castillo Carlos Gómez Lojero. CINVESTAV, IPN. Unidad Zacatenco

B-64
Effect of Mixed Inoculation on the Yield and Content of Phenols in the Common Bean Seed (*Phaseolus Vulgaris* L.). **Miguel Jacinto Hurtado Contreras**, Lourdes Macías-Rodríguez, Eduardo Valencia-Cantero, José López-Bucio, Josué Altamirano-Hernández. Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana

B-65
Rol of autophagy in altered hydrotropic response (*ahr1*) mutant of *Arabidopsis thaliana*. **Gladys Edith Jiménez Nopala**, Diego Cevallos Porta, Gladys Cassab López, Helena Porta Ducoing. Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

B-66
Carbonic anhydrases and their role in mammalian sperm physiology. **Omar José Ramírez**, Paulina Torres Rodríguez, Rafael Baltiérrez Hoyos, Fabrizio Carta, Joachim W Deitmer, Claudiu Supuran, Claudia L Treviño Santa Cruz. Instituto de Biotecnología, UNAM

B-67
Structural studies of Mu-class glutathione S-transferase from shrimp *Litopenaeus vannamei.* **Juárez-Martínez Ariadna B,** Sotelo-Mundo Rogerio, Rudiño-Piñera Enrique. Facultad de Ciencias, UAEM

B-68
Flavonoids acacetin, chrysin and 4´,7-dimethyl narangenin as photosynthesis and seedling growth inhibitors. **Beatriz King-Díaz**, José Fausto Rivero-Cruz and Blas Lotina-Hennsen. Facultad de Química, UNAM

B-69
Importance of the GTPase Gpn3 in the nuclear accumulation of RNA polymerase II in breast cells with increasing degrees of malignancy. **Bárbara Lara Chacón**, Mónica R Calera, Roberto Sánchez Olea. Instituto de Física, UASLP

B-70
Effect of methanol extracts of *Lentinula edodes* on the gelatinases A and B (MMP2 and MMP9) and urokinase (PLAU) in breast cancer cells MCF7. **Jacqueline Viridiana Lara Espinosa**, María Guadalupe Martínez Hernández, Carmen Elizabeth Zamora Mancera, Víctor Manuel Esparza Martínez, Luis Arturo Baiza Gutman. Laboratorio de Biología del Desarrollo, UMF. Jardín Botánico. UNAM

B-71
Structure and function characterization of SdrP protein from *Thermus thermophilus*. **Kevin Eder Gamboa Ramírez**, José G. Sampedro, Ruben Paul Gaytan Colin, Samuel Lara González. Laboratorio de Biología Estructural, IPICyT

B-72
A derivative-based steroidal androstane skeleton compound (OM4) inhibits FcεRI-dependent degranulation after IgE/Antigen stimulation in mast cells . **Alfredo Ibarra-Sánchez**, Mariana Garrido González, Alejandro Padilla-González, Cabeza M, Bratoeff E and Claudia González-Espinosa. CINVESTAV – IPN. Zona Sur

B-73
Expression of MUC1 glycoprotein in HEK293 cells for studying the role of MAL and GGA2 proteins on its cellular trafficking. **Antonio Roberto Lara Lemus** and Ana Karina Saldaña Villa. Investigación en Bioquímica, Instituto Nacional de Enfermedades Respiratorias

B-74
Quality Control Test for Sequence-Phenotype Assignments. **Maria Teresa Lara Ortiz**, Pablo Benjamín Leon Rosario, Pablo Luna-Nevarez, Alba Savin Gamez, Ana Martínez-del Campo, Gabriel Del Rio Guerra. Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

B-75
Functional Divergence of Genes Implicated in the Leucine Biosynthesys in *Sacharomyces cerevisiae* and the Ancestor Type Yeast *Kluyveromyces lactis*. **Mijail Lezama,** Geovani López and Alicia González. Bioquímica y Biología Estructural. Instituto de Fisiología Celular, UNAM

BIOTECHNOLOGY

BT-41
The nanoparticles of gold destroy the capsule of *Streptococcus pneumoniae*; the principal virulence factor that this pathogen has been developing to evade the immune system. **Edgar Augusto Ortiz Benítez**, Mariana Carrillo Morales, Alejandra Stephany Rodríguez Leviz, Norma Velázquez Guadarrama, Jesús Fandiño Armas y José de Jesús Olivares Trejo. Posgrado en Ciencias Genómicas. Universidad Autónoma de la Ciudad de México

BT-42
Expression and chromatographic purification of full-length and a truncated form of the GTPase Gpn1 in bacteria for structural and functional studies. **Rogelio González González**, José Alberto Guerra Moreno, Gabriela M. Montero Morán, Sonia Griselda Peña Gómez, Jorge Jaime Juárez Lucero, Samuel Lara González, Mónica R Calera y Roberto Sánchez Olea. Instituto de Física, Universidad Autónoma de San Luis Potosí.

BT-43
Evaluation of extraction techniques of grain amaranth albumin (*Amaranthus hypochondriacus* L.) for increased efficiency. **Aldo Rodrigo González Luna**, Diana Caballero Hernández, Jesús Iván Luevano Fernández, Deyanira Quistián Martínez, Sergio Moreno Limón. Facultad de Ciencias Biológicas UANL.

BT-44
Development of recombinant strains of *Bacillus thuringiensis* expressing the endochitinase ChiA74 as inclusion bodies or chimeric crystals with Cry1Ac. **Karen Stephania González-Ponce,** Luz Edith Casados-Vázquez, Rubén Salcedo-Hernández and J. Eleazar Barboza-Corona. División Ciencias de la Vida. Universidad de Guanajuato

BT-45
Solid Phase Microextraction of Volatile Compounds in Basil (*Ocimum basilicum* L.). **Salvador González-Palomares**, Luis Humberto Rivera-Cambero, Gabriel Sánchez-Zaragoza, Juan García-Estrada and Héctor Manuel González-Sánchez. Instituto Tecnológico de Ocotlán

BT-46
Mechanical properties of thermoplastic sorghum starch films reinforced with lignin from *Agave tequilana* Weber. **Rosa Angélica Guillén-Garcés**, Tania Itzel Sánchez-Gómez Andrea Cristina Hernández-Molina,

Julián Martínez-Salgado, Diana Karina Díaz de Anda, José Luis Rivera-Corona. Universidad Politécnica del Estado de Morelos

BT-47

Overexpression of fatty acid synthesis and its effect over the biosynthesis of medium-chain-length polyhydroxyalkanoates (mcl-PHAs) in *Azotobacter vinelandii*. **Claudia Velázquez-Sánchez,** Fanny A. Flores, Josefina Guzmán, Guadalupe Espín, Daniel Segura. Instituto de Biotecnología, UNAM

BT-48

Selection and Characterization of a Native *Psathyrella* Strain as a Producer of Laccases Exhibiting Significant Activity under Near-Neutral pH. **Carlos Eduardo Hernández Luna**, Juan Francisco Contreras Cordero, Michelle Ramírez-Plascencia, Guadalupe Gutiérrez-Soto, Sergio Manuel Salcedo-Martínez. Facultad de Ciencias Biológicas, UANL

BT-49

Measurement of the enzymatic activity of laccase in the wild strain 4287 and R*ho1::hyg* strains of *Fusarium oxysporum* f. sp. *lycopersici* under induction conditions and presence of a chelating agent. **Wendy S Hernández-Monjaraz**, César Caudillo-Pérez, Karla L Macías-Sánchez. Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato, IPN

BT-50

Studying of the phenotype from the mutants with deletion ape2 gene and function of leucine aminopeptidase yspII from *Schizosaccharomyces pombe*. **Irma Herrera-Camacho**, Nessie Gutiérrez-Díaz, José Luis Cerriteño-Sánchez, Lourdes Millán-PerezPeña, Sandra Reyes-Carmona, Nora Rosas-Murrieta. Centro de Química-ICUAP-BUAP

BT-51

Obtaining mutants haploids and diploids by deletion of *ape2* gene of leucine aminopeptidase yspII from *Schizosaccharomyces pombe.* **Irma Herrera-Camacho**, Nessie Gutiérrez-Díaz, José Luis Cerriteño-Sánchez, Lourdes Millán-Pérez Peña, Felix Luna, Nora Rosas-Murrieta. Centro de Química-ICUAP-BUAP

BT-52

cDNA obtaining and three-dimensional structure of Maradol Papaya (*Carica papaya* L.) endoxylanase. **J Jesús Iniestra González**, Robert E Paull, Helen Turano, Ana Paulina Barba de la Rosa and Juan Alberto Osuna Castro. Instituto Tecnológico Superior de Tamazula de Gordiano

BT-53

Expression and biochemistry characterization of a hyaluronidase from the Scolopendra viridis Say VENOM. **Erika Yazmin Ibarra Rojas**, Fernando Martínez Morales, María del Carmen Gutiérrez Villafuerte. Centro de Investigación en Biotecnología, UAEM

BT-54

Transcriptional Analysis of the *Arabidopsis thaliana* TFIIB*2* mutant. **Dulce Jared Jaime Gallardo**, José Antonio Miranda Ríos, José Augusto Ramírez Trujillo, Ramón Suarez Rodríguez. Centro de Investigación en Biotecnología. UAEM

BT-55

Antimicrobial peptides and commercial antibiotics synergistic effect evaluation in bacteria from diabetic foot ulcer. **Katia Esmeralda Jiménez Sánchez,** Gerardo Corzo, Alexis Jiovani Rodríguez Solis, Elba Villegas. Centro de Investigación en Biotecnología, UAEM

BT-56

Protein engineering with immunoglobulin-like fold. **Pedro Jiménez-Sandoval**, Luis Gabriel Brieba-de Castro. LANGEBIO – CINVESTAV – IPN Irapuato

BT-57

Generation of recombinant strains of *Bacillus thuringiensis* that synthesize the endochitinase ChiA74 and mosquitocidal Cry proteins. **Estefania Odemaris Juárez-Hernández**, Luz Edith Casados-Vázquez, Gustavo Hernández-Guzmán and J. Eleazar Barboza-Corona. División Ciencias de la Vida, Universidad de Guanajuato, Campus Irapuato-Salamanca

BT-58
Identification and cDNA cloning of a hyaluronidase from theskin secretion of *Trachycephalus typhonius.* **Selma Jurado**, Francia García, Herlinda Clement, Gerardo Corzo. Instituto de Biotecnología, UNAM

BT-59
Kinetics of fatty acids accumulation in *Chlorella vulgaris* jointly immobilized with *Azospirillum brasilense* at three temperatures. **Luis Leyva**, Mariana Díaz Tenorio, Luz Estela de Bashan, Yoav Bashan. Northwestern Center for Biological Research. Instituto Tecnológico de Sonora

BT-60
Proteomic approach to the early stages of pozol fermentation. **Cynthia Leyva-Arguelles**, Catalina Cárdenas, Carmen Wacher, Rosario Vera and Romina Rodríguez-Sanoja. Instituto de Investigaciones Biomédicas, UNAM

BT-61
Relationship between the increase in the oxidative state and the improvement of infectivity in the *Isaria fumosorosea* conidia. Francisco Miranda-Hernández, Katiushka Arévalo-Niño, Gerardo Saucedo-Castañeda. **Octavio Loera.** Universidad Autónoma Metropolitana - Iztapalapa

BT-62
Identification of a peroxidase related to post –harvest insect resistance of maize (*Zea mays* L) by activity–directed proteomics. **L. Margarita López – Castillo**, Luis Gabriel Brieba, Robert Winkler. LANGEBIO. CINVESTAV – IPN Irapuato.

BT-63
Hydrolytic enzymes of membrane and cell wall assay secreted by endophytic bacteria as an antifungal factor against *Mycosphaerella fijiensis*. **Gloria Margarita Macedo Raygoza,** Miguel Juan Beltrán García. Universidad Autónoma de Guadalajara

BT-64
Influence of Rho1 in the expression of *Icc2*, *Icc3* and *Icc5* in strains of *F. oxysporum* f. sp. *Lycopersici*. **Karla L. Macías-Sánchez**, Alejandra Reyes-Medina. Unidad Profesional Interdisciplinaria de Ingeniería. Campus Guanajuato, IPN.

BT-65
Application of marine yeast for biological control of the phytopathogenic fungus *Aspergillus flavus*on post-harvested *P. vulgaris* L. **Rubí Alejandra Martínez Camacho**, Felipe Ascencio. Centro de Investigaciones Biológicas del Noroeste

BT-66
Synthetic production of a Teparybean lectin: generation of the genetic construction and insertion into *Agrobacterium tumefaciens.* **Martínez-Alarcón D**, Castro-Guillén JL, Cruz-Hernández A, Blanco-Labra A, Mora-Áviles A, Espinoza-Núñez AM, García-Gasca T. Facultad de Química, Universidad Autónoma de Querétaro

BT-67
Characterization of biologically active cyclic dipeptides from bacterial isolates originating in pools of Cuatro Ciénegas, Coahuila. **Enrique Martínez-Carranza**, Valeria Souza-Saldívar, Alma Laura Díaz-Pérez, Victor Meza-Carmen, Jesús Campos-García. IIQB, Universidad Michoacana

BT-68
Alkaloid synthesis during seedling development in *Argemone mexicana* L. **Felipe Vázquez-Flota**, Jorge Xool-Tamayo, Miriam Monforte-González, UBBMP, CICY

BT-69
Analysis of *Trichoderma atroviride* –plant interaction with fungal strains that overexpress the *SwoI* gene. **Richa Mehta,** Edgar Balcázar, Karina Atriztán Hernández, Alfredo Herrera-Estrella, y Jorge Luis Folch Mallol. Centro de Investigación en Biotecnología. UAEM

BT-70
Generation and characterization of truncated versions of MelA tyrosinase from *Rhizobium etli* expressed in *Escherichia coli.* **María Alejandra Mejía Caballero,** Paul Gaytán Colín, Luz María Martínez Mejía, Alfredo Martínez Jiménez, Guillermo Gosset Lagarda. Instituto de Biotecnología, UNAM

BT-71
The physicochemical characterization and antioxidant potential of *Bromelia karatas* fruits. **Karen Elizabeth Meza Vázquez,** Juan Florencio Gómez Leyva, José Pedro Castruita Domínguez, Julia Zañudo Hernández, Rudy Antonio García Reyes, Lucila Méndez Morán. CUCBA, Universidad de Guadalajara

BT-72
Metabolic characterization of tomato plants using low-temperature plasma (LTP) as a new ambient ionization technique. **Josaphat Miguel Montero-Vargas**, John Délano-Frier and Robert Winkler. Biotecnología y Bioquímica. CINVESTAV - Unidad Irapuato

BT-73
Elaboration of fermented fruit beverages. **Sandra Morales Arrieta**, Ana Verónica Hernández Cervantes, Yarely Lizeth Palacios Martínez, Eduardo Rodríguez Díaz, Iván Ocampo Abraján, Zamri Miamin Ramírez Carbajal and Antonio Lagunas Figueroa. Universidad Politécnica del Estado de Morelos

BT-74
Expression and preliminary characterization of amarantin acidic subunit and amarantin acidic subunit modified with bioactive peptides. **Jocksan Ismael Morales Camacho**, Silvia Luna Suárez, Daniel Alejandro Fernández Velasco. CIBA - Instituto Politécnico Nacional, Tlaxcala

BT-75
Isolation of lactic acid bacteria which produce bacteriocins from ranchero cheese. **Aurea Itzel Morales-Estrada**, Rosa María Ahidé López-Merino, Minerva Georgina Araiza-Villanueva, Araceli Contreras-Rodríguez. Escuela Nacional de Ciencias Biológicas, IPN

BT-76
Isolation and characterization of fungi for the biocontrol of *Phytophthora capsici L*. and their protective effect in *Capsicum annuum L*. plants. **Montzerrat Betzabé Muñoz Medina**, Saúl Fraire-Velázquez Victor Balderas Hernández. Universidad Autónoma de Zacatecas

BT-77
Peptides of different protein fractions from Chia seeds (*Salvia hispanica*) produced inhibitory activity against Angiotensin I-converting enzyme. **Blanca Nieto-Rendón**, Domancar Orona-Tamayo, María Elena Valverde, Talía Hernández-Pérez and Octavio Paredes-López. CINVESTAV – IPN Irapuato

BT-78
Analyzing the function the*arg2* gene encoding the small subunit of carbamoyl-phophatesynthetaseas a transformation marker in *Trichoderma atroviride*. **Omar Nieves Ugalde**, Fidel Landeros Jaime, José Antonio Cervantes Chávez y Edgardo Ulises Esquivel Naranjo. Universidad Autónoma de Querétaro

BT-79
Cloning and recombinant expression of Elongation Factor 1-α of *Leishmania infantum.* **Ana Cristina Olivas Bejarano**, Annete Itzel Apodaca Medina, Sergio Alonso Durán Pérez, Claudia del Rosario León Sicairos, Jeanett Chávez Ontiveros, Vianney Francisco Ortiz Navarrete, Evangelina Beltrán López and Héctor Samuel López Moreno. Universidad Autónoma de Sinaloa

BT-80
Insecticidal activity of δ-PaluIT1 in *Heliothis virescens* and *Apis mellifera*. **Giovanna Patricia Olivos Trejo**, Ninfa María Rosas García Alexis Joavany Rodríguez Solís, Gerardo Corzo and Elba Villegas. Centro de Investigación en Biotecnología, Facultad de Biología UAEM

BT-81
Peptides with antihypertensive functions of protein fractions from the canary seeds (*Phalaris canariensis* L.). **Domancar Orona-Tamayo**, Blanca Nieto-Rendón, María Elena Valverde, Talía Hernández-Pérez and Octavio Paredes-López. CINVESTAV – IPN Irapuato

BT-82
Stability evaluation of bioemulsifier by *Acinetobacter bouvetii* in polluted water with diesel. **Ortega de la Rosa N. D**, Motolinia Alcántara E. A,Gutiérrez Rojas M. Universidad Autónoma Metropolitana Unidad Iztapalapa

BT-83
AtGRDP2, a novel glycine-rich domain protein, involves in plant growth and development. **María Azucena Ortega-Amaro,** Aída Araceli Rodríguez-Hernández, Margarita Rodríguez-Kessler, Eloísa Hernández-Lucero, Sergio Rosales Mendoza, Alejandro Ibáñez-Salazar, Pablo Delgado-Sánchez, Juan Francisco Jiménez-Bremont. División de Biología Molecular, IPICyT

BIOCHEMISTRY EDUCATION

E-1
Validation of the questions of an "extraordinay examination" in the subject of Biochemistry and Molecular Biology of the medical curriculum at the School of Medicine, UNAM. **Héctor Javier Delgadillo-Gutiérrez** and Yolanda Saldaña Balmori. Departamento de Sistemas Biológicos, UAM-X

E-2
Validation of the conversion rate of weight live weight processed sea cucumber, *Isostichopus badionotus,* through the moisture content. **Cynthia Paulina Lucio Ramírez**, Aurea Luisa May Ruiz, Luis Alfonso Rodríguez Gil, Carlos Francisco Reyes Sosa. Instituto Tecnológico de Mérida

E-3
The use of clinical scenarios in biochemistry and the development of competencies (communication and collaborative work. **Deyamira Matuz Mares**, Diego Rolando Hernández Espinosa, María Alicia del Sagrado Corazón Cea Bonilla. Biochemistry. Faculty of Medicine, UNAM

E-4
Analysis of the accreditation criteria for the students in the first year of the medicine school in the matter of biochemistry and molecular biology. **Rebeca Milán Chávez**, Deyamira Matuz Mares, Eugenia Flores Robles. Juan Pablo Pardo Vázquez. Biochemistry. UNAM

E-5
Correlation between the grades attained at the high school of origin and the final grades reached in the subject of Biochemistry and Molecular Biology of the medical curriculum in a group of students at the School of Medicine, UNAM. **Yolanda Saldaña-Balmori** and Héctor Javier Delgadillo-Gutiérrez. Departamento de Bioquímica, Facultad de Medicina, UNAM

OXYGEN REACTIVE SPECIES

OR-1
Effect of senescence- associated secretory phenotype (SASP) from primary mice fibroblasts on cellular migration, proliferation and senescence on the transformed cell line L929. **Angélica Alejandra Aquino Cruz**, Gibrán Pedraza Vázquez, Luis Ángel Maciel Barón, Mina Konigsberg, Norma Edith López DíazGuerrero, Armando Luna López. Ciencias Básicas y de la Salud. UAMI

OR-2
Neuroprotective effect of *Spirulina (Arthrospira) platensis*in intracellular redox state in rats undergoingendotoxic shock. **Bañuelos-León Janeeth**, Ramírez-Anguiano Ana Cristina, Sánchez-Peña María Judith, Pacheco-Moisés Fermín, Gabriel-Ortiz Genaro, Bitzer-Quintero Oscar Kurt, Rosales-Rivera Lizet Yadira, Velasco-Ramírez Sandra Fabiola. Centro Universitario de Ciencias Exactas e Ingenierías (CUCEI), Universidad de Guadalajara

OR-3

Phospholipid Hydroperoxide Glutathione Peroxidase 2 (GPX2) preserves yeast mitochondrial function and viability during chronological aging in the presence of PUFA. **Melina Canizal García**, Elizabeth Calderón Cortés, Renato Canizal García, Marissa Calderón Torres, Luis Alberto Madrigal Pérez, Alfredo Saavedra Molina Christian Cortés Rojo. Instituto de Investigaciones Químico-Biológicas. Universidad Michoacana de San Nicolás de Hidalgo

OR-4

HSP70 protein levels in young and old monosodium glutamate (MSG) female and male treated mice: a link between obestiy and aging. **Hernández-Bautista RJ**, Cazares-Ordoñez S, Konigsberg M, López-DiazGuerrero NE. Departamento de Ciencias Básicas y de la Salud. UAMI

OR-5

Karwinskia humboldtiana administration as a model of the induction of oxidative stress in *Sprague Dawley* rat: evaluation of melatonin and fish oil as preventive treatment. **Margarita Cid Hernández**, Ana C. Ramírez Anguiano, Ana L. Briones Torres, Luis J. González Ortíz, Genaro Gabriel Ortiz y Fermín P. Pacheco-Moisés. CUCEI. Universidad de Guadalajara

OR-6

Dynamic of reactive oxygen species in roots from *Arabidopsis* under Aluminum treatment. **Lidia Díaz**, Jesús S. Lopez Bucio, Alejandra Hernández-Barrera, Rosana Sánchez, Eric Johnson, Hen-ming Wu, Alice Cheung**,** Federico Sánchez, Rosana Sánchez, Carmen Quinto and Luis Cárdenas. Instituto de Biotecnología, UNAM

OR-7

Tissular and biochemical changes in maize scutellum during transition of germination to the postgermination. **David Manuel Díaz-Pontones,** Sandra González-Martínez, José Isaac Corona-Carrillo, Coral Viruel, Gerardo Chávez-Nájera y Mitzi Flores-Ponce. UAMI

OR-8

Effect of the specific NADPH oxidase inhibitor (VAS2870) on intracellular calcium changes: a connection between ROS and calcium signaling. **Román Espinal**, Ana-María Velarde-Buendía, Alejandra Hernández-Barrera, Rosana Sánchez, Federico Sánchez, Carmen Quinto and Luis Cárdenas. Instituto de Biotecnología, UNAM

OR-9

Comparative DNA repair capacity of human adipose derived mesenchymal stem cells to remove oxidative damage induced by different oxidative insults. **Jonathan Lozano Salgado**, Emilio Rojas del Castillo, Mahara A Valverde Ramírez. Instituto de Investigaciones Biomédicas, UNAM

OR-10

Malfunctioning assembly of the iron–sulfur cluster machinery in *Saccharomyces cerevisiae* produces mitochondrial oxidative stress by an iron-dependent mechanism, affecting respiratory function and increases sensitivity to ethanol and ROS. **Mauricio Gómez Gallardo**, Rocío Pérez-Gallardo, Luis A. Sánchez Briones, Alma Díaz-Pérez, Christian Cortes-Rojo and Jesús Campos-García. IIQB, Universidad Michoacana de San Nicolás de Hidalgo

OR-11

Effects of DPI (NADPH oxidase inhibitor) on post-fatigue tension of slow skeletal muscle. **Ana María Guzmán Ambriz**, Elizabeth Sánchez-Duarte, Xóchitl Trujillo, Miguel Huerta, Alain Rodríguez-Orozco, Christian Cortés-Rojo, Rocío Montoya-Pérez. IIQB, Universidad Michoacana

OR-12

Maize polyamine oxidases are important for tumor formation in the maize-*Ustilago maydis* interaction. **Ignacio Jasso Robles,** Alicia Becerra Flora, Margarita Juarez-Montiel, Juan Francisco Jiménez-Bremont, Ramón Fernando García de la Cruz, Maria Azucena Ortega-Amaro, Margarita Rodríguez-Kessler. Facultad de Ciencias, Universidad Autónoma de San Luis Potosí

OR-13
Antioxidant Compounds of Timbe (*Acaciella angustissima*) and Hypoblycemic Effect. **Sandra N Jimenez-Garcia**, Wendy M Carmen-Sandoval, Adriana Jheny Rodríguez-Méndez, Ramón G Guevara-González, Irineo Torres-Pacheco, María Elena Villagran-Herrera, Adrés Cruz-Hernández, Ana A Feregrino-Perez. Facultad de Ingenieria, Universidad Autonoma de Queretaro

OR-14
Influence of Different Elicitors on phenolic compounds in sweet peppers (*Capsicum annuum* L.) cultivated in greenhouse conditions. **Sandra N. Jimenez-Garcia**, Moises A. Vazquez-Cruz, Lina Garcia-Mier Ramón G. Guevara-Gonzalez, Irineo Torres-Pacheco, Rosalía V. Ocampo-Velázquez, Adrés Cruz-Hernández, Ana A. Feregrino-Perez. Universidad Autónoma de Querétaro

OR-15
Ascorbate-glutathione cycle in mitochondria of tomato fruit in two different ripening stages. **Omar R López-Vidal**, Ana M Jiménez Hurtado, Mina Konigsber Fainstein, Laura J Pérez-Flores, Fernando Rivera Cabrera , José A Mendoza Espinoza y Fernando Díaz de León-Sánchez. Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana

OR-16
Determination of antioxidant activity in larvae and vermicompost of the insect *Tenebrio molitor*. **José de Jesús Luna-Díaz**, Ana Cristina Ramírez-Anguiano, Fermín Paul Pacheco-Moisés, Lizet Yadira Rosales-Rivera, Oscar Kurt Bitzer-Quintero, María Judith Sánchez-Peña, Luis Javier González-Ortiz, Sandra Fabiola Velasco-Ramírez. CUCEI. Universidad de Guadalajara

OR-17
Chronic administration of S-allycysteine (SAC), do not causes histological damage in eight tissues, and induces Nrf2 factor and antioxidant enzymes in brain of male rats. **Perla D Maldonado**, Urzula Franco-Enzástiga, Sandra Bautista-Pérez, Laura Garza-Montoya, Diana Barrera-Oviedo. Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suarez"

OR-18
Effect of moderate physical exercise as Tai chi and Walking on Biological markers of Oxidative stress and chronic inflammation in the elderly. **Juana Rosado-Pérez**, Mirna Ruiz-Ramos, Víctor M. Mendoza-Núñez. Laboratorio de Investigación en Gerontología, FES Zaragoza UNAM

IMMUNOLOGY

I-1
Role of ZnT1 in the regulation of hepatic Zinc after an experimental inflammatory process. **Violeta Aburto Luna**, Gerardo Santos López, Samuel Treviño Mora, Diana Moroni González, Alfonso Daniel Díaz Fonseca, Patricia Aguilar Alonso, and Eduardo Miguel Brambila Colombres. Facultad de Ciencias Químicas, BUAP

I-2
Effect of oestradiol and testosterone on the activity of GPx and levels of MDA in CBA/Ca mice infected with *P. berghei* ANKA. **Jesús Aguilar Castro** and Martha Legorreta Herrera. FES Zaragoza, UNAM

I-3
Glycosylation pathways in T lymphocyte-associated signal transducer and activator of transcription-6 in a mouse model knockout. **Janette Arias Escobedo**, Francisco Javier Urrea Ramírez. Instituto Nacional de Enfermedades Respiratorias

I-4
Effect of oestradiol and testosterone on the activity of catalase and the levels of haemoglobin in CBA/Ca mice infected with *P. berghei* ANKA. **Fidel Orlando Buendía González** and Martha Legorreta Herrera. FES Zaragoza, UNAM

I-5

Analysis of Branched-chain amino acid metabolism and mTOR activation during macrophage polarization. **Ana E. Bunt Alatorre**, Armando R. Tovar Palacio, Nimbe Torres y Torres, Vianney Ortiz Navarrete, Lilia G. Noriega López. Fisiología de la Nutrición, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

I-6

Effect of sexual hormones on the variation of weight and temperature in CBA/Ca mice infected with *P. berghei* ANKA. **Luis Antonio Cervantes Candelas** and Martha Legorreta Herrera. Laboratorio de Inmunología Molecular. FES Zaragoza, UNAM

I-7

Cortactin regulates intestinal epithelial permeability by stabilizing tight junctions. **Alí Francisco Citalán-Madrid**, Alexander García-Ponce, Hilda Vargas-Robles, Abigail Betanzos, Klemens Rottner, Rudolf Mennigen, Michael Schnoor. Molecular Biomedicine. CINVESTAV - IPN

I-8

Virulence, immunopathology and transmissibility of selectedstrains of *M. tuberculosis* from epidemiological studies in Colombia evaluated in a murine model. **María Fernanda Cornejo**, Jaime Robledo, Dulce Mata, Brenda Marquina, Jorge Barrios, Luis García and Rogelio Hernández-Pando. National Institute of Medical Sciences and Nutrition Salvador Zubirán

I-9

Effect of the E6 and E7 oncoproteins of HPV16 on dendritic cells in skin of transgenic mice models. Gabriela Damián-Morales, Nicolás Serafín-Higuera, Enoc Mariano Cortés-Malagón, Genaro Rodríguez-Uribe, Mario Adán Moreno-Eutimio, José Bonilla-Delgado, Rodolfo Ocadiz –Delgado, Ana Alejandra Ramírez-Rodríguez and Patricio Gariglio-Vidal. Genética y Biología Molecular. CINVESTAV – IPN Zacatenco

I-10

Effects of plant antimicrobial peptides on the innate immune response of bovine mammary epithelial cells infected with *Staphylococus aureus*. **Violeta Díaz-Murillo**, Joel E López-Meza y Alejandra Ochoa-Zarzosa. Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, UMSNH

I-11

Activity of GPx and MDA levels in mice knockout for the prolactin receptor infected with *P. berghei* ANKA. **Azrael Estrada Sosa,** Carmen Clapp and Martha Legorreta Herrera. FES Zaragoza, UNAM

I-12

Plant Defensins: Structure, Function and Heterologous Expression. **Georgina Estrada Tapia**, Adolfo Guzmán Antonio, Ignacio Islas, Ernesto Ortiz, Gerardo Corzo. Unidad de Bioquímica y Biología Molecular de Plantas. CICY

I-13

Immunomodulatory effect of an adenosine derivative (IFC-305) in isoproterenol induced myocardial infarction. **Jessica Abigail Feria-Pliego**, Mariana Domínguez López, Victoria Eugenia Chagoya de Sánchez. Instituto de Fisiología Celular UNAM

I-14

Regulation of steroid hormones on the secretion of proMMP-9 produced from human fetal membranes during infection with *Escherichia coli*. **Héctor Flores Herrera**, Raúl Mendieta Flores, Denise Acuña González, Yuridia Paredes Vivas, Guadalupe García López, Iyari Morales Méndez, Irma E Sosa González, Anayansi Molina Hernández, Fabían N Díaz. Departamento de Inmunobioquímica, Instituto Nacional de Perinatología

I-15

Proinflammatory cytokines responses induced by *Pseudomonas aeruginosa* in human type II pneumocyte cells. **Fuentes Zacarías Paulina,** Barrientos Galeana Edgar, Mendieta Flores Raúl, Ortega González D Katherine, Díaz N. Fabián, Iyari Morales Méndez, García López Guadalupe, Arenas Huertero Francisco, Molina Hernández Anayansi, Flores Herrera Héctor. Instituto Politécnico Nacional

I-16
Identification and characterization of allergenic proteins in latex gloves. **Christian Galicia Díaz Santana**, Guillermo Mendoza Hernández, José Federico Del Río Portilla, Adela Rodríguez Romero. Instituto de Química, UNAM

MEDICINE, HEALTH AND NUTRITION

M-1
Searching potential inhibitors of arginase II through virtual screening. The search for new drugs against endothelial dysfunction. **Miriam Leticia Aguirre-Raudry**, María Irene Betancourt-Conde, Alejandra GuadalupeVazquez-Raygoza, Lluvia Iveth Ríos-Soto, Claudia Avitia-Domínguez, Marie Jazmín Sarabia-Sánchez, Artemisa Luévano-De la Cruz, Alfredo Téllez-Valencia. CIAN, Facultad de Medicina y Nutrición, UJED

M-2
Effect of sucrose-induced metabolic syndrome upon amp-activated protein kinase (ampk) and insulin signaling in the rat skeletal muscle. **Blanca Viridiana Alcantar González**, Eduardo Rodríguez Correa, Karla Carvajal. Nutrición Experimental, Instituto Nacional de Pediatría.

M-3
Antifibrotic activity of flavonoids and saponins extracted from the seed coat of black bean (*Phaseolus vulgaris*) in fibroblasts from rat heart. **Gilberto Amaro-Osejo**, Carlos Enrique Guerrero-Beltrán, José Luis González-Guerra, Guillermo Torre-Amione and Gerardo García-Rivas. Instituto de Cardiología y Medicina Vascular, Escuela de Medicina, Tecnológico de Monterrey

M-4
Reduced activity and Ca^{2+} sensitivity of ryanodine receptors in cardiomyocytes from metabolic syndrome rats. **Ma. Dolores Bello Sánchez**, Jaime Balderas-Villalobos, Hugo Aldana-Quintero, Tzindilu Molina-Muñoz, Maritza Mayorga, Karla Carvajal and Norma L Gómez-Viquez. Departamento de Farmacobiología. CINVESTAV – IPN

M-5
MT-ND3 sequence analysis in tumor tissue and peripheral blood from patients with prostate cancer. **Jesús Benítez Granados,** Juan P Méndez Blanco, André Tapia Vázquez, Mónica A Martínez Ramírez, Lisandro Vazquez Niño, Edgardo Reyes Gutiérrez, Guillermo Feria Bernal, Ramón M Coral Vázquez, Ileana P Canto Cetina. UNAM

M-6
Docking studies in shikimate dehydrogenase from *Enterobacter cloacae* to find potential inhibitors. **María Irene Betancourt-Conde**, Alejandra Guadalupe Vázquez-Raygoza, Claudia Isela Avitia-Domínguez, Lluvia Iveth Ríos-Soto,Miriam Leticia Aguirre-Raudry, Edna Madai Méndez-Hernández, Alfredo Téllez-Valencia. Medicina y Nutrición, Universidad Juárez del Estado de Durango

M-7
Effects of steam cooking on the proximate and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) from Huasca de Ocampo, Hidalgo, México. **Edith Araceli Cano Estrada**, Jair Onofre Sánchez, Javier Añorve Morga. Universidad Autónoma del Estado de Hidalgo

M-8
The Post-conditioning in the Treatment of Acute Myocardial Infarction in an Experimental Model with Metabolic Syndrome. **Juan Carlos Cantellano Degante**, Francisco Correa y Cecilia Zazueta. Instituto Nacional de Cardiología-Ignacio Chávez

M-9
Antineoplastic copper coordinated complexes (Casiopeínas) uncouple oxidative phosphorylation and induce mitochondrial permeability transition in cardiac mitochondria and cardiomyocytes. **Mariana Carrancá,** Luis E Martínez, Christian Silva-Platas, Carlos E Guerrero-Beltrán, Elena Cristina Castillo, Jesús R Garza,

Noemí García and Gerardo García-Rivas. Hospital Zambrano Hellion

M-10

Identification of Enteropathogenic Bacteria with DNA Microarrays. **Lorena Chávez González**, José Luis Santillán Torres, Simón Guzmán León, Jorge Ramírez Salcedo. Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, UNAM

M-11

Effect of chronic immobilization stress and sucrose consumption on the development of hepatic steatosisin male infant rat. **Adriana Corona Pérez,** Ida Soto Rodríguez, Francisco Castelán, Margarita Martínez Gómez, Jorge Rodríguez Antolín y Leticia Nicolás Toledo. Universidad Autónoma de Tlaxcala.

M-12

Molecular identification of respiratory viruses in patients with clinical diagnosis of influenza. **Iliana Alejandra Cortés Ortiz**, Edith Violeta Martínez Martínez, Juan Carlos Bravata Alcántara, Gustavo Acosta Altamirano, Mónica AlethiaCureño Díaz, Mónica Sierra-Martínez. Hospital Juárez de México

M-13

Sleep restriction induces glucosetolerance in rats. **Andrea Cortés Panameño**, Beatriz Gómez González, Javier Velázquez Moctezuma and Emilio Domínguez Salazar. Biología de la Reproducción, UAMI

M-14

Evaluation of the effect of avocado oil on renal vascular reactivity and mitochondrial dysfunction induced by angiotensin II. **Lucio Hernández de la Paz**, Elizabeth Calderón Cortés, Rocío del Carmen Montoya Pérez, Rafael Salgado Garciglia, Alfredo Saavedra Molina, Daniel Godinez Hernández, Christian Cortés Rojo. IIQB. Universidad Michoacana de San Nicolás de Hidalgo

M-15

Proteomic Analysis of Rat Serum Treated with DNAse I and Papain (preliminary results). **Desiree Ariadna De La Cruz Sigüenza**, Catalina Trejo Becerril, Humberto Gonzales Marques, Alfonso Dueñas Gonzales. Instituto Nacional de Cancerología

M-16

Expression and distribution of tight junction proteins in peritoneal cells of diabetic rats. **Yazmín Debray García,** Elsa Irene Sánchez Montes de Oca, Rafael Rodríguez Muñoz, José Luis Reyes Sánchez. Fisiología Biofísica y Neurociencias, CINVESTAV – IPN

M-17

Sucrose consumption plus and chronic stress induces histological changes in the adrenal gland in the male rat. **Yadira Díaz-Aguila***,* Francisco Castelán, Mauricio Díaz-Muñoz, Estela Cuevas, Margarita Martínez-Gómez, Jorge Rodríguez-Antolín and Leticia Nicolás-Toledo. Universidad Autónoma de Tlaxcala

M-18

Low ADAR 1, ADAR 2 and ADAR 3 levels are associated with a different expression of microRNAs in Idiophatic Pulmonary Fibrosis. **Gabriela Díaz-Piña**, Eduardo Montes, Alfonso Salgado, Pedro Zamudio, Iliana Herrera, José Cisneros, Luis Felipe Dominguez, Carina Becerril, Marco Checa, Victor Ruiz. Instituto Nacional de Enfermedades Respiratorias

M-19

Effect of Omega 3 Fatty Acids in Diabetic Female Rats. **María del Consuelo Figueroa García**, Francisco Alfredo Saavedra Molina, Christian Cortés Rojo and Ricardo Mejía Zepeda. UMSNH

M-20

Effect of plant lectins of *Ruta graveolens* on breast cancer cells. **Laura Cecilia Flores García**, Francisco Martínez Flores y Catalina Machuca Rodríguez. Facultad de Estudios Superiores Zaragoza. UNAM

M-21

Treatment with Pirfenidone produces an important reduction in necroinflammation score, fibrosis index and increased gene expression of CB2 in patients with liver cirrhosis. **Lucia Flores-Contreras**, Ana Sandoval-Rodríguez A, Silvia Lucano-Landeros, Inmaculada Arellano-Olivera, Juan Armendáriz-Borunda. Instituto de Biología Molecular y Terapia Génica, CUCS, Universidad de Guadalajara

M-22
Development of an in vitro experimental model for human amniochorion rupture. **Miriam K García-Cid**, Elisa García-Vences,Victor G Perea-Santillán, Felipe Vadillo-Ortega, Carmen Canchola-Sotelo, Noemí Meraz-Cruz. Vinculación Científica. Facultad de Medicina, UNAM / INMEGEN

M-23
Transcriptomic profiling of adipose tissue in response to ACET/moxibustion treatment in obese women. **Jessica M. Garcia-Vivas**, Ma. Del Carmen Garcia Cardona, Carlos Galaviz-Hernandez, Francisco Lozano-Rodriguez, Cesar Lopez-Camarillo, Laurence A Marchat. Biotechnology Programy Molecular Biomedicine Program, ENMH-IPN

MICROBIOLOGY AND PARASITOLOGY

MP-1
Association between nonspecific febrile illness and leptospirosis. **Norma Patricia Adan Bante**, Danilo Manuel González Román, Adolfo Virgen Ortiz, Ramona Icedo García, José Guadalupe Soñanez Organis, Ana Paola Balderrama Carmona. Ciencias Químico-Biológicas. Universidad de Sonora

MP-2
Study of the role of Avin03910 depolymerase in the metabolism of Polyhydroxybutyrate polyester (PHB) in *Azotobactervinelandii.* **Libertad Adaya**, Josefina Guzmán, Soledad Moreno,Daniel Pfeiffer, Dieter Jendrossek, Guadalupe Espín y Daniel Segura. Instituto de Biotecnología, UNAM

MP-3
Estudio de la Diguanilatociclasa E putativa de *Azospirillumbrasilense*, que participa en crecimiento y estrés. **JJ Aguilar-Piedras**, A Gamboa-Pérez, A Ramírez-Mata, Ma. L Xiqui Vázquez, y BE Baca. Centro de Investigaciones en Ciencias Microbiológicas, BUAP

MP-4
Frequency of mutations in the *rpoB, katG* and *inhA* gene that confer drug resistance to rifampin and isoniazid in patients with Tuberculosis in the State of Jalisco: identification molecular basis. **Daniel Aguirre-Chavarria**, Guadalupe González Palomar, Martin López-Rodríguez, Manuel Sandoval-Díaz, Juan Carlos Villanueva-Arias and Ikuri Álvarez-Maya. Medical and Pharmaceutical Biotechnology Unit. CIATEJ

MP-5
Role of the putative domain, of the MucG protein, for the degradation of c-di-GMP, involved in alginate synthesisin *A. Vinelandii.* **Carlos L Ahumada-Manuel**, Josefina Guzmán, Guadalupe Espín and Cinthia E Núnez. Microbiología Molecular, Instituto de Biotecnología, UNAM

MP-6
Isolation, identification and characterization of cyanobacteria from biofilms of the archaeological site of Yohualichan, Puebla. **Raúl Alcalde-Vázquez**, Isela Aguila-Maldonado, Nora Beatriz Medina-Jaritz y Roxana Olvera-Ramírez. Escuela Nacional de Ciencias Biológicas. IPN

MP-7
Family characterization of Cyclin dependent kinasesin *Trichomonas vaginalis.* **Erick Amador Gaytán**, Karla López Pacheco, Nataly Morales Galeana y María Imelda López Villaseñor. Instituto de Investigaciones Biomédicas, UNAM

MP-8
The role of *Avin08930* in biosynthesis of Poly-β-Hydroxybutyrate and alkylresorcinols in *Azotobacter vinelandii*. **Leidy Patricia Bedoya-Pérez**, Soledad Moreno, Guadalupe Espín. Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM

MP-9
Effect of dichloromethane-methanol extract of *Parthenium hysterophorus* on enteropathogenic *Escherichia coli* (EPEC) adherence. **Ana Patricia Beltrán Ogazón**, Jazmín P Espinosa Rivero, Erika P Rendón Huerta and Irma Romero Álvarez. Bioquímica. Facultad de Medicina. UNAM

MP-10
Exploring the molecular mechanisms that maintain the *Rhizopus microsporus–Burkholderia rhizoxinica* symbiosis. **José Roberto Bermúdez-Barrientos**, Cei Abreu-Goodger and Laila Pamela Partida Martínez. LANGEBIO – CINVESTAV IPN Unidad Irapuato

MP-11
The flavonoid (-)-epicatechin affects erythrophagocytosis in *Entamoeba histolytica*. **Verónica Bolaños-Suárez,** Jacqueline Soto-Sánchez, Laurence Marchat, Esther Ramírez–Moreno. Laboratorio de Biomedicina Molecular, ENMyH- IPN

MP-12
Identification of a gene encoding putative flagellin in *Haemophilus influenzae*. **Alejandro Carabarin Lima**, Elías Campos de la Cruz, Patricia Lozano Zarain, Ygnacio Martínez Laguna, Sirenia González Posos, Rosa del Carmen Rocha Gracia. Instituto de Ciencias. BUAP

MP-13
Modification of the host cell cytoskeleton during *Toxoplasma gondii*encystment. **Kitzia Nohemí Castro Elizalde,** Pedro Hernández Contreras, Sirenia González Pozos, Ricardo Mondragón Flores. Unidad de Microscopía Electrónica(LaNSE). CINVESTAV-IPN, Zacatenco

MP-14
Potato bacterial endophytes as biocontrol and biofertilize ragents. **Claudia I Cisneros-Reyes**, Enrique Tinajero-Cortés, Miguel A Ibarra-Arias, Francisco R Torres-Guardado, Luis A Plascencia-Correa, Marcela de la Mora-Amutio, Julia del C Martínez-Rodríguez and Tetsuya Ogura. CIBA Tlaxcala-IPN

MP-15
Biodiversity and community structure associated torhizosphere of *Quercussp*. in Sierra de Lobos, Guanajuato. **Juan Gualberto Colli Mull**, Daniela Iturriaga Garcidueñas, Francisco Alejo Iturbide, Victoria Hernández Hernández y Gustavo Alberto de la Riva de la Riva. Instituto Tecnológico Superior de Irapuato

MP-16
Functional characterizationof the heterodimerization domain of GrlR, a LEE-encoded negative regulator of Enteropathogenic *Escherichia coli*. **Emma Aurora Cruz Gómez** y José Luis Puente García. Microbiología Molecular, Instituto de Biotecnología, UNAM

MP-17
Implementing the BioDAgeneas a transformation marker in *Trichoderma atroviride*. **Vincent Anthony Czarnowski Corona**, Fidel Landeros Jaime, José Antonio Cervantes Chávez y Edgardo Ulises Esquivel Naranjo. Universidad Autónoma de Querétaro

MP-18
Dense granule protein 12 (GRA12) is a virulence factor associated to subpelicular cytoskeleton in *Toxoplasma gondii.* **Rubén Díaz-Martín,** Carmen Gómez-de León, Sirenia González-Pozos, David Bzik, Ricardo Mondragón-Flores. Unidad de Microscopía Electrónica (LaNSE), CINVESTAV-IPN

MP-19
Microbial Diversity and Community Structure, by ARDRA Analysis to soils of Natural Protected Areas in Guanajuato Mexico. **Juan Alberto Esquivel Ramírez**, Claudia Isela Gonzáles López, Francisco Alejo Iturbide, Gustavo Alberto de la Riva de la Riva y Juan Gualberto Colli Mull. Instituto Tecnológico Superior de Irapuato

MP-20
Antibacterial activity of extracts and lectins from leaves of Capitaneja (*Verbesinacrocata*). **Bertha Fenton Navarro**, Anahí Luisa Reyes Virrueta, Edgar Vázquez Contreras, Graciela Letechepía Vallejo Sergio Gutiérrez Castellanos. Laboratorio de Glicobiología. Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Universidad Michoacana

MP-21
Binding of extracellular matrix proteins to *Acanthamoeba castellanii* is modulated by surface molecules. **Lérida Liss Flores-Villavicencio,** J Alberto Juárez-Rodríguez, M AngelesSotelo-Olague, Gloria Barbosa-Sabanero, J Jesus Serrano-Luna, Mineko Shibayama-Salas, Patricia Talamas–Rohana, Myrna Sabanero-López. Biology Department, DCNyE Campus Guanajuato

MP-22
Isolation of beer spoiling bacteria *Lactobacillus* and *Pediococcus* from environmental samples. **Jorge Hugo García García**, Luis Damas Buenrostro, Luis Galán Wong and Benito Pereyra Alférez. Instituto de Biotecnología. Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo León

GENETIC REGULATION & EPIGENETIC

GR-23
Down regulation of SERCA3 expression in gastrointestinal cancer cells is modulated by Sp transcription factors. **Lucía Flores-Peredo**, Gabriela Rodríguez, Jonathan González, Ariel Velarde and Ángel Zarain-Herzberg. Departamento de Bioquímica, Facultad de Medicina, UNAM

GR-24
Proximal CAAT box mediates SERCA2 increased transcription by thapsigargin. **Jorge Alberto Fragoso Medina**, Gabriela Rodríguez Rodríguez, Ana Catalina Arreola Tapia and Ángel Zarain Herzberg. Biochemistry. Department, School of Medicine, UNAM

GR-25
Analysis of human eIF3f phosphorylation by CDK1/cyclin B and its role in the control of protein synthesis. **César Adrián Gómez Correa**, Ana Edith Higareda Mendoza and Marco Aurelio Pardo Galván. Facultad de Ciencias Medicas y Biológicas "Dr. Ignacio Chavez"

GR-26
Towards the functional characterization of Brf1, a subunit of transcription factor TFIIIB, in the parasite *Leishmania major*. **Maricarmen Gómez-García**, Luis E. Florencio-Martínez, Rebeca Manning-Cela and Santiago Martínez-Calvillo. Facultad de Estudios Superiores Iztacala, UNAM

GR-27
Expression of MicroRNA-143 In Epithelial Cell Lines. **Alejandro González Torres,** Luis Marat Alvarez Salas. Departamento de Genética y Biología Molecular, CINVESTAV - IPN

GR-28
Analysis of the histone deacetylation on *Ustilago maydis* dimorphism.**Juan Manuel González-Prieto**, Nubia Andrea Villota-Salazar, José Ruiz-Herrera. Biotecnología Vegetal, Centro de Biotecnología Genómica, IPN

GR-29
Transcription Factor Sp1 Regulates T-Type Ca^{2+}ChannelCa$_V$3.1 Gene Expression. **Ricardo González-Ramírez**, Elizabeth Martínez Hernández, Alejandro Sandoval y Ricardo Félix Grijalva. Hospital General Dr. Manuel Gea González

GR-30
The role of the histone demethylase KDM4A in a cancer model**. Lissania Guerra-Calderas**, Ernesto Soto-Reyes, Iliana Alcalá Moreno, Rodrigo González Barrios, Héctor Aquiles Maldonado-Martínez, David Cantú de León, Clementina Castro, Luis A Herrera. Unidad de Investigación Biomédica en Cáncer - Instituto Nacional de Cancerología

GR-31
Interaction of lncRNA transcribed from α satellite repeats and the protein HP1α in a colorectal cancer model. **Nancy Gutiérrez Hernández**, Victor Manuel Del Castillo Falconi, Rodrigo González- Barrios, Ernesto Soto-Reyes, Clementina Castro H., Luis A Herrera. Unidad de Investigación Biomédica en Cáncer - Instituto Nacional de Cancerología

GR-32
Regulation of ABA-INSENSITIVE (ABI) 4 transcription factor in *Arabidopsis thaliana*. **Alma Fabiola Hernández-Bernal**, Josefat Gregorio, Elizabeth Córdoba and Patricia León. Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-33
Quantitative analysis of the telomere position effect on gene expression. **Jhonatan A. Hernández-Valdés**, Alejandro Juárez-Reyes & Alexander de Luna. LANGEBIO – CINVESTAV IPN

GR-34
Analysis of the small RNA RgsA in *Azotobacter vinelandii.* **Jesús Manuel Huerta Campos**, Liliana López Pliego, Daniel Segura González, Ricardo Carreño López, Miguel Castañeda Lucio. CICM-IC. BUAP

GR-35
Effect of resveratrol in the expression of SERCA2b and SERCA3 in breast cancer cell lines. **Eduardo de Jesús Izquierdo Torres**, Gabriela Rodríguez y Ángel Alfonso Zarain Herzberg. Molecular Biology Laboratory, Department of Biochemistry, School of Medicine, UNAM

GR-36
Transcriptional regulation of *ipdC*and*hisC1* genes involved in indole-3-acetic acid production in *Azospirillum brasilense* Sp7 strain. **Saúl Jijón Moreno and** Beatriz E Baca. Centro de Investigaciones en Ciencias Microbiológicas, BUAP

GR-37
Genetic regulation of nitrogen metabolism in *Lachancea kluyveri*. **José Ángel Jiménez Benítez** y Lina Raquel Riego Ruiz. División de Biología Molecular. IPICYT

GR-38
Pseudogenization and reactivation of gene expresión by gain or erosion within promoter regions during the expansion of gene families in plants. **Estela Jiménez Morales**, Víctor Aguilar Hernández, Laura Aguilar Henonin and Plinio Guzmán. CINVESTAV Unidad Irapuato

GR-39
A new lncRNA is regulated by the bidirectional promoter of *CatSper1*. **Salma E Jiménez-Badillo**, Norma A Oviedo de Anda, Javier Hernández Sánchez. Departamento de Genética y Biología Molecular, CINVESTAV, IPN

GR-40
Functional characterization of polyamine oxidase-2 (PAO2) Uorf from *Arabidopsis thaliana.* **Juan Francisco Jiménez-Bremont**, Alicia Becerra Flora and María de la Luz Guerrero-González. División de Biología Molecular. IPICyT

GR-41
Effect of arsenic exposure on DNA methylation in brain áreas.**Verónica Jiménez-Suárez**, Lesly Doniz-Padilla, Roberto González-Amaro, María E Jiménez-Capdeville. Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de San Luis Potosí

GR-42
Analysis of *Macrophomina phaseolina* (Tassi) Goid. pathogenicity genes considering several epigenetic backgrounds. **Nubia Andrea Villota-Salazar**, Sanjuana Hernández-Delgado, Juan Manuel González-Prieto. Biotecnología Vegetal, Centro de Biotecnología Genómica, IPN

GR-43
Role of the RNA binding protein UmRrm75 from *Ustilagomaydis*. **Margarita Juárez-Montiel**, Margarita Rodríguez-Kessler, Alicia Becerra-Flora, Juan Francisco Jiménez-Bremont. Instituto Potosino de Investigación Científica y Tecnológica AC

GR-44
Can miR396 and its target genes regulate leaf development in *Phaseolus vulgaris* L. under drought conditions?. **María Beatriz Pérez-Morales**, Cecilia Contreras-Cubas, Alejandra Covarrubias and José Luis Reyes-Taboada. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-45
Functional study of neutral trehalase "*NTH1*gene" in *Ustilago maydis*. **Alonso López Cabrera**, Laura Valdés Santiago, Claudia Geraldine León Ramírez, Edgardo Ulises Esquivel Naranjo, Fidel Landeros Jaime, José Ruiz-Herrera and José Antonio Cervantes Chávez. Facultad de Química-UAQ

SIGNAL TRANSDUCTION

ST-33
In vitro and in vivo endothelial activation mediated by tumor soluble factors secreted by different breast cancer cell lines. **César Alejandro Guzmán Pérez**, Alberto Jose Cabrera Quintero, Alfredo Ibarra Sánchez, José Luis Ventura Gallegos, Claudia González Espinosa, Alejandro Zentella Dehesa. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

ST-34
Scaffold activity of the MAPKK Pbs2p during the endoplasmic reticulum stress response in *Saccharomyces cerevisiae*. **Mariana Hernández Elvira**, Laura Kawasaki Watanabe, Roberto Coria Ortega. Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

ST-35
Physical interaction between the GTPases Gpn1 and Gpn3 takes place in the cytoplasm**. Gema Rosa Cristóbal Mondragón**, Victor De la Rosa Jiménez, Gisela E. Rangel Yescas, Ana Estela Pérez Mejía, León Islas, Roberto Sánchez Olea, and Mónica R Calera. Instituto de Física, UASLP

ST-36
FGF-2 induces epithelial to mesenchymal transition in MDA-MB-231 cell line. **José Antonio Hernández Ortiz**, Eduardo Monjaraz-Guzmán. Instituto de Fisiología. BUAP

ST-37
YMCA: Yeast MultiColor Assay. **Cynthia Hernández-Garduño,** Francisco Manuel Amezola-Chávez, Beatriz Cruz-Castañeda, Gerardo Cruz-Castañeda, Minerva Estefanía Núñez-Manjarrez, Fernanda Zamora-Cortina, Francisco Torres-Quiroz. Instituto de Fisiología Celular, UNAM

ST-38
TEM5/GPR124, a tumor endothelial marker orphan GPCR, modulates the signaling effects of cancer cell-secreted factors. **Magda Nohemí Hernández-Vásquez**, Guadalupe Reyes-Cruz and José Vázquez-Prado. Department of Pharmacology. CINVESTAV-IPN

ST-39
Hyperactivation rescue in spermatozoa from potassium channel *Slo3*$^{(-/-)}$ knockout mice. **Francisco Fabio Herrera Rodríguez**, Celia Santi and Claudia L. Treviño Santa Cruz. Instituto de Biotecnología, UNAM

ST-40
Role of protein kinase Fyn on the regulation of TNF secretion stimulated by LPS in mast cells. **Alfredo Ibarra-Sánchez**, Martín Avila, Ulrich Blank, Marina Macías-Silva and Claudia González-Espinosa. CINVESTAV-IPN

ST-41
"G-protein receptor magnitude of signaling depends on the glycocalyx and flow". **Azucena E. Jiménez-Corona** & Rafael Rubio García. Facultad de Medicina, UASLP

ST-42
Novel estrogen signaling pathway in breast cancer cells migration. **Javier Esteban Jiménez-Salazar**, Mina KönigsbeGR-Fainstein, Luis Enrique Gómez-Quiroz & Pablo Damián-Matsumura. Biology of Reproduction and Health Sciences. UAMI

ST-43
Leptin induces FAK activation and cell migration in a Src-dependent pathway in breast cancer cells. **Juan C Juárez Cruz**, Bernabé Visoso Torres, Eduardo Castañeda Saucedo, Mercedes Calixto Gálvez, Miguel A

Mendoza Catalán, Napoleón Navarro Tito. Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

ST-44

High doses of IL-2 inhibit cell proliferation by inducing an arrest in the G1 phase of the cell cycle in cervical cancer cell lines CALO and INBL. **María del Carmen Lagunas Cruz**, Arturo Valle Mendiola, Benny Weiss-Steider, Isabel Soto Cruz. Facultad de Estudios Superiores Zaragoza, UNAM

ST-45

Innate and adaptive IL-9 orchestratetype 2 immunity. **Paula Licona-Limón**, Jorge Henao-Mejía, Angela U. Temann, Nicola Gagliani, Ileana Licona-Limón, Harumichi Ishigame, Liming Hao, De' Broski R Herbert, Richard A Flavell. School of Medicine. Yale University

ST-46

Tissue inhibitor of metalloproteinase-4 regulates stemness and apoptosis in cervical cancer cells. **Floria Lizárraga**, Magali Espinosa, Gisela Ceballos, Iván Bahena, Angela Shwarz, Montserrat Vega, Vilma Maldonado and Jorge Meléndez. National Institute of Genomic Medicine

ST-47

Electrophysiological and pharmacological characterization of *Slo3* channels in mouse spermatogenic cells. **Verónica Loyo Celis**, Alberto Darszon and Gerardo José Orta Salazar. Instituto de Biotecnología, UNAM

ST-48

The function of the Syk and STAT3 in the regulation of Interleukin 10 expression in human macrophages infected with *Mycobacterium bovis*. **Edgardo Madrid-Paulino**, Tomás Villaseñor-Toledo, Leonor Pérez-Martínez and Gustavo Pedraza-Alva. Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

ST-49

The identification of kinases, cyclases and phosphatases in mitochondria from the human placenta. **Federico Martínez Montes**, Sofia Olvera Sanchez, Oscar Flores Herrera, Mercedes Esparza Perusquia, Erika Gomez Chang. Bioquímica, Facultad de Medicina, UNAM

ST-50

Importance of the nucleocytoplasmic transport cycle of the GTPase Gpn1 on the subcellular localization of RNAPII and RNAPII. **Mayra Martínez Sánchez**, Esmeralda Nolasea Cruz, Mónica Calera y Roberto Sánchez Olea. Instituto de Física, Universidad Autónoma de San Luis Potosí

ST-51

Injury signal molecules activate MAPK pathways to trigger asexual reproduction in *Trichoderma atroviride*. **Elizabeth Medina-Castellanos**, Edgardo Ulises Esquivel-Naranjo, Martin Heil, Alfredo Herrera-Estrella. LANGEBIO. CINVESTAV- IPN Irapuato

ST-52

Disection of the protein-protein interaction between Sdo1 and Efl1 using yeast two-hybrid. **Alfonso Méndez Godoy**, Nuria Sánchez Puig. Instituto de Química, UNAM

ST-53

Gpn1 and Gpn3, GTPases involved in nuclear import of RNA polymerase II, mutually determine their subcellular distribution and protein levels. **Lucía E Méndez Hernández**, Ana E Pérez Mejía, Bárbara Lara Chacón, Ángel A Barbosa Camacho, Sonia G Peña Gómez, Mayra Martínez Sánchez, Angélica Y Robledo Rivera, Roberto Sánchez Olea, and Mónica R Calera. Instituto de Física, UASLP

ST-54

Transcriptional profiling of pirfenidone reveals immunomodulatory pathways associated to Nrf2 activation in primary culture of human Hepatic Stellate Cells. **Margarita Montoya-Buelna**, José Macías-Barragán, Alessandra Caligiuri, Erica Novo, Maurizio Parola, Jesús García-Bañuelos, Massimo Pinzani, Juan Armendáriz-Borunda. CUCS, Universidad de Guadalajara

ST-55

Analysis of the effect of G. duodenalis trophozoites treated with TPCK on epithelial homeostasis and on signaling pathways using a model of duodenal loop in gerbils (*Meriones unguiculatus*). **Mora-Hernández**

Yaremit, Fonseca-Liñán Rocío, Galindo-Gómez Silvia, Quezada-Lázaro Rodrigo, Silva-Olivares Angélica, Nava-Domínguez Porfirio, Shibayama-Salas Mineko, Ortega-Pierres Guadalupe. CINVESTAV – IPN Zacatenco

ST-56

Expression profile of Hsp90α and Hsp90β, and their involvement in signalling mediated by β-catenin on cervical cancer cell lines. **Silvia Gabriela Morales-Guadarrama**, José de la Luz Díaz-Chávez, Carlos Pérez-Plasencia, Luis Alonso Herrera-Montalvo, Carlo César Cortés-González. Instituto Nacional de Cancerología, Unidad de Investigación Biomédica en Cáncer, IIB-UNAM

ST-57

The AMP-activated protein kinase (AMPK) is involved in the regulation of lipid metabolism in *Caenorhabditis elegans.* **Elizabeth Moreno Arriola**, Daniel Ortega Cuellar, Mohammed El Hafidi Bentlakder, Karla Carvajal Aguilera. Instituto Nacional de Pediatría

ST-58

Phospholipases C and D modulate vanillin production elicited by salicylic acid in *Capsicum chinense* Jacq. **Armando Muñoz-Sánchez**, Beatriz Adriana Rodas-Junco, Felipe Vázquez-Flota and SM Teresa Hernández-Sotomayor. Centro de Investigación Científica de Yucatán

ST-59

The mating heterotrimeric G protein participates in the yeast endoplasmic reticulum stress response. **Rocío Navarro-Olmos**, Citlalli Rodríguez-Cervantes, Laura Kawasaki-Watanabe, Laura Ongay-Larios and Roberto Coria. Genética Molecular, Instituto de Fisiología Celular, UNAM

ST-60

The role of protein E6 and its small isoform E6*I of HPV-18 in the Wnt signaling pathway. **Leslie Olmedo Nieva**, Jesús Omar Muñoz Bello, Alma Mariana Fuentes González, Adriana Contreras Paredes and Marcela Lizano Soberón. Unid. Investigación Biomédica Cáncer, INCan

ST-61

Biotin deficiency induces a decrease in mitochondrial mass mediated by the activation of Akt in liver. **Estefania Ochoa-Ruiz**, José Corella-Vázquez, Lucy Camberos-Luna, Luis Alberto Luévano-Martínez, Salvador Uribe-Carvajal, Rodrigo Díaz-Ruiz, Antonio Velázquez-Arellano. Instituto de Investigaciones Biomédicas. Torre de Investigación INP

ST-62

The yeast two-component response regulator Skn7 participates in the response to tunicamycin induced stress. **Abiram Eliab Olivares-Reséndiz**, Eunice Alejandra Domínguez-Martín, Laura Kawasaki-Watanabe, and Roberto Coria. Genética Molecular, Instituto de Fisiología Celular, UNAM

ST-63

Interaction of Gonadotropin-releasing hormone receptor with protein calnexin depends of the species. **Alejandro Cabrera-Wrooman**, Jo Ann Janovick, P Michael Conn. Oregon National Primate Research Center, Oregon Health and Science University

BASIC BIOCHEMISTRY

B-76
Influence of MIF absence on hepatic mitochondrial function using a MURINE model of diabetes mellitus Type two. **Alejandra Lira-León**, Cabellos-Avelar Tecilli, Gutiérrez-Cirlos Madrid Emma Berta, Zamora Sánchez Yuriko, Juárez Avelar Imelda, Rodríguez-Sosa Miriam. FES Iztacala UBIMED, UNAM

B-77
Polyphosphate increases cadmium resistance in Methanosarcina acetivorans. **Elizabeth Lira-Silva**, Ricardo Jasso-Chávez, Biochemistry, INC "Ignacio Chávez"

B-78
Expression, purification and characterization of CGI58/ABHD5 enzyme. **Miriam Llamas García**, Gabriela Montero Morán, Samuel Lara González. Laboratorio de Biología Estructural, IPICYT

B-79
Characterization of cyclin proteins implied in cell cycle regulation of *Trichomonas vaginalis*. **Karla Concepción López Pacheco**, Erick Amador Gaytán y María Imelda López Villaseñor. Instituto de Investigaciones Biomédicas, UNAM

B-80
Role of S-adenosylmethionine and Methionine Adenosyl transferases in a sequential model of cirrhosis-hepatocellular cancer induced by DEN treatment. **María Guadalupe Lozano-Rosas**, Nora Gabriela Velasco-Loyden, Mariana Domínguez López and Victoria Chagoya de Sánchez. Instituto de Fisiología Celular, UNAM

B-81
Triosephosphate isomerases of *Arabidopsis thaliana*. **Elvy Daniela Lucio Hernandez**, Renata Elizarraraz Morrill, Margarita Lopez, Pedro Jimenez, Karla Rojas, Alfredo Torres Larios, Samuel Lara, Luis G Brieba. LANGEBIO, CINVESTAV - IPN Irapuato

B-82
Membrane supercomplexes and complexes of oxygenic photosynthesis in the marine cyanobacterium *Prochlorococcus* MIT 9313. **Jesús Barrera Rojas**, Luis E González de la Vara, Lourdes E Leyva Castillo, Jorge Zarco Mendoza and Carlos Gómez Lojero. Bioquímica. CINVESTAV IPN. Unidad Zacatenco

B-83
Energetic basis of molecular recognition of guanine nucleotides by the ribosomal GTPase Efl1. **Axel Luviano**, Nuria Sánchez Puig, Enrique García Hernández. Instituto de Química. UNAM

B-84
Aminopeptidases as potential markers of celular aging in *Schizosaccharomyces pombe*. **Mario Maciel Rodríguez**, Laura Morales Lara, Sandra Raquel Reyes Carmona, Lourdes Millán Pérez Peña, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. CQ-IC. BUAP

B-85
Preliminary localization by confocal microscopy of a protein DING in seeds of habanero chili (*Capsicum chinense* Jacq.). **Dianeli de Jesús Madera Piña**, Ligia Brito Argáez, Rita Ramona Uc-Ku, Angela Kú González, Marco Antonio Villanueva Méndez, Ignacio Islas-Flores. Unidad de Bioquímica y Biología Molecular de Plantas, CICY

B-86
The structure, stability and fibrillogenesis of a model amyloidogenic protein as a function of pH. **Ma. Isabel Velázquez López**, Sergio Romero Romero, Miguel Costas Basín, Yazmín Frías Lucio, Héctor Ceceña Álvarez, Roberto Maya Martínez, Carlos Amero, Nina Pastor, and D Alejandro Fernández Velasco. Facultad de Medicina, UNAM

B-87
Structural and functional characterization of two chitinase-like proteins from Hevea brasiliensis belonging to the glycosil-hydrolase family (GH19). **Carol Siseth Martínez-Caballero**, Patricia Cano-Sánchez, Israel Mares-Mejía, Martha L Macías-Ruvalcaba, Juan A Hermoso and Adela Rodríguez-Romero. Instituto de Química, UNAM

B-88
The glucose transporter 1 -Glut1- plays an important role in the white shrimp *Litopenaeus vannamei* in the response to hypoxia. **José A Martínez-Quintana**, Alma B Peregrino-Uriarte, L Adrián Gámez-Alejo, Silvia Gómez-Jiménez, Gloria Yepiz-Plascencia. Centro de Investigación en Alimentación y Desarrollo, A.C.

B-89
Posttranslational Regulation of maize Spermine Synthase 1: A Key Enzyme In Polyamine Metabolism. **Maruri-López Israel**, Rodríguez-Kessler Margarita, and Jiménez-Bremont Juan Francisco. División de Biología Molecular, IPICyT

B-90
Identification of beta-glucosidase isoenzymes (*TdGlu1 y TdGlu2*) from teosinte *Zea diploperennis*. **Carlos Alberto Matias Cervantes**, Sergio Espíndola Mateos, Liliana Hernández García. Eduardo Pérez Campos, Manuel Sánchez Rubio, Nora Hilda Rosas Murrieta and Margarito Martínez Cruz. Instituto Tecnológico de Oaxaca

B-91
Identification of an allosteric GlcNAc6P-binding site with a novel structure. **Andrea Celeste Medina García,** Jorge Marcos Víquez, Mario L. Calcagno. Facultad de Medicina, UNAM

B-92
MDM2 E3 ubiquitin ligase activity is regulated by inter and intra molecular interactions. **Ixaura Medina-Medina**, Angel Torres-Rosales, Yolanda Rebolloso-Gómez, Gregorio Alvarez-Fuentes and Vanesa Olivares-Illana. Instituto de Física, Universidad Autónoma de San Luis Potosí

B-93
Mmp8-Mmp13 double deficient mouse is more sensitive to experimental pulmonary fibrosis. **Fabián Vergara Ovalle**, Sandra Cabrera Benítez, Mariana Maciel Herrerías, Miguel Gaxiola Gaxiola, Moisés Selman Lama, Annie Pardo Cemo. Facultad de Ciencias, UNAM

B-94
A histological and immunohistochemical study of beta cells in streptozotocin diabetic rats treated with *Carica papaya* leaf extract chloroform. Isela Esther Juárez Rojo **Rodrigo Miranda**, Pedro Hilario Miranda Osorio, Carlos A Tovilla Zarate, Teresa Ramón Frias, Andrés E Castell-Rodríguez, Juan C Díaz-Zagoya. DACS, Universidad Juárez Autónoma de Tabasco

B-95
A new experimental model tostudy primary and secondary metabolism in *Capsicum chinense* Jacq.: cell suspension from placentas. **María de Lourdes Miranda-Ham,** Jemina Tuyub-Che, Fray M Baas-Espinola and Lizbeth A Castro-Concha. Unidad de Bioquímica y Biología Molecular de Plantas, CICY

B-96
Antiproliferative activity of *Tournefortia mutabilis*VENT. in cell line MCF-7 of breast cancer. **Zoila Mora-Guzmán,** Ma. Del Socorro Pina-Canseco, Ruth Martínez-Cruz, Rebeca López-Marure, Eduardo Pérez-Campos, Luis Bernardo Flores-Cotera, Ma. Teresa Hernandez-Huerta, Laura Juárez-Chávez. Facultad de Medicina y Cirugía. UABJO

B-97
Antibacterial activity of *Lophocereusschottiii* (Muso) and *Pachycereuspecten- aboriginum* (Etcho) against *Escherichia coli* (ATCC 23922**). Edgar F Moran-Palacio**, Luis Alberto Zamora-Alvarez, Adolfo Virgen-Ortiz, and Norma Patricia Adan-Bante. Ciencias Químico Biológicas y Agropecuarias. Universidad de Sonora

B-98
Antifungal activity of *Zizyphus obtusifolia* (Jutuki) against *Aspergillus niger* (NRRL3). **Edgar F. Moran-Palacio**, Paulina Sayas-Galindo, Luis Alberto Zamora-Alvarez, Ema Carina Rosas-Burgos, Adolfo Virgen-Ortiz and José Guadalupe Soñanez-Organis. Ciencias Químico Biológicas y Agropecuarias. Universidad de Sonora

B-99
Direct analysis of biomolecules from microorganisms by Low-Temperature Plasma Ionization coupled to Mass Spectrometry Imaging (LTP-MSI). **Abigail Moreno Pedraza** & Robert Winkler. CINVESTAV .Irapuato

B-100
Energy disturbances in metabolic syndrome are related to development of neurodegenerative diseases. **Joceline Aidee Baires López**, Bryan Phillips, Karla Carvajal. Instituto Nacional de Pediatría

B-101
Mass Spectrometry Characterization of Trypanothione and Novel Peptides of Medical Importance Isolated from *Acanthamoeba polyphaga***. Raúl N Ondarza-Vidaurreta,** Eva Hernández-Márquez, Gerardo Hurtado Sil, Mathew Woolery and Francisco Hernandez-Sandoval. UNAM–INSP–University of California-CIBNOR

B-102
Characterization of the forms of U / DAPC complex in vascular smooth muscle cells of human. **Ariadna Jazmín Ortega Lozano**, Jessica Márquez Aniceto, Mario Edgar Tena Sanabria, Nayeli Suarez Reyes, Juan Fernando Sanmiguel Minauro. UIM. Genética humana, Hospital de Pediatría, CMN S XXI IMSS

B-103
Evaluation of the possible herbicide activity of an alkaloid isolated of *Ricinus communis* L. **Valeria Ortega Mendoza**, Karina Zarco Tovar, Beatriz King Díaz, María Isabel Aguilar Laurents, Blas Lotina Hennsen. Bioquímica, Facultad de Química. UNAM

B-104
Effect of protein phosphatases secreted by promastigotes and amastigotes of *Leishmania mexicana* in human macrophages. **Dalia Margarita Ortiz-Lozano**, Alma Reyna Escalona-Montaño, Arturo Wilkins-Rodríguez, Araceli Rojas-Bernabé, Laila Gutiérrez-Kobeh and M Magdalena Aguirre García. Facultad de Medicina, UNAM

B-105
Putative Transcription Factors In *Trichomonas vaginalis*. **Olivia Parra Marín,** Lluvia Rosas Hernández, Imelda López Villaseñor. Instituto de Investigaciones Biomédicas, UNAM

B-106
Inhibition of amyloid fiber formation for the protein 6aR24G, associated with light chain amyloidosis disease. **Ángel Enrique Peláez Aguilar,** Roberto Carlos Maya Martínez, Lina Andrea Rivillas Acevedo, Gilberto Valdés García, Nina Pastor Colón, Carlos Amero Tello. Centro de Investigaciones Químicas, UAEM

B-107
Digestive physiology and characterization of the enzymatic activity during larval development of spotted rose snapper *Lutjanusguttatus*. **Renato Peña,** Ivette Moguel-Hernández, Héctor Nolasco-Soria, Silvie Dumas, Mauricio Contreras-Olguín, Laura Flores-Montijo y Margarita Rangel-Durán. Centro Interdisciplinario de Ciencias Marinas-IPN

B-108
Bio-guided isolation of antibacterial compounds from *Rhizophora mangle* L. (mangle rojo) against *Staphylococcus aureus* (ATCC 6538P). **Luis Alberto Zamora-Alvarez**, Grelda Acela Yañez-Farías, Eduardo Ruíz-Bustos, Ema Carina Rosas-Burgos, Jesús A. Rosas-Rodríguez and Edgar F. Moran-Palacio. Departamento de Ciencias Químico Biológicas y Agropecuarias. Universidad de Sonora

B-109
New functions for known proteins involved in mitochondrial protein import. **José Carlos Ponce Rojas**, Roberto Yañez, Fabiola Jaimes Miranda, Alexander de Luna y Soledad Funes. Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

B-110
Protein phosphatase PP2C in *Leishmania mexicana* parasites. **Mariana Zuñiga-Fabian**, Alma Escalona-Montaño, Laila Gutiérrez-Kobeh, Ruy Pérez-Monfort, Nallely Cabrera-González, Ingeborg Becker, Ricardo Mondragon-Flores and M. Magdalena Aguirre-García. Departamento de Medicina Experimental, Facultad de Medicina, UNAM

B-111
Binding thermodynamics in periplasmic binding proteins: A comparison between HisJ and LAOBP. **Nancy O Pulido Mayoral**, F Aarón Cruz Navarrete, Haven A López Sánchez, Alejandro Sosa Peinado. Enrique García Hernández and D Alejandro Fernández Velasco. Facultad de Medicina, UNAM

B-112
Effect of β3-adrenergic agonist (carazolol) on calcium regulation of Sarcoplasmic Reticulum from rat slow skeletal muscle. **Ibrahim Arley Ramirez-Soto**, Eduardo Rodríguez Correa, Aura Jiménez Gaduño, Rocío Álvarez y Alicia Ortega. Facultad de Medicina, UNAM

B-113
Kinetic mechanism of thioredoxin-glutathione reductase from *Taenia crassiceps*. **Juan L Rendón**, Alberto Guevara-Flores, Oscar Flores-Herrera y Juan P Pardo. Facultad de Medicina, UNAM

SYSTEMS BIOLOGY & BIOINFORMATICS

SB-17
Characterization of pathogenic organisms using next generation sequencing and bioinformatic. **Edgar Omar Fragoso García**, Alejandra García Molina y Abraham Itzcoatl Acatzi Silva. Subdirección de Secuenciación y Bioinformática. SENASICA

SB-18
Study of reductive genomic evolution in prokaryotes. **David Martínez Cano**, Luis Delaye. Department of Genetic Engineering, CINVESTAV-IPN Irapuato

SB-19
Genome assembly of *Burkholderia* sp. and analysis of the functional annotation of genes involved in the degradation of organophosphorus compounds. **Fernando Martínez-Ocampo**, Elida C Popoca-Ursino, Luis F. Lozano-Aguirre, Janette Onofre-Lemus, Ma. Laura Ortiz-Hernández, Armando Hernández-Mendoza, Edgar Dantán-González. Centro de Investigación en Biotecnología, UAEM

SB-20
Metagenomic analysis of root-associated bacteria in bean plants. **Francisco Medina-Paz**, Cei Abreu-Goodger and Martin Heil. CINVESTAV-IPN Irapuato

SB-21
A dynamical model of the regulatory network that controls terminal differentiation of B lymphocytes. **Akram Sharim Méndez Rangel**, Luis Antonio Mendoza Sierra, Instituto de Investigaciones Biomédicas, UNAM

SB-22
The Regulatory Network of Lymphopoiesis in Mammals. **Luis Mendoza**. IIB, UNAM

SB-23
Meta-analysis of DNA microarrays for generate a progression model of melanoma. **Daniel Ortega-Bernal**, Elena Aréchaga-Ocampo, Claudia Haydée González de la Rosa and Claudia Rangel-Escareño. Departamento de Ciencias Naturales, UAM, Unidad Cuajimalpa

SB-24
Molecular dynamics simulation of EhCFIm25 protein and analysis of its ability to BIND RNA. **Juan David Ospina-Villa,** Jorge Luis Rosas-Trigueros, Absalom Zamorano-Carrillo, César López-Camarillo, Laurence Marchat Marchau. Escuela Nacional de Medicina y Homeopatía, IPN

SB-25
Proteomic analysis of Entamoeba histolytica in vivo assembled pre-mRNA splicing complexes. **Jesús Valdés**, Tomoyoshi Nozaki, Emi Sato, Yoko Chiba, Kumiko Nakada-Tsukui, Nicolás Villegas-Sepúlveda, Robert Winkler, Elisa Azuara-Liceaga, MaríaSaraí Mendoza-Figueroa, Natsuki Watanabe, Herbert J. Santos, Yumiko Saito-Nakano, José Manuel Galindo-Rosales. CINVESTAV – IPN Zacatenco

SB-26
The role of molecular hinges in protein stability and folding. **Jaqueline Padilla Zúñiga**, UAMI

SB-27
Structural modelling of the N463D, R275G mutations in FKRP. **Tobias Portillo-Bobadilla**, Carmen Navarro Fernández-Balbuena, Rosa Elena Escobar-Cedillo, Luz Berenice López-Hernández, Alexandra

Berenice Luna-Angulo, Francisco Javier Estrada-Mena, Guillermina Ávila-Ramírez, Elisa Martínez-Coria, Benjamín Gómez-Díaz. UNAM. Instituto Nacional de Rehabilitación

SB-28

Effect of pH on the Affinity of Chymopapain to Chicken Cystatin.An enzyme-inhibitorassociation modulated by electrostatics. **Francisco Reyes Espinosa**, Rafael A. Zubillaga, Alfonso Arroyo Reyna, Iris N. Serratos and Ponciano García Gutiérrez. Departamento de Química. UAMI

SB-29

Transcriptional regulation of coding and non-coding RNAs by the Cyclin Dependent Kinase 8 (CDK8) module of Arabidopsis Mediator. **Joel Rodríguez-Medina**, Daniel Lepe-Soltero, Claudia Silva-Ortega, Cei Abreu-Goodger and Stewart Gillmor. LANGEBIO – CINVESTAV – Irapuato

SB-30

Phylogenetic study of sperm-specific Na+/H+ exchanger. **Francisco Romero Corpus**, Takuya Nishigaki Shimizu. Departamento de Genética del Desarrollo y Fisiología Molecular. IBT, UNAM

SB-31

GMOseq: A Next Generation System for GMO characterization. **Salvador Ángel Romero Martínez**, ZhailaIsaura Santana Hernández, Claudia Castillo Mercado y Abraham Itzcoatl Acatzi Silva. Subdirección de Secuenciación y Bioinformática. SENASICA

SB-32

Phylogenetic and biogeographic inference from six cpDNA loci reveals Neotropical origin of grammitid ferns. **Viacheslav Shalisko**, José Antonio Vázquez-García, Miguel Angel Muñiz-Castro, Cinthia Karina Pérez-Espejo, Alma Rosa Villalobos-Arámbula. Instituto de Botánica, Departamento de Botánica y Zoología, CUCBA, Universidad de Guadalajara

SB-33

Systematic identification of signal integration by Protein Kinase A. **Francisco Torres-Quiroz**, Marie Filteau, Guillaume Diss, Alexandre K Dubé, Isabelle Gagnon-Arsenault, Andrea Schraffl, Andrée-Ève Chrétien, Anne-Lise Steunou, Ugo Dionne, Jacques Coté, Nicolas Bisson, Eduard Stefan, Christian R Landry. Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

PHARMACOLOGY

PH-1

Sexual hormones and their influence over mitochondria: estrogens. **Raúl Fabián Alvarez Pimentel**, Alfredo Cabrera, Salvador Uribe, Nadia Rivero-Segura, Marco Cerbón, Juan C Torres-Narvaez, Natalia Pavón. Departamento de Farmacología, Instituto Nacional de Cardiología

PH-2

Isoindoline-1,3-dione derivatives analogous to dopamine as potential agonist toD_2 receptor. **Erik Andrade Jorge**, José Raúl Bahena Herrera, Jesús García Gamez, Marvin A Soriano Ursúa and José G Trujillo Ferrara. Departamento de Bioquímica, Escuela Superior de Medicina, IPN

PH-3

Aliskiren modifies (Pro)renin receptor associated with canonical Wnt/β-catenin signaling expression on heart and kidney in 5/6 nephrectomy-induced hypertension in rats. **Massiel Alfonsina Ávila Ramírez**, Liliana Anguiano Robledo, Pedro López Sánchez. Laboratorio de Farmacología Molecular, ESM-IPN

PH-4

Evaluation and synthesis of a series of 2-substituted isoindolines with probably antineoplastic activity and their action with histone deacetylase 8. **José Raúl Bahena Herrera**, Jesús García Gamez, Erik Andrade Jorge, Yudibeth Sixto López, José G Trujillo Ferrara. Escuela Superior de Medicina, IPN

PH-5

Synthesis and characterization of lipophilic bismuth dimercaptopropanol nanoparticles and their effects on oral microorganisms growth and biofilm formation. **Claudio Cabral Romero**, Appala Raju Badireddy,

Shankararaman Chellam, Rene Hernández Delgadillo, Valentin Zaragoza Magaña y Gustavo Israel Martínez González. Facultad de Odontología, UANL

PH-6

Influence of sex hormones on mitochondrial function in male rat heart. **Montserrat Gpe Cárdenas Regules**, Alfredo Cabrera, Salvador Uribe, Nadia Rivero-Segura, Marco Cerbón, Juan C Torres- Narvaez, Natalia Pavón. Farmacología, Instituto Nacional de Cardiología

PH-7

Purification and evaluation of the effect of *scammonin I* y *tyrianthin C* isolated from the *Ipomoea tyrianthina* root on gabaergic transmission system. **José Manuel Castro García**, Lucero Valladares Cisneros, Ismael León Rivera, María Del Carmen Gutiérrez Villafuerte. Centro de Investigación en Biotecnología, UAEM

PH-8

The CB1-receptoragonist PhAR-DBH-Me inhibits the proliferation of SKOV-3, MCF-7, A549 and HepG2 tumor cell lines. **Roberto Cruz-Castañeda**, Vladimir Santiago-Ortega, Iván Monsalvo, Mario Trejo-Pérez, Ignacio Regla, Isabel Soto-Cruz, Arturo Valle-Mendiola, Axel Luviano. Oncología Molecular, UIDCC, UMIEZ, FES Zaragoza, UNAM

PH-9

Design and synthesis of a novel anti-tripanosome drug and description of the binding pocket on TcTIM of fluorine containing benzothiazole. **Roberto I Cuevas-Hernández,** Sarai Martínez-Cerón, Mónica G Arellano-Mendoza, José Correa-Basurto and José G Trujillo-Ferrara. Modelado Molecular y Bioinformática y Laboratorio de Bioquímica, Escuela Superior de Medicina, IPN

PH-10

Caspase 3 activation through 7-Hydroxicoumarin in lung adenocarcinoma A-549 cells performing in vivo microinjection. **Azucena Díaz-Morales,** Maribel Soto-Núñez, Patricia Cuautle Rodríguez, Juan Molina-Guarneros. Pharmacology. Department. Faculty of Medicine, UNAM

PH-11

On the mechanism of action of the relaxing effect of the 5,4´-Dihidroxi-6,7,8,3´-Tetrametoxi-Flavone, Flavone A, on vascular smooth muscle of the rat. **Ricardo Espinosa-Tanguma**, Martha Patricia Navarro-Huerta, J Roberto Valle-Aguilera, María Dolores Brito-Orta, Aldo Azmar Rodríguez-Menchaca, Ivan Arael Aréchiga-Figueroa, Paola Algara-Suárez and Marco Martín González-Chávez. Fisiología y Biofísica, Facultad de Medicina. UASLP

PH-12

Vasorelaxant effect from chloroform extract of *Justicia spicigera*. **Edgar R. Esquivel-Gutiérrez,** Salvador Manzo-Avalos, Rafael Salgado-Garciglia, Maximiliano Ibarra-Barajas, Mónica Clemente-Guerrero, Rocío Montoya-Pérez, Alfredo Saavedra-Molina. IIQB. Universidad Michoacana

PH-13

Natural neuroprotective compounds enhance autophagy to increase cells survival in Parkinson's disease model. **Yoandy Ferrer Marcelo**, René Hernández Vargas, Alejandro Olea Martínez, Angel Carvajal Oliveros, Enrique Reynaud Garza. Instituto de Biotecnología, UNAM

PH-14

New isoindoline-1,3dione as possible selective ligands for $ß_2$AR (*in silico*study). **Jesús García Gamez**, José Raúl Bahena Herrera, Erik Andrade Jorge, Marvin A Soriano Ursúa and José G Trujillo Ferrara. Departamento de Bioquímica, Escuela Superior de Medicina, IPN

PH-15

CaMKII-dependent mitocondrial calcium uniporter phosphorylation and its potential role in ventricular arrthymias. **Jesus R Garza López** and Gerardo de J Garcia Rivas. Instituto de Cardiología y Medicina Vascular, Escuela de Medicina, Tecnológico de Monterrey

PH-16

Synergistic effect of α-mangostin on the cytotoxicity of cisplatin in a cell line of cervical cancer. **Raquel González-Macías,** Patricia Gracía-López, Jazmin Pérez-Rojas. Facultad Química, UNAM

PH-17
Antimicrobial activity of the venom of scorpion fish *Scorpaena mystes (Scorpaenidae)*. **Cintia Gutiérrez Villegas**, Georgina Ivette López Cortés, María de Lourdes Delgado Aceves, Cristian Moises Galvan Villa, and Alfonso Enrique Islas Rodríguez. Department of Ecology, University of Guadalajara

PH-18
Rational design of boroarylamines as agonists of beta 3 adrenergic receptor. **Christian F Hernández-Martínez**, Marvin A Soriano-Ursúa, José G Trujillo-Ferrara. Escuela Superior de Medicina, IPN

PH-19
Estradiol enhances gold nanoparticles incorporation in mcf-7 breast cancer cells by modifying membrane roughness. **Carlos Lara-Cruz**, Javier Esteban Jiménez Salazar, Nikola Batina, Eva Ramón-Gallegos, Pablo Damián-Matsumura. Biology of Reproduction and Nanotechnology and Molecular Engineering Laboratory, Metropolitan Autonomous University

PH-20
Evaluation of acute and sub-acute oral toxicity of *Callistemon citrinus* extract in male Wistar rats. **Alejandro López Mejia**, Patricia Rios-Chavez, Daniel Godínez Hernández, Blanca Nateras Marin. Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo

PH-21
Role of Rho and Rac proteins in renal vascular reactivity to angiotensin II in hypertension. **Andrea Martínez Cruz**, Alfredo Saavedra Molina, Maximiliano Ibarra Barajas y Salvador Manzo Avalos. Instituto de Investigaciones Químico Biológicas, UMSNH

PH-22
Ambroxol hydrohcloride antimicrobial and antibiofilm activity and the study of its mechanism of action. **Juan José Martínez Sanmiguel**, Juan Francisco Contreras Cordero, Rene Hernández Delgadillo, Valentin Zaragoza Magaña, Claudio Cabral Romero. Facultad de Odontología, UANL

PH-23
Pharmaceutical Thesaurus: unity, structure and the relationship of biomedical terminology. **Vanessa Sánchez Delgadillo**, Eniak Hernández Alarcón, Daniel Ramírez Martínez, Diana Ramírez Álvarez, LaylaMichán Aguirre. Biología Comparada, Facultad de Ciencias, UNAM

PH-24
Antiproliferative and apoptotic effects of Bcr-Abl second generation inhibitors in SKOV-3 and MCF-7 tumor cell lines. **Vladimir Santiago-Ortega**, Roberto Cruz-Castañeda, Mario Trejo-Pérez, Enrique García-Hernández, Isabel Soto-Cruz, Axel Luviano, Arturo Valle-Mediola. Laboratorio de Oncología Molecular, UIDCC, UMIEZ, Facultad de Estudios Superiores Zaragoza, UNAM

IMMUNOLOGY

I-17
Analysis of gene expression and activity of MMP9 in human corio-amniotic membranes. **Edna Elisa García Vences**, Noemi Meraz Cruz, María del Rocio Maldonado Guzmán, Yunuén Pruneda Padilla, Carmen Chancola-Sotelo, Felipe Vadillo Ortega. Facultad de Medicina, UNAM. INMEGEN

I-18
Inhibitory effect of Galectin-1 over IL-6 expression in LPS-treated decidual cells by reducing IκBζ. Fernando Gómez-Chávez, Violeta Castro-Leyva, Aurora Espejel-Núñez, Guadalupe Estrada-Gutierrez, Juan Carlos Cancino-Díaz, Mario Eugenio Cancino-Díaz, **Sandra Rodríguez-Martínez**. Department of Immunology, Escuela Nacional de Ciencias Biológicas, IPN

I-19
Role of the α7 nicotinic receptor on the LPS-induced TNF production in bone marrow-derived mast cells. **Fabiola Guzmán Mejía**, Carolina López Rubalcava and Claudia González Espinosa. Farmacobiología, CINVESTAV-IPN

I-20
Intestinal innate immune response against rotavirus infection: Role of dendritic cells from mesenteric lymph nodes. **Luis Enrique Hernández Téllez**, Vanessa López-Guerrero and Fernando Esquivel Guadarrama. Facultad de Medicina, UAEM

I-21
Recognition sites for murine IgG and IgE in the allergen Hev b 8 profilin. **José Israel Mares-Mejía,** Enrique Ortega-Soto, Adela Rodríguez-Romero. Instituto de Química, UNAM

I-22
Dissociation of immunosuppressive and nociceptive effects of fentanyl, but not morphine, after repeated administration in mice: Fentanyl-induced sensitization to LPS. **Luz María Molina Martínez,** Claudia González Espinosa, Silvia Cruz. Farmacobiología. CINVESTAV - IPN, Sede Sur

I-23
Immune sensing of *Candida non-albicans* and members of the *Sporothrix schenckii complex*. **Héctor Manuel Mora-Montes**, José Ascensión Martínez-Álvarez, María Navarro-Arias, Luis Antonio Pérez-García, Eine Estrada-Mata, and Edgar Cordero-Roldán. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

I-24
Effect of cobalt chloride-induced hypoxia on the secretory machinery and the cytokine profile secreted by mast cells activated through TLR-4 and FcεRI receptors. **Itzel Guadalupe Ramírez-Moreno** y Claudia González-Espinosa. Farmacobiología, CINVESTAV - IPN

I-25
Production mechanisms of anti-Non-bilayer phospholipid arrangements IgG Antibodies. **Claudia Albany Reséndiz Mora,** Sandra Sánchez Barbosa, Luz Ángela Zárate Neira, Carlos Wong Ramírez, María Isabel Baeza Ramírez, Leopoldo Flores Romo y Carlos Wong Baeza. Bioquímica. Escuela Nacional de Ciencias Biológicas, IPN

I-26
Effects of *Bacillus thuringiensis*-derived Cry1Acproteins and identification of putative receptors inmurine macrophages. **Nestor Rubio-Infante,** Damaris Ilhuicatzi-Alvarado, Ana Lilia García-Hernández, Raúl Nava-Acosta, Marilú Torres-Martínez, Leticia Moreno-Fierros. FES Iztacala, UNAM

I-27
Effect of high glucose conditions on classical and alternative activation of human macrophages *in vitro*. **Israel Torres Castro**, Úrsula Dinorah Arroyo Camarena, Guadalupe Itzel Azuceno García Galileo Escobedo González. Medicina Experimental, Hospital General de México

I-28
Toll-like Receptor-2 and Toll-like Receptor-4 Expression on Human CD16+ and CD16- Monocyte Subsetsfrom Peritoneum in Homeostatic and Inflammatory Conditions. **Xareni R Valle Jiménez**, Sergio R Aguilar Ruiz, Joel Pinacho Jiménez. Instituto Tecnológico de Oaxaca. Centro de Investigación en Ciencias Médicas Biológicas, UABJO

I-29
Participation of germinal centers in mice with Chagas disease treated with NIPOx-B. **Carlos Wong Baeza**, Claudia Albany Reséndiz Mora, Carla Elizabeth Landa Saldivar, Lourdes Sarai Sánchez Dávila, Juan Carlos Santiago Hernández, Benjamin Nogueda Torres, María Isabel Baeza Ramírez, Carlos Wong Ramírez. Escuela Nacional de Ciencias Biológicas, IPN

I-30
The Effect of BCG Vaccination on the Virulence of Different Genotypes of *Mycobacterium tuberculosis*. **Zyanya Lucia Zatarain-Barrón**, Kristin Kremer, Camilo Molina, Yadira Rodríguez, Dulce Adriana Mata-Espinosa, Brenda Marquina, Jorge Barrios-Payan, Rogelio Hernández-Pando, Dick von Soolingen. Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"

I-31
Interaction of neutrophil extracellular traps with *Trichomonas vaginalis*. **Eva E Ávila**, Yordan Jhovani Romero Contreras, Mayra C. Rodríguez and Julieta Pulido. División de Ciencias Naturales y Exactas, Universidad de Guanajuato

MEDICINE, HEALTH AND NUTRITION

M-24
The effect of aqueous extracts of *Hibiscus sabdariffa* L. and NSAIDs on the expression of circulating cytokines in brewer's yeast-induced fever. **Adrián Gómez Baltazar**, Luz Torner Aguilar, Martha Eva Viveros Sandoval, Víctor Manuel Farías Rodríguez and Bertha Fenton Navarro. Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez"

M-25
DNA microarrays making for identification of genetically modified organisms. **Simón Guzmán León**, José Luis Santillán Torres, Lorena Chávez González, Jorge Ramírez Salcedo. Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, UNAM

M-26
CDP-choline ameliorates heart reperfusion damage strengthen by hyperthyroidism. **María de la Luz Hernández Esquivel**, Natalia Pavón, Mabel Buelna-Chontal, Héctor Hernández-Pacheco, Javier Belmont, Edmundo Chávez. Instituto Nacional de Cardiología "Ignacio Chávez"

M-27
Inhibitory Activity of *Reishi Medicinal Mushroom, Ganodermalucidum*on Transformed Cells by Human Papillomavirus. **Hernández–Márquez E**, Lagunas-Martínez A, Bermudez-Morales VH, Burguete-García AI, León-Rivera I, Montiel-Arcos E, García-Villa E, Gariglio Patricio, Madrid-Marina V, and Ondarza-Vidaurreta RN. Instituto Nacional de Salud Pública

M-28
Effects of the direct factor Xa inhibitor rivaroxaban on platelet function in Mexican patients with thrombosis. **Jesús Hernández Juárez**, Hugo Guillermo Espejo Godínez, Nidia Julieta Espinoza Islas, Rodrigo Mancilla Padilla, Abraham Majluf Cruz. Hospital Gral. Reg. No. 1. Dr Carlos Mac Gregor Sánchez Navarro, IMSS

M-29
Insulin sensitivity is inversely related to cellular energy status, as revealed by biotin deprivation**. Hernández-Vázquez Alain de J**, Salvador-Adriano Ana, Vargas-Chávez Sonia, Ortega-Cuellar Daniel, Sánchez-Salvador Alan, Tovar Armando R y Velázquez-Arellano Antonio**.** Instituto de Investigaciones Biomédicas, UNAM

M-30
Development and evaluation of a novel panel of tumor-associated antigens with applications in breast cancer diagnosis. **César López Camarillo,** Cecilia Pagaza Straffon, Luis Herrera, José Díaz Chávez, Mauricio González Avante, Rosalba Carmona, Elena Arechaga Ocampo, Mauricio Castañon, Yadira Palacios, Laurence A Marchat. Universidad Autónoma de la Ciudad de México

M-31
Spoligotyping of resistant *M. tuberculosis* isolates in Jalisco México. **Gladys Guadalupe López-Avalos,** Martin López-Rodríguez, Manuel Sandoval-Díaz, Juan Carlos Villanueva-Arias and Ikuri Álvarez-Maya. Medical and Pharmaceutical Biotechnology Unit, CIATEJ

M-32
Matrix Metalloproteinase (MMP)-28 increases growth rate and migration of lung alveolar epithelial cells and localizes in the nuclei of alveolar epithelial cells in Idiopathic Pulmonary Fibrosis. **Mariel Maldonado**, Iliana Herrera, Jorge García Álvarez, Remedios Ramírez, Blanca Ortíz Quintero, José Cisneros, Moisés Selman, Annie Pardo. Laboratorio de Bioquímica, Facultad de Ciencias, UNAM

M-33
Effect of nicotinamide on cell viability and secretion of plasminogen activators in invasive MDA-MB-231 breast cancer cells. **María Guadalupe Martínez Hernández**, Rubí Araceli Viedma Rodríguez, Margarita Díaz Flores, Luis Arturo Baiza Gutman. Morfofisiología, FES-Iztacala, UNAM

M-34
Increased autophagic flux in A549 lung epithelial cells after tunicamycin treatment. **David Medina Perez**, Moisés Selman Lama, Annie Pardo Cemo, Cabrera Benítez Sandra. Facultad de Ciencias, UNAM

M-35
Omega-3 Fatty acids on Diabetic Pregnant Rats: Effects on Placental Mitochondrial Function and Fatty Acids Composition. **Ricardo Mejía Zepeda**, María del Consuelo Figueroa García, Francisco Alfredo Saavedra Molina, Christian Cortés Rojo, and Martín Palomar Morales. FES Iztacala - UNAM

M-36
Truncated receptor type II TGF-β (TβRIIΔcyt) decreases hepatic fibrosis and steatosis modulating gene expression of TGF-β, Col-1, PAI-1 and Cannabinoids receptors. **Mayra Guadalupe Mena Enriquez**, Ana Soledad Sandoval Rodríguez, Juan Armendáriz Borunda. Ciencias de la Salud. Centro Universitario de Tonalá. Universidad de Guadalajara

M-37
Antioxidant effect of Stevia rebaudiana Bertoni in heart of rats feed with a hyper-glycemic/hyper-calorie diet. **Diana Moroni González**, Patricia Aguilar Alonso, Eduardo Miguel Brambila Colombres, Alfonso Daniel Díaz Fonseca, Samuel Treviño Mora. Laboratorio de Investigaciones Químico Clínicas, BUAP

M-38
Role of R-Spondin2 in the pathogenesis of idiopathic pulmonary fibrosis. **Adrian Munguía Reyes**, Remedios Ramírez Rangel, Criselda Mendoza Milla, Annie Pardo Cemo, Moisés Selman Lama. Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas

M-39
Transforming Growth Factor beta induces down regulation of DNA methyltransferase 1 and up regulation of ten-eleven-translocation 3 in human lung fibroblasts. **Miguel Negreros**, James Hagood, Celia Espinoza, Moisés Selman, and Annie Pardo. Facultad de Ciencias, UNAM

M-40
Effects of avocado oil on lipid profile, kidney mitochondrial function and protein uriain type 2diabetes. **Omar Ortiz Avila**, María del Consuelo Figueroa García, Claudia Isabel García Berumen, Elizabeth Calderón Cortés, Alfredo Saavedra Molina, Christian Cortés Rojo. IIQB. Universidad Michoacana

M-41
Generation of an apta sensorbased on gold nanoparticles for detection of HPV-16 L1 VLPs. **Giovanni Palomino-Vizcaino**, Ana G Leija-Montoya, María L Benitez-Hess, Luis M Álvarez-Salas. Terapia Génica, Departamento de Biología Molecular, CINVESTAV – IPN

M-42
Cecropine, an antibacterian peptide and its effects over heart function. **Natalia Pavón**, Juan Carlos Torres-Narváez, Humberto Lanz Mendoza, Rebeca López-Marure, Mabel Buelna Chontal, Leonardo del Valle Mondragón, Francisco Javier Roldán Gómez. Instituto Nacional de Cardiología Ignacio Chávez

M-43
Relevance of Notchcell signaling pathway in cellular subpopulation with ALDH high activity enriched in cancer stem cells derived from cervical cell lines. **Pérez Díaz Leny Palma**, Alejandro García Carrancá, Elizabeth Ortiz Sánchez, Instituto Nacional de Cancerología

M-44
S-allylcysteine in diabetic nephropathy in rat. **María Antonieta Ramos Vega**, Perla Deyanira Maldonado Jiménez, Diana Barrera Oviedo. Facultad de Medicina, UNAM

M-45
Evaluation of the cardioprotective effect of Citicolione and Erythropoietin against Doxorrubicin-induced cell death. **Reséndiz Castillo**, Luis J Rocha Zavaleta, Leticia. Instituto de Investigaciones Biomédicas. UNAM

M-46
Characterization of an intrauterine microenvironment conditioned by choriodeciduals cells around the onset of human labor. **Yasser E Reyes-Martínez**, Marisol Castillo-Castrejón, Yirham Echegoyen-Alvárez, Felipe Vadillo-Ortega, Carmen Canchola-Sotelo, Noemí Meraz-Cruz. Vinculación Científica Facultad de Medicina, UNAM /INMEGEN

M-47
Screening of the antibacterial, cytotoxicity, antioxidant activities and chemical composition of *Plumbago auriculata* roots. **Patricia Rios-Chavez**, Romel González Hernández , Enrique Ramírez -Chávez and Jorge Molina-Torres. Facultad de Biología, Universidad Michoacana

MICROBIOLOGY AND PARASITOLOGY

MP-23
Prevalence of virulence markers cagA and vacA in Mexican patients infected with *Helicobacter pylori*. **Sigfrid García Moreno Muito**, Juan Carlos Bravata Alcantara, José Bonilla Delgado, Mónica Sierra Martínez and Enoc Mariano Cortés Malagón. Research Unit, Hospital Juárez de México

MP-24
Frequency of *Entamoeba gingivalis* in two groups of Mexican patients. **Gabriela García Pérez,** Fernando Ramos Reyes, Lilian Hernández Mendoza, Antonio Fernández López, Juan Maldonado Martínez, Josué Adad Ronquillo Gómez, Jorge Yañez Ponce de León, Paul Gaytán Colin. Departamento de Microbiología y Parasitología, Facultad de Medicina, UNAM

MP-25
Study of the interaction of SltF and FlgJ, proteins that are involved in the biogenesis of the type 1 flagellar system of *Rhodobacter sphaeroides***. Mariela García-Ramos**, Javier de la Mora, Teresa Ballado, Laura Camarena and Georges Dreyfus. Instituto de Fisiología Celular, UNAM

MP-26
Detection, mapping and phylo genetic analysis of flavor hemoglobins from the genome of rhizobial bacteria. **Reinier Gesto Borroto,** Elena I Villarreal Moguel, Raúl Arredondo Peter. Facultad de Ciencias, UAEM

MP-27
Characterization of Tg Articulin in *Toxoplasma gondii* tachyzoites. **Carmen T Gómez de León**, Sirenia González, Mónica Mondragón, Ricardo Mondragón Flores. Bioquímica. CINVESTAV – IPN

MP-28
Phylogenetic profile of the carotenoid biosynthesis across sequenced bacterial genomes. **Oscar Francisco González Gutiérrez** and Luis David Alcaraz Peraza. Ecología de la Biodiversidad. Instituto de Ecología. UNAM

MP-29
STIM1 and Orai1 Identification of *Entamoeba histolytica*. Alejandra Arreola Martínez, **Omar González Maldonado**, Mario Alberto Rodríguez Rodríguez, Carlos Vázquez Calzada, Diana Jiménez López, Isela Pérez Galarza, Andrés Monsalvo Villagómez, Guadalupe Salazar Enríquez, Ana Laura Cano Martínez y Andrés Salas Casas. Universidad Politécnica de Pachuca

MP-30
Understanding copper uptake in GraMP-negative bacteria. **Antonio González Sánchez,** Ciro Alberto Cubillas Ramírez, Araceli Dávalos and Alejandro García de los Santos. Centro de Ciencias Genómicas, UNAM

MP-31
Proteins with protease activity are secreted by *Leishmania mexicana*, *Entamoeba histolytica* and *Trypanosoma cruzi*. **Augusto González-Canto**, Lorena Sánchez-García, Magdalena Aguirre-García, Alma R Escalona-Montaño, Arturo A Wilkins-Rodríguez, Laila Gutiérrez-Kobeh Rosario López-Vancell, Bertha Espinoza, Ignacio Martínez, Adriana Rodríguez-Guzmán, Mario Nequíz-Avendaño and Ruy Pérez-Tamayo. Medicina Experimental, Facultad de Medicina, UNAM

MP-32
Functional Analysis of *MNN4*–Like family of *Candida albicans.* **Roberto de J. González-Hernández** and Héctor M Mora-Montes. Biología, DCNyE. Universidad de Guanajuato

MP-33
Identification of nitrogen-fixing bacteria associated with the rhizosphere of different varieties of *Zea mays.* **Jairo Eder Guerra-Camacho**, Bibiana Ríos-Galicia, Catalina Villagómez-Garfias, Ramón Ignacio Arteaga-Garibay and César Hugo Hernández-Rodríguez. Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas. IPN

MP-34
Effect of a semi-purified extract of cruzipain on endocytic capacity and cytokine expression in murine macrophage. **Lilian Hernández Mendoza,** María Guadalupe Maldonado Mercado, José Luis Molinari Soriano,Gabriela García Pérez, Fernando Ramos Reyes, Rocío Gabriela Tirado Mendoza, Sandra Solano Gálvez, Natalia Copitin, Ivanova Niconova, Enrique Meléndez Herrada, Araceli González Esteban and Patricia Tato Zaldivar. Facultad de Medicina, UNAM

MP-35
Antibiotic Resistance to First and Second Choice Antibiotics of *E. coli* isolated from Mexican Population with Urinary Trac Infection and its Relationship to Serotype and Phylogenetic Group. **Marco Antonio Ibarra - Valencia**, Margarita María De La Paz Arenas-Hernández, Armando Navarro-Ocaña, Abraham Medrano López, Rosa Del C Rocha Gracia, Ygnacio Martínez-Laguna. Facultad de Medicina, BUAP

MP-36
Study of the antimicrobial activity of the venom from honeybee *Apismellifera*. **Georgina Ivette López Cortés**, María de Lourdes Delgado Aceves, Cintia Gutiérrez Villegas, Bruno Casciaro, Antonio Di Grazia, Víctor Rubén Padilla Salas, Citlalli Arlae Rojas López, Rafael Ordaz Briseño, María Luisa Mangoni, Alfonso Enrique Islas Rodríguez. Cellular and Molecular Biology. University of Guadalajara

MP-37
The diguanylate cyclase DgcA encoding by *dgcA* has role in biofilm formation in *Azospirillum brasilense.* **Lilia I López-Lara**, Alberto Ramírez-Mata, Angelica Romero-Osorio, Felipe J Castañeda and Beatriz E Baca. Instituto de Ciencias. BUAP

MP-38
Motility in *Klebsiella pneumoniae* mediated by flagellum. **Patricia Lozano Zarain,** Rosa del Carmen Rocha Gracia, Libia Adriana León Izurieta, Nataly Olivar Espinosa, Carmen Torres, Sirenia González Pozos, Zita Gutiérrez Cazares, Miguel Castañeda Lucio, Alejandro Carabarin Lima. Microbiología. Instituto de Ciencias. BUAP

MP-39
*In silico*designof a chimericproteinfor a vaccine generated against urinary infections by Uropathogenic *Escherichiacoli.* **Victor M. Luna Pineda**, Sara A. Ochoa Pérez, Zeus Saldaña Ahuatzi, Juan Xicohtencatl Cortes. Hospital Infantil de México Federico Gómez

MP-40
Characterization of new genes regulated by HilD in *Salmonella entérica* serovar Typhimurium. **Rubiceli Manzo Durán**, Aoife Colgan, José Luis Puente García, Jay CD Hinton, and Víctor Humberto Bustamante Santillán. Microbiología Molecular, Instituto de Biotecnología, UNAM

MP-41
Molecular characterizationandIndole-3-acetic acid production of native *Azospirillum*strains from diverse origin. **Cynthia Marcos Jiménez**, Claudia A. Vásquez Hernández, Saúl Jijón Moreno, María L. Xiqui Vázquez and Beatriz E. Baca. Centro de Investigaciones en Ciencias Microbiológicas, BUAP

MP-42
Role and subcellular localization of EGL-1 and EGL-, two putative GPI anchored cell wall β (1-3) endoglucanasas, in *Neurosporacrassa.* **Leonora Martínez-Núñez** and Meritxell Riquelme. Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada

MP-43
Taxonomic diversity of the carnivorous plant microbiome *Utricularia gibba*. **Shamayim Martínez-Sánchez**, Enrique Ibarra-Laclette, Luis Herrera-Estrella and Luis David Alcaraz. Departamento de Ecología de la Biodiversidad. Instituto de Ecología, UNAM

MP-44
Mannheimia haemolytica expresses an OmpP2-likeamyloid protein. **Juan Fernando Montes García**, Sergio Vaca, Francisco Aguilar, Tomas Villamar, Candelario Vázquez y Erasmo Negrete Abascal. Facultad de Estudios Superiores Iztacala UNAM

MP-45
Enhancement of insecticidal activity of *Bacillus thuringiensis* Cry1Ab toxin against *Spodoptera frugiperda* (*Lepidoptera:Noctuidae*) through alanine substitution in domain III. **Josué Ocelotl Oviedo**, Jorge Sánchez Quintana, Guadalupe Peña Chora, Alejandra Bravo de la Parra, Mario Soberón Chávez, Isabel Gómez Gómez. Instituto de Biotecnología /UNAM

NEUROSCIENCE AND NEUROBIOLOGY

N-1
Evaluation of the glutathione system associated to enzymes in brain ischaemia. **Patricia Aguilar Alonso**, Norma Judith Cruz Ortega, Alfonso Daniel Díaz Fonseca, Eduardo Miguel Brambila Colombres, Addí Rhode Navarro Cruz, Raúl Ávila Sosa Sánchez, Gonzalo Flores, Samuel Treviño Mora. Facultad de Ciencias Químicas, BUAP

N-2
Assessment of major depressive disorder model in rat.**Venus Berenice Aparicio Díaz**, Glenda Gladis Cazalez Linares, Eduardo Miguel Brambila Colombres, Gonzalo Flores, Héctor Armando Rubio Zapata. Irma Herrera Camacho,Israel Camacho Abrego, Patricia Aguilar Alonso. Facultad de Ciencias Químicas, BUAP

N-3
The mitochondria: an integrator of damage signals that result inneurodegeneration in an Alzheimer's disease model. **Tonali Blanco-Ayala**, Gustavo Pedraza-Alva and Leonor Pérez-Martínez. Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

N-4
Short-term high-fat-and-fructose feeding produces hippocampal insulin resistance, dendrite and spine reduction, tau and MAP-2 alterations, synaptic mitochondrial dysfunction and neuro inflammation. **Erika Calvo-Ochoa**, Karina Hernández-Ortega, Patricia Ferrera, Ricardo Quiroz-Báez, Clorinda Arias. Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, UNAM

N-5
Effect of Ketone bodies on glucose deprivation-induced autophagy in rat cultured cortical neurons. **Lucy Camberos**, Cristian Gerónimo, Teresa Montiel and Lourdes Massieu. Instituto de Fisiología Celular, UNAM

N-6
Conjugation of Sema3C with a biodegradable hydrogel causes enhanced axonal growth of dopamine neurons. **Oscar Alejandro Carballo Molina**, Iván Velasco Velázquez. División de Neurociencias, Instituto de Fisiología Celular, UNAM

N-7
Standardizing a Method Based on qPCR to Determine the Loss of Dopaminergic Neurons in *Drosophila melanogaster*. **Luis Ángel Carvajal Oliveros**, René Hernández Vargas, Yoandy Ferrer Marcelo, Verónica Narváez Padilla, Enrique Alejandro Reynaud Garza. Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM

N-8
Expression profile of cytokine mRNAs in the retina of Fyn-/- mice: Possible involvement of Fyn during the process of reactive gliosis. **Marbella Chávez-S,** Claudia González-E, and Mónica Lamas-G. Pharmacobiology. Department, CINVESTAV - IPN

N-9
Análisis de los mecanismos de daño del 27-hidroxicolesterol: Implicaciones para la Enfermedad de Alzheimer. **Hélène Clémente Montoya**, Aydé Mendoza Oliva, Patricia Ferrera Boza, Clorinda Arias Álvarez. Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, UNAM

N-10
KChIP3 mediates cholinergic SN56 neuronal cell death induced by oxidative stress. **Javier Cortés-Mendoza,** Eunice Gezabel Zuñiga Hinojosa, Gustavo Pedraza-Alva & Leonor Pérez-Martínez. Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

N-11
Sensibilidad diferencial de células de neuroblastoma humano a los anti-inflamatorios no esteroideos y al péptido β-amiloide: efectos sobre la sobrevivencia y expresión de ciclina D. **Patricia Ferrera** y Clorinda Arias. Instituto de Investigaciones Biomédicas. UNAM

N-12
Expression of connexins 32, 36 and 43 in rathippocampus during seizures induced by 4-aminopyridine. **Xóchitl Natalia Flores-Ponce**, Anne Santerre, Laura Guadalupe Medina-Ceja. Laboratorio de Neurofisiología y Neuroquímica, Departamento de Biología Celular y Molecular, CUCBA, U. de G.

N-13
The role of glutathione and the reactive oxygen species in postnatal development of rat cerebellar cortex. **Brenda Vianey García Hernández**, Erick Ariel Martínez Chávez. Instituto de Fisiología Celular, UNAM

N-14
Searching new compounds with better selectivity intoβA in α-helix than β-sheet conformation under *In silico* studies for targeting the Alzheimer disease. **Iohanan Daniel GarcíaMarín**, Maricarmen Hernández Rodríguez, José Correa-Basurto, Martha Cecilia Rosales-Hernández. Escuela Superior de Medicina, IPN

N-15
Early activation of autophagy during glucose deprivation contributes to cortical neuronal death. **Cristian Gerónimo-Olvera** & Lourdes Massieu. Instituto de Fisiología Celular, UNAM

N-16
Role of the NADPH oxidases during the excitotoxic damage in the striatum in mice. **Diego Rolando Hernández Espinosa**, Lourdes Massieu Trigo, Edgar Zenteno Galindo, Julio Morán Andrade. Neurodesarrollo y Fisiología, Instituto de Fisiología Celular, UNAM

GENETIC REGULATION & EPIGENETIC

GR-46
Differential effect of the members of mir-34 family on siha cell proliferation. **Jesús Adrián López** and Angelica Judith Granados López. Laboratorio de microRNAs. Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas

GR-47
Transcriptional study of non-coding small RNAs genes *rsm Z1, Z2, Z3, Z4, Z5, Z6, Z7, Z8* and *Y* in *Azotobactervinelandii.* **López-Pliego L,** Castañeda Lucio-M. Centro en Ciencias Microbiológicas, BUAP

GR-48
Down-regulation of *Phaseous vulgaris PvRbohD* gene during the symbiotic interaction with rhizobia. **José Luis López-Angeles**, Manoj Kumar Arthikala, Noreide Nava, Jesús Montiel and Carmen Quinto. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-49
Transcriptomic analysis of photoconidiation process in *Trichoderma atroviride.* **López-Hernández** José Fabricio and Herrera-Estrella Alfredo Heriberto. LANGEBIO. CINVESTAV – IPN Irapuato

GR-50
Pirfenidone down regulates inflammatory mediators and modulates endo cannabinoids receptors in non-alcoholic steatohepatitis induced by highfat/carbohydrate diet. **José Macías-Barragán**, José Vera-Cruz, Krista Rombouts, Margarita Montoya-Buelna, David López-de-la-Mora, Cibeles Sánchez-Roque, Jesús García-Bañuelos, Massimo Pinzani, Juan Armendáriz-Borunda. Departamento de Ciencias Naturales y Exactas, Universidad de Guadalajara

GR-51
Effect of the different sizes of ds RNA in the efficiency of NADH oxidase gene silencing in *Giardia lamblia*. **Jaime Marcial-Quino**, Ignacio De la Mora-De la Mora, Adriana Castillo-Villanueva, Sara Méndez-Cruz, Saúl Gómez-Manzo, Itzhel García-Torres, Sergio Enríquez-Flores, Angélica Torres-Arroyo, América Vanoye-Carlo, Gabriel López-Velázquez, Jesús Oria-Hernández, Horacio Reyes-Vivas. Instituto Nacional de Pediatría

GR-52
Regulatory divergence in paralogous genes ALT1 and ALT2 in *Saccharomyces cerevisiae*. **Dariel Márquez Gutiérrez**, James González, José Carlos Campero and M Alicia González Manjarrez. Instituto de Fisiología Celular, UNAM

GR-53
Functional characterization of the *Arabidopsis thaliana WIP2* gene in the meristematic regions of vegetative tissues. David Díaz Ramírez, Humberto Herrera Ubaldo, Daniela Ramos Cruz, Víctor Zúñiga Mayo, Vicente Balanza, Pieter Ouwerkerk, Martin Sagasser, Cristina Ferrándiz, Stefan de Folter, **Nayelli Marsch Martínez**. Biotecnología y Bioquímica, CINVESTAV - IPN

GR-54
Tissular localization of transcripts and proteins induced by water deficit in *Arabidopsis thaliana*: group 4 LEA proteins. **Coral Martínez Martínez**, Yadira Olvera-Carrillo and Alejandra A. Covarrubias Robles. Biología Molecular de Plantas. Instituto de Biotecnología, UNAM

GR-55
Molecular characterization of TbZ5, a protein with multiple zinc-finger domains in *Trypanosoma brucei.* **María de Lourdes Mejía-Hernández**, Daniel E Vélez-Ramírez, Luis E Florencio-Martínez and Santiago Martínez-Calvillo. Biomedicina, FES Iztacala, UNAM

GR-56
Study of Azotobacter vinelandii small non-coding RNARsmY. **Giselda Mena Muñoz**, Liliana Lopez Pliego, Cinthia Nuñez López, Luis Ernesto Fuentes Ramírez y Miguel Castañeda Lucio, CICM-IC. BUAP

GR-57
Ethylene-auxin interaction modulates primary root growth and cell division in *Arabidopsis thaliana*. **Alejandro Méndez Bravo,** Miguel Martínez Trujillo, José López Bucio. Biología del Desarrollo Vegetal. IIQB. Universidad Michoacana

GR-58
Intronic circles as pre-mRNA splicing products in *Entamoeba histolytica.* **María Saraí Mendoza-Figueroa**, Elisa Azuara-Liceaga and Jesús Valdés. CINVESTAV – IPN Zacatenco

GR-59
Effect of over-expression of the members of mir-34 family on siha cell proliferation. **Julieta Moreno Longoria**, Jesús Adrián López. Unidad Académica de ciencias Biológicas, Universidad Autónoma de Zacatecas "Francisco García Salinas"

GR-60
Population density regulates *Arabidopsis thaliana* growth and development through modulating auxin transport. **Edith Muñoz Parra**, Ramón Pelagio Flores, Javier Raya González, Randy Ortíz Castro, León Francisco Ruíz Herrera, José López Bucio. IIQB, Universidad Michoacana

GR-61
Actin depolymerizing factor is required during the early stages of the rhizobia-legume symbiosis. **Yolanda Ortega-Ortega**, David Jáuregui, Marco A Juárez-Verdayes, Xóchitl Alvarado, Olivia Santana and Carmen Quinto. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-62
Gene expression profiling of DKK1 and patterns of methylation in osteoblast biology. **Alma Parra-Torres**, Diana García-Peralta, Karina Albarrán-Casildo, Nelly Patiño-Uriostegui, Rafael Velázquez-Cruz. Instituto Nacional de Medicina Genómica

GR-63
Merlin negative regulation by mir-146a promotes cell transformation. **Pedraza-Alva Gustavo**, Pérez-García Erick, López-Sevilla Yaxem, Meza-Sosa Karla, Camacho-Concha Nohemí and Pérez-Martínez Leonor. Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

GR-64
In *Candida glabrata* Gln3 GATA factor is a nitrogen assimilation key regulator. **Francisco Javier Pérez de los Santos** and Lina Raquel Riego Ruiz. División de Biología Molecular, IPICYT

GR-65
Transcript profiling distinguishes complete treatment responders with locally advanced cervical cancer. **Carlos Pérez-Plasencia,** Jorge Fernández-Retana, Federico Lasa-Gonsebatt. Laboratorio de Genómica, UBIMED. FES Iztacala, UNAM

TOXICOLOGY

T-18
Kinetics of toxicity of aluminum 3^{+} and free radicals in evaluating (ATCC ® CCL-81 ™) Vero cells. **Ofelia Juárez-Carrillo**, Marcela Galar-Martínez, Amparo Celene Razo-Estrada, Ricardo Pérez-Pastén Borja. Toxicología Mecanística, Escuela Nacional de Ciencias Biológicas, IPN

T-19
Toxicological evaluation of ethanolic extract of *Justicia spicigera* in30 days-treated Wistar rats. **Balderas-Plascencia S**, Alvarado-Sánchez, Hernández-Morales A, Del Toro-Herrera J, Hernández-Aguilar JA, León-Buitimea A. Unidad Académica Multidisciplinaria, UASLP

T-20
Evaluation of the Effect of Freeze Dried *Thalassia testudinum* in the cell Proliferation and Viability of Human Tumor Cell Lines. **Itzel Leyva-Bahena**, Elizabeth Estrada-Muñiz, Idania Rodeiro, Libia Vega Loyo. Toxicology. CINVESTAV-IPN

T-21
Evaluation of methyl parathion to determine LC_{50} bullfrog tadpole *Lithobates catesbeianus* (Shaw, 1802). **Laura Méndez-Alcántara**, Ofelia Juárez-Carrillo, Mario Cárdenas-León, Amparo Celene Razo-Estrada. Toxicología Acuática, Escuela Nacional de Ciencias Biológicas - IPN

T-22
Study of the metabolism effects of arsenic by expression of arsenic (+3 oxidation state)-methyltransferase in human bladder transfected cells (HTB-1) on cell cycle regulation. **Jeshua Donamis Molina Aguilar**, Araceli Hernández Zavala, José Efraín Garrido Guerrero. Escuela Superior de Medicina, IPN

T-23
Differential expression of drugs transporters associated with chemoresistance in childhood rhabdomyosarcoma: Comparison between tumoral and normal tissue. **Dora Molina-Ortiz**, Araceli Vences-Mejía, Rafael Camacho-Carranza, José González-Zamora, Rocío Cárdenas-Cardós, Jaime Shalkow-Klincovstein. Instituto Nacional de Pediatría

T-24
Effect of the biotransformation of arsenic on cell cycle regulation in human bladder cells. **Teresita Rocio Moore-Ambriz**, Pedro Chávez-Olmos, José Efraín Garrido Guerrero, Araceli Hernández-Zavala. Escuela Superior de Medicina, IPN

T-25
Bergamottin as an inhibitor of CYP1A during embryonic development in the chicken. **Diego Arturo Ornelas Ayala**, Jesús Javier Espinosa Aguirre, Jesús Chimal Monroy. Instituto de Investigaciones Biomédicas, UNAM

T-26
Gestational exposure to inorganic arsenic (iAs) modulates cysteine transport in mouse brain. **LA Ramos-Chávez**, CRR Rendón-López, P Petrosyan, & ME Gonsebatt. Instituto de Investigaciones Biomédicas, UNAM

T-27
Study of toxic effects of biotin pharmacological concentrations. **Leticia Riverón-Negrete,** Carolina Álvarez-Delgado, Cristina Fernández-Mejía. Biomedical Research Institute, National University of Mexico

T-28
Human and rat CYP1A1 enzyme inhibition by grapefruit juice compounds. **Rebeca Santes Palacios**, Antonio Romo-Mancillas, Rafael Camacho Carranza, Jesús Javier Espinosa Aguirre. Instituto de Investigaciones Biomédicas, UNAM

T-29
Effect of Metforminon the metabolic regulation of carbohydrates and lipids in Wistar rats chronically administered with Cd. **Victor Enrique Sarmiento Ortega**, Samuel Treviño Mora, Alfonso Daniel Diaz Fonseca, Patricia Aguilar Alonso, Eduardo Miguel Brambila Colombres. Facultad de Ciencias Químicas, BUAP

T-30
Modulation of the GSH and Trx systems by arsenic in the mouse brain. **Irma Daniela Silva Adaya**, Lucio Antonio Ramos Chávez, Gabriel Gutiérrez Ospina, María E Gonsebatt. Instituto de Investigaciones Biomédicas. UNAM

T-31
Venom of the centipede *Scolopendra polymorpha* causes muscular and nervous tissue alterations in mouse. **Judith Tabullo De Robles**, Lucero Valladares Cisneros, Juana Villeda Hernández, Francisca Fernández Valverde, Ma. Del Carmen Gutiérrez Villafuerte. CEIB. UAEM

T-32
Evaluation of plasmatic concentrations of natriuretic peptides in children environmentally exposed to arsenic. **José M. Torres Arellano**, Guadalupe Aguilar Madrid, Laura Arreola-Mendoza, Ángel Barrera Hernández, Luz C Sánchez Peña, Citlalli Osorio Yáñez, Luz M Del Razo. Toxicología, CINVESTAV-IPN

T-33
Systemic nerve growth factor modulates the transcription of amino acid transporters and glutathione synthesis in mouse striatum. **Valdovinos-Flores,** Cesar, Petrosyan Pavel y Gonsebatt Bonaparte María Eugenia. Instituto de Investigaciones Biomédicas, UNAM

T-34
Cytotoxic effects and antimicrobial activity of Cuachalalate (*Amphipterygium adstringens*) extracts, prepared in a traditional way. **JanetteVillarruel Muñoz**, Zaira López, Diana Solís Padilla, José Rico, Joel Salazar Flores and Peter Knauth. Centro Universitario de la Ciénega, UDG

SIGNAL TRANSDUCTION

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Interferon Stimulated Gene 12 is a negative regulator of the Estrogen Receptor Alpha/Estradiol signaling. **Bibiana Ortega Domínguez**, Angeles C Tecalco Cruz, Marcela Sosa Garrocho and Alfonso León Del Río. Instituto de Investigaciones Biomédicas, UNAM

ST-65
Akt behavior in different tissues at early stages of an experimental model of renal carcinogenesis and effect of a tamarind seed extract. **Ignacio Pacheco-Bernal**, Francisco Aguilar-Alonso, José D Solano, Patricia Curiel-Muñiz, Telma Pariente-Pérez, Chabetty Vargas-Olvera, María Elena Ibarra-Rubio. Biología. Facultad de Química, UNAM

ST-66
The other side of the moon: H_20_2 as a second messenger for adrenaline. **Enrique Piña Garza**, María Magdalena Vilchis Landeros, Raquel Guinzberg Perrsuquía, Disraeli Díaz-Guinzberg, Antonio Díaz-Cruz. Facultad de Medicina, UNAM

ST-67
Analysis of the damage to intestinal epithelial cells by purified cathepsin B like from *Giardia duodenalis* and its effect on signaling pathways that modulate intestinal epithelial homeostasis using the duodenal loop ligation model in gerbils (*Meriones unguiculatus*). **Rodrigo Quezada-Lázaro**, Yaremit Mora-Hernández, Rocio Fonseca-Liñán, Silvia Galindo-Gómez, Angélica Silva-Olivares, Mineko Shibayama, Porfirio Nava-Domínguez. Guadalupe Ortega-Pierres. CINVESTAV-IPN Zacatenco

ST-68
Identification of an arginine-phospho tyrosine based endoplasmic retention motif in a G-protein activated potassium channel. **Claudia Iveth Rangel-García**, Beatriz Díaz-Bello, Carolina Salvador, Laura Escobar. Facultad de Medicina, UNAM

ST-69
The proto-oncoprotein SnoN is downregulated by the antibiotics anisomycin and puromicyn via proteasome. **Diana G Ríos-López**, Jacqueline Hernández-Damián, Angeles C Tecalco-Cruz, Genaro Vázquez-Victorio, Aleida Vázquez-Macías, Cassandre Caligaris, Marcela Sosa-Garrocho, Blas Flores-Pérez, Margarita Romero-Avila, Marina Macías-Silva. Instituto de Fisiología Celular, UNAM

ST-70
Study of the histidine protein kinase RpfC2 in *Azotobacter vinelandii*. **Rodríguez Antonio Ana Laura**, Castañeda Lucio Miguel. Instituto de Ciencias, BUAP

ST-71
HER3/HER2 heterodimer activates PI3K but not the Akt signaling pathway in cervical cancer cells. **Octavio Zerecero-Carreón**, Arturo Valle-Mendiola, Benny Weiss-Steider, Isabel Soto-Cruz. Unidad de Investigación en Diferenciación Celular y Cáncer. FES Zaragoza, UNAM

ST-72
Phosphorylation and internalization of short splicing variant of the omega 3 fatty acid sensor, GPR120. **María Teresa Romero-Avila**, Omar Benoni Sanchez-Reyes, Jean A. Castillo-Badillo, Yoshinori Takei, Akira Hirasawa, Gozoh Tsujimoto, Rafael Villalobos-Molina and J. Adolfo García-Sáinz. Instituto de Fisiología Celular, UNAM

ST-73
Adipocytes show alterations in the signaling pathways that control glucose transport in a model of metabolic syndrome. **Jorge Rosas-García** J, Karla Carvajal-Aguilera KG. Nutrición experimental, Instituto Nacional de Pediatría

ST-74
Toll-like receptor 4 activation promotes epithelial-mesenchymal transition and progression in breast cancer. **Karina Ruiseco-Flores**, Eduardo Monjaraz-Guzman. Instituto de Fisiología, BUAP

ST-75
Characterization of signaling pathways that allow to mast cells to respond against bacterial compounds. **Elizabeth Sánchez-Miranda,** Alfredo Ibarra-Sánchez, Damaris A. Hernández- Sánchez, Ulrich Blank, Marina Macías-Silva and Claudia González-Espinosa. Instituto de Fisiología Celular, UNAM

ST-76
Role of Wnt signaling into cellular subpopulation with increased ALDH activity from cervical cell lines. **Miguel Ángel Sarabia Sánchez**, Alejandro García Carrancá and Elizabeth Ortiz Sánchez, Instituto Nacional de Cancerología

ST-77
GPR40 phosphorylation is induced by free fatty acids and activation of Protein Kinase C. **Carla Sosa-Alvarado**, Omar B Sánchez-Reyes, M Teresa Romero-Ávila, Yoshinori Takei, Akira Hirasawa, Gozoh Tsujimoto, J Adolfo García-Sáinz. Instituto de Fisiología Celular, UNAM

ST-78
The expression of progesterone induced blocking factor is hormonally regulated and it increases the growth of human astrocytoma cells through IL-4R/STAT6 pathway. **Paulina Valadez-Cosmes**, Carolina Arellano-Jiménez, Mónica López-Sánchez, Aliesha González-Arenas and Ignacio Camacho-Arroyo. Biología, Facultad de Química, UNAM

ST-79
Evaluation of heat shock proteins in human gastric adenocarcinoma cells that overexpress claudins. **Priscila J. Torres Granados**, Rosalba Pacheco Bautista, Luis F Montaño Estrada, Erika P Rendón Huerta. Biología Celular y Tisular, Facultad de Medicina, UNAM

ST-80
Identification of proteins involved in signaling pathways induced protein from *Bacillus thuringiensis* Cry1Ac activation in RAW 264.7 macrophages. **Marilu Torres Martínez**, Ana Lilia García Hernández, Nestor Rubio Infante y Leticia Moreno Fierros. UBIMED. FES Iztacala. UNAM

ST-81
Retinol Binding Protein type 4 (RBP4) induced insulin resistance in skeletal muscle cells of rats (L6), through the reduction in gene expression of elements of the signaling pathway activated by insulin. **Rafael Torres Montiel**, Antonio Hernández Ortiz, Eduardo Monjaraz Guzmán. Neuroendocrinología. Instituto de Fisiología. BUAP

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Study of pH changes during human sperm capacitation. **Paulina Torres- Rodríguez** , Mariano G Buffone, Lis del C Puga Molina, Alberto Darszon, Claudia L Treviño Santa Cruz. Instituto de Biotecnología. UNAM

ST-83
Claudins -6 and -9 regulate the activation of MMP-2 and MMP-9 in human gastric adenocarcinoma cells. **Ana C Torres-Martínez**, Luis F Montaño-Estrada, Erika P Rendón-Huerta. Biología Celular y Tisular Facultad de Medicina, UNAM

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Real time monitoring of the acrosomal reaction in human sperm. **Esperanza Mata Martínez**, Claudia Sánchez-Cárdenas, Alberto Darszon y Claudia L Treviño. Instituto de Biotecnología-UNAM

ST-85
Participation of ERK1/2, JNK and P38 in the formation of benzo[a]pireno metabolites in BEAS-2B cells. **Gerardo Vázquez Gómez**, Leticia Rocha Zavaleta, Miriam Rodríguez Sosa, Julieta Rubio Lightbourn. Instituto de Investigaciones Biomédicas. UNAM

ST-86
The presence of proinflammatory cytokines exacerbates the proliferative and migratory capacity of breast cancer cells. **Monserrat Vázquez Rojas,** Eduardo Monjaraz Guzmán. Instituto de Fisiología, BUAP

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Leptin increase migratory activity of MDA-MB-231 breast cancer cells through voltage gated sodium channels overexpression and Na^+/H^+ exchanger. **Venancio-García E**, Monjaraz-Guzman E. Instituto de Fisiología. BUAP

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Regulation of ribosome biogenesis in maize embryonic axes during germination. **Juan Manuel Villa Hernández**, Tzvetanka Dimitrova Dinkova, Estela Sánchez de Jiménez, Fernando Rivera Cabrera, Fernando Díaz de León Sánchez, Pérez Flores Laura Josefina. Ciencias de la Salud, UAM

ST-89
Leptin induces epithelial-mesenchymal transition in a FAK-ERK dependent pathway in mammary non-tumorigenic cells MCF10A. **José Alfredo Villanueva Duque**, Eduardo Castañeda Saucedo, Mercedes Calixto Galvez, Carlos Ortuño Pineda, Eugenia Flores Alfaro, Napoleón Navarro Tito. Unidad Académica de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

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Molecular characterization of the Receptor for Activated C Kinase 1 (RACK1) in the jellyfish *Cassiopea xamachana.* **Patricia Cabrales Arellano**, Tania Islas Flores, Claudia Morera Román and Marco A Villanueva Méndez. Instituto de Ciencias del Mar y Limnología, UNAM

ST-91
Leptin induces cell migration in an EGFR transactivation-dependent pathway in MDA-MB-231 breast cancer cells. **Bernabé Visoso Torres**, Juan C Juárez Cruz, Eduardo Castañeda Saucedo, Sócrates Villegas Comonfort, Daniel Hernández Sotelo, Napoleón Navarro Tito. Unidad Académica de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

ST-92
Shh, Wnt and Notch pathways activation in acute lymphoblastic leukemia and their correlation to proliferative rate and chemoresistance. **Zárraga Vargas Laura**, Collí Magaña Dianelly, Acevedo Fernández Juan José, González Nava Gabriela , Chávez Carreño Adriana Santa-Olalla Tapia Jesús. Facultad de Medicina. UAEM

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Kinetic mechanism of thioredoxin-glutathione reductase from *Taenia crassiceps*. **Juan L Rendón**, Alberto Guevara-Flores, Oscar Flores-Herrera y Juan P Pardo. Bioquímica, Facultad de Medicina, UNAM

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Stability analysis of the perchloric acid-soluble protein (Tv-PSP) from *Trichomonas vaginalis.* **Alma Villalobos-Osnaya**, Laura Itzel Quintas-Granados, Laura Isabel Vazquez-Carrillo, María Elizbeth Alvarez-Sanchez. Universidad Autónoma de la Ciudad de México

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Plant proteasomes. **Daniel Aristizábal,** Viridiana Rivas, y Fernando Lledías. Biología Molecular de Plantas. Instituto de Biotecnología, UNAM

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High-performance planar chromatography (HPTLC) and low temperature plasma ionization mass spectrometry (LTP-MS) for the analysis of bioactive compounds in guava fruit (Psidium guajava). **Fabián Heriberto Rivera Chávez**, Jorge Molina Torres, Robert Winkler. Bioquímica y Biotecnología. CINVESTAV - IPN - Unidad Irapuato

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Effect of omega fatty acids (3, 6, or 9) against diabetes. **Mónica Rivera Valencia,** Ricardo Mejía Zepeda. Facultad de Estudios Superiores Iztacala, UNAM

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Contribution of tryptophan residues on the Human Gamma D-Crystalin stability: Real Time NMR folding study. **Lina Rivillas-Acevedo**, Jonathan King, Carlos Amero. Lab. RMN. Centro de Investigaciones Químicas, UAEM

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Relevant aminoacids for the differences in reactivation of the triosephosphate isomerases from *Trypanosoma brucei* and *T. cruzi*. **Mónica Rodríguez Bolaños**, Nallely Cabrera González and Ruy Pérez Montfort. Bioquímica y Biología Estructural. Instituto de Fisiología Celular, UNAM

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Expresion de peptido amiloide β en los vasos de las leptomeninges cerebrales en individuos con enfermedad de Alzheimer y su relación con el genotipo ApoE. **Emma Rodriguez Maldonado**, Chera L Maarouf, Ian D Daugs, Alex E Roher. Instituto Nacional de Cardiología "Ignacio Chávez"

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Increase in pH causes conformational changes in laccase from bacteria *Thermus thermophilus* producing loss in activity. **Claudia Rodríguez-Almazán**, Edith Flores Hernández and Enrique Rudiño Piñera. Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

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Detection of the genes which encode to the ICp55 peptidase, methionine aminopeptidase 1 and methionine aminopeptidase 2 in the yeast *Schizosaccharomyces pombe.* **Patricia Leany Segundo Ibañez**, Xadeni Burgos Gamez, Lourdes Millán Pérez Peña, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. Bioquímica y Biología Molecular, CQ-IC. BUAP

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Cytochrome C oxidase Cox2 subunit expression from the nucleus and its import into yeast mitochondria. **Diana Rubalcava Gracia Medrano**, Xochitl Pérez-Martínez and Diego González Halphen. Instituto de Fisiología Celular, UNAM

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Expression and isotopic labelling of sea anemone toxin Bcs Tx1. **Jessica N Villegas Moreno**, Carlos Amero Tello. Centro de Investigaciones Químicas, UAEM

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Aluminum induces low phosphate adaptive responses and modulates primary and lateral root growth by differentially affecting auxin signaling in *Arabidopsis* seedlings. **León Francisco Ruíz Herrera** and José López Bucio. IIQB Universidad Michoacana de San Nicolás de Hidalgo

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Respiratory complex and supercomplexes arrangement of mitochondriopathy primary skin fibroblasts cultures. **Karina Olivia Salvador Severo**, Diana Lashidua Fernández Coto, Edgar Tena-Sanabria, Carlos Castañeda-Reséndiz, Martha Elisa Vázquez-Memije, Juan Fernando Minauro Sanmiguel. UIM Genética Humana. Hospital de Pediatría CMN S XXI IMSS

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Characterization of proteolytic phenomenon in the starch binding domain (SBD) of the α-amylase of *Lactobacillus amylovorus*. **Zaira Esmeralda Sánchez Cuapio**, Alejandra Hernández Santoyo, Sergio Sánchez Esquivel & Romina Rodríguez-Sanoja. Instituto de Investigaciones Biomédicas, UNAM

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Role of amino acid F763 in the function of *Saccharomyces cerevisiae* mitochondrial polymerase *MIP1*. **Dulce María Vazquez-Zavala**, Diana Fabiola Díaz-Jiménez and Luis Gabriel Brieba de Castro. DCNyE, Universidad de Guanajuato

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Protein-ligand interactions to understand the role of the mutations in the Shwachman-Diamond Syndrome. **Eugenio de la Mora,** Abril Gijsbers Alejandre, Adrián García Márquez, Alfonso Méndez Godoy, Nancy Marcial Bazaldúa, y Nuria Sánchez Puig. Instituto de Química, UNAM

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High pH and salt affects growth and energetic metabolism in *Debaryomyces hansenii.* **Norma Silvia Sánchez Sánchez**, Martha Calahorra Fuertes y Antonio Peña Díaz. Departamento de Genética Molecular. Instituto de Fisiología Celular. UNAM

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Characterization of the hydrophobic subunits of the peripheral arm of the ATP synthase from the colorless alga *Polytomella* sp. **Lorenzo Sánchez Vásquez**, Alejandra Jiménez Suárez, Héctor Miranda Astudillo, Francisco Javier de la Mora Bravo, Georges Dreyfus y Diego González Halphen. Genética Molecular, Instituto de Fisiología Celular UNAM

B-132
Structural Contributions to the Thermal Adaptation of the TATA-Binding Protein. **José Ángel Santiago Terrones**, Nina Pastor Colón. Facultad de Ciencias, UAEM

B-133
Heterologous Expression of an Immunogenic Peptide of *Helicobacter pylori*. Vacuolating Cytotoxin A. **Sauceda Arellano E**, Hernández Márquez E, Flores-Luna L, Sánchez Castillo J, Ayala Aguilar G. Desarrollo Biotecnológico. CISEI. Instituto Nacional de Salud Pública

B-134
Analysis of changes in the transcription of genes involved in autophagy and apoptosis in aged cells of *Schizosacharomyces pombe.* **Patricia Leany Segundo Ibañez**, Guadalupe Rojas Sánchez, Juan Carlos Benitez Serrano, Diana Lancho Zahuantitla, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. Bioquímica y Biología Molecular, CQ-IC. Microbiología. Fac. Ciencias Químicas. BUAP

B-135
Interaction between the receptor for advanced glycation end products (RAGE) domains and quinolic acid (QUIN). **Iris N Serratos,** Nina Pastor , Pilar Castellanos, César Millán-Pacheco, Ruy Pérez-Montfort, Nallely Cabrera, Francisco Reyes-Espinosa, Ambar López-Macay, Karina Martínez, Laura Castañeda, Ermilo Haas Dzib, Alberto López, Ponciano García-Gutiérrez y Abel Santamaria. Departamento de Química, UAM-I

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Advances in protein-protein interface design: grafting, docking and *de novo* approaches. **Daniel-Adriano Silva,** Alex Ford, David La, William Sheffler, Lei Shi, Shawn Yu, Michelle Scalley-Kim and David Baker. University of Washington

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Isolation and characterization of bioactive peptides from conditioned media by vegetal cells of various species. **Carmen Nayeli Soto Vargas**. Facultad de Química, UNAM

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Canine Tracheal Cartilage Cryopreservation: Freezing Injury is not related to Caspase-3 Expression. **Avelina Sotres-Vega**, Jaime Villalba-Caloca, Isabel Guadarrama-Sánchez, José Luis Torre-Jaime, Jazmín Azareel García-Montes, Miguel Gaxiola-Gaxiola, J Alfredo Santibañez-Salgado, Carlos Ramos-Abraham, Ana M Rosales-Torres, L Felipe Jiménez-García. Lung Transplantation Research Unit. National Institute of Respiratory Diseases / UAMX

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Effect of the proteic fraction G10P1.7.57 from the habanero chili (*Capsicum chinense* Jacq.) on growth of the tumoral cell lines Hep-2, SiHa, PC-3, and VERO. **José Aarón Tamayo-Sansores**, Ligia G. Brito-Argaez y Rosa E. Moo-Puc, Andrés H. Uc-Cachón, Ignacio Islas Flores. Unidad de Bioquímica y Biología Molecular de Plantas. CICY

B-140

Search S6 ribosomal protein not associated with ribosomes in nuclei of *Saccharomyces cerevisiae.* **Reynaldo Tiburcio-Felix**, Arnulfo Bautista-Santos, Josue Romero, Arturo Ortega-Soto, Bulmaro Cisneros-Vega, Samuel Zinker-Ruzal. Genética y Biología Molecular. CINVESTAV-IPN

B-141

In vitro import of the cytochrome C oxidase COXIII subunit in *Polytomella* sp. mitochondria. **Miriam Vázquez-Acevedo**, Diana Rubalcava Gracia Medrano, Diego González-Halphen. Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM.

B-142

Native electrophoresis studies of different aggregation states of triosephosphate isomerase. **Paola Toledo Ibelles**, Edgar J Pérez Castañeda, Mónica Rodríguez Bolaños, Alejandra Jiménez Suárez, Héctor V Miranda Astudillo, Diego González Halphen, Ruy Pérez Montfort. Instituto de Fisiología Celular, UNAM

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NaTrxh: its possible role in the cell-cell interaction that lead to pollen rejection in *Nicotiana.* **María Daniela Torres Rodríguez** y Felipe Cruz García. Departamento de Bioquímica. Facultad de Quimica, UNAM

B-144

In vitro evaluation of antioxidant activity of essential oil of *Satureja macrostema.* **Rafael Torres-Martínez**, Yolanda García-Rodríguez, Alejandra Hernández-García, Patricia Ríos-Chávez, Alfredo Saavedra-Molina, Joel Edmundo López-Meza, Alejandra Ochoa-Zarzosa and Rafael Salgado-Garciglia. IIQB, Universidad Michoacana

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Comparative analysis of the synthesis and accumulation of dhurrin in sorghum plants associated with mycorrhizal fungi under different water stress conditions. **Silvia Edith Valdés Rodríguez**, Magaly Perez Lara, Rosalinda Serratos Flores, Victor Olalde Portugal. CINVESTAV-Irapuato

B-146

Daily variations of liver 5-tryptophan hydroxylase in rats under daytime restricted feeding. **Marlen Valdés-Fuentes**, Isabel Méndez, Dalia de Ita-Pérez and Mauricio Díaz-Muñoz. Laboratorio de Fisiología Celular. Instituto de Neurobiología, Campus Juriquilla. UNAM

B-147

Low pH induces changes in the dynamic properties of Ig light chain variable domain 6aJL2, impacting both unfolding pathways and fibril formation. **Gilberto Valdés-García**, Cesar Millán-Pacheco, Nina Pastor. Facultad de Ciencias, UAEM

B-148
Transcriptomic analysis of two *Arabidopsis* mutants with altered expression of inorganic soluble pyrophosphatase isoforms under phosphate starvation. **Lilián Gabriela Valencia-Turcotte**, Yahir Jaramillo-Díaz, Yazmin Martínez-Casales, Miguel Carranza-Brito, Carlos Páez-Franco y Rogelio Rodríguez-Sotres. Facultad de Química, UNAM

B-149
Analysis of storage proteins in native maizes of Oaxaca by electrophoresis. **Carlos Francisco Varapizuela Sánchez**, Marco Sánchez-Medina, Socorro Pina-Canseco, Alma Dolores Pérez-Santiago. Instituto Tecnológico de Oaxaca

B-150
Thermostability of *Phaseolus lunatus* Lectin. **Miguel Ángel Vásquez Calleja**, Lucia Francely Flores Carlos, Mario Valera Zaragoza, Yadira Gochi Ponce, Alma Dolores Pérez Santiago. Instituto Tecnológico de Oaxaca

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Dominant Negative Phenotype of *Bacillus thuringiensis* Cry1Ab, Cry11Aa and Cry4Ba Mutants Suggest Hetero-Oligomer Formation among Different Cry Toxins. **Daniela Carmona**, Claudia Rodríguez-Almaza, Carlos Muñoz-Garay, Leivi Portugal, Claudia Pérez, Ruud A de Maagd, Petra Bakker, Mario Soberón, Alejandra Bravo. Instituto de Biotecnología, UNAM

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BT-84
Quantification of genetically modified MON810 maize (Zea mays L.) leaves by qPCR and Dpcr. **Blanca Gómez C**, Lizbeth Gutiérrez A, Luis Castillo D, Felipe Arguijo P, Abraham Acatzi S. CNRDOGM-SENASICA

BT-85
Identification of microRNAs responsive to chronic drought, during three growth stages of *Brachypodium distachyon* Bd21. **Amado Ortiz-Yescas**, Mariana E. Cesario-Solis, Julián M. Peña-Castro, Blanca E. Barrera-Figueroa. Instituto de Biotecnología, Universidad del Papaloapan

BT-86
Antimicrobial peptide expresion in *E. Coli*. **Gina Pacheco Arredondo**, Herminia Loza Tavera, Carlos Regalado González, Romina Rodríguez Sanoja. Instituto de Investigaciones Biomédicas, UNAM

BT-87
The overexpression of the *Amaranthus hypochondriacus NFY-C* gene modifies the growth rate and confers abiotic stress resistence in Arabidopsis. **Paola Andrea Palmeros-Suárez** y John Paul Délano-Frier. CINVESTAV – IPN Unidad Irapuato

BT-88
AtNTC, a novel arabidopsis gene involved in development and stress responses. **David Paz Cabrera,** Nadia Celene Rodríguez Felipe, Aida Araceli Rodríguez-Hernández, Maria Azucena Ortega-Amaro, Juan Francisco Jiménez-Bremont, Margarita Rodríguez-Kessler. Facultad de Ciencias, UASLP

BT-89
Isolation and characterization of microorganisms with tolerance to toxic concentrations of lead and zinc, and their effect on the growth of *Arabidopsis thaliana* under conditions of lead toxicity. **Daniel Alejandro Perea de Ávila**, Víctor Emmanuel Balderas Hernández. Universidad Autónoma de Zacatecas

BT-90
A new transformation vector to increase the accumulation of trehalose in *Nicotiana tabacum* transgenic plants. **Martha Berenice Pérez Mendoza**, José Oscar Mascorro-Gallardo, José Augusto Ramírez Trujillo, Ramón Suárez Rodríguez. Centro de Investigación en Biotecnología. UAEM

BT-91

Characterization of protein extracts from two strains able to hydrolyze methyl parathion. **Elida Carolina Popoca-Ursino**, Maikel Gilberto Fernández-López, Ma. Laura Ortiz-Hernández. Biotechnology Research Center, Autonomous University of the State of Morelos

BT-92

Role of potassium uptake systems in *Azospirillum brasilense* Sp245 osmo adaptation. **Indalecio Prieto-Sánchez**, Ramón Suárez Rodríguez, José Augusto Ramírez-Trujillo. Centro de Investigación en Biotecnología. UAEM

BT-93

Antimicrobial and Biochemical Characterization of Actinomycetes Antagonists of Phytopathogenic Microorganisms Isolated from Soils of Michoacán. **Evangelina Quiñones-Aguilar**, Jesús Trinidad-Cruz, Gabriel Rincón-Enríquez, Zahaed Evangelista-Martínez, Joaquín Qui-Zapata. Línea de Biotecnología Vegetal. CIATEJ

BT-94

Different speract gradients attract *Strongylocentrotus purpuratus* spermatozoa. **Héctor Vicente Ramírez Gómez**, Vilma Jiménez Sabinina, Adán Oswaldo Guerrero Cárdenas y Alberto Darszon Israel. Consorcio de la fisiología del espermatozoide. Departamento de Genética del Desarrollo y Fisiología Molecular. Instituto de Biotecnología, UNAM

BT-95

I*n Vivo* assessment of probiotic characteristics indigenous lactic acid bacteria. **Alejandra Ramírez Torres**, Lucía María Cristina Ventura Canseco, Dolores Guadalupe Vidal López, Miguel Abud Archila, Sandy Luz Ovando Chacón. Instituto Tecnológico de Tuxtla-Gutiérrez

BT-96

Electrophoretics patterns of pregerminatedmaize and field performance. **Juan Carlos Raya-Pérez,** Fco. Chablé Moreno, Cesar L Aguirre-Mancilla, J Gabriel Ramírez-Pimentel, Jorge Covarrubias-Prieto, Gpe. García-Rodríguez. Instituto Tecnológico de Roque

BT-97

The ISC Genetic System Involved in the Virulence of Pathogenic Bacteria of Plant. **Gabriel Rincón-Enríquez**, Diego Eloyr Navarro-López, Julio Cesar Juarez-Garcia, Evangelina Esmeralda Quiñones-Aguilar, Joaquin Alejandro Qui-Zapata. Línea de Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C.

BT-98

Biochemical and immunological studies of mannitol dehydrogenase in strain YR-1 of *Mucor circinelloides* isolated from petroleum contaminated soil. **Marlene Gabriela Rivas Paulin,** Roberto Zazueta-Sandoval. División de ciencias Naturales y Exactas. Universidad de Guanajuato.

BT-99

Purification and characterization of antimicrobial peptides derived from the venom of *Scolopendra polymorpha*. **Carmen Itzamatul Rodríguez Alejandro**, Lucero Valladares Cisneros y María del Carmen Gutiérrez Villafuerte. Centro de Investigación en Biotecnología, UAEM

BT-100

Cloning and bioinformatic and biochemical characterization of a novel glycosyl hydrolase family 10 of *Bjerkandera adusta*. **María del Rocío Rodríguez Hernández**, Ramón Alberto Batista García, Claudia Martínez Anaya, Jorge Luis Folch Mallol. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos.

BT-101

Viability and germination of seeds of *Swietenia macrophylla King* mediated by tetrazolium staining. **Juan Rodríguez Santiago,** E Hernández-Domínguez, Alejandro G Nila-Méndez, F Gabino. Unidad de Investigación en Biotecnología Vegetal, Instituto Tecnológico Superior de Acayucan

BT-102

Design and biological comparison between alpha helical and beta-defensin antimicrobial peptides. **Alexis Joavany Rodríguez Solís**, Elba Cristina Villegas Villarreal, Gerardo A. Corzo Burguete Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

BT-103

Exposure Effects of toxic dinoflagellates on gene expressions related to cell cycle regulation in *Crassostrea gigas*. **Reyna Romero-Geraldo**, Norma García-Lagunasand Norma Yolanda Hernández-Saavedra. Centro de Investigaciones Biológicas del Noroeste

BT-104

Changes in polyamine profile of *Arabidopsis* in the interaction with *Trichoderma atroviride*. **Fatima Berenice Salazar Badillo**, Diana Sánchez Rangel, Artemio Mendoza Mendoza, Alicia Becerra Floraand Juan Francisco Jiménez Bremont. Department of Molecular Biology, The Institute for Scientific and Technological Research of San Luis Potosi, IPICYT

BT-105

Evaluation of antibiotics in milk content of the main producing areas of Chiapas, Mexico. **Patricia G Sánchez Iturbe**, Pedro T Ortiz y Ojeda, Pedro Alfonso Gpe Ortiz S. Instituto Tecnológico de Tuxtla Gutiérrez

BT-106

Novel thioesterase for degradation of toxic aromatic compounds. Functional and structural implications. **Ayixon Sánchez Reyes**, Ramón Batista García and Jorge Luis Folch Mallol. Facultad de Ciencias. UAEM

BT-107

Evaluation of antiviral and toxic effect of secondary metabolites present in fractions and methanol extracts of *Rhoeo discolor* (L'HerHance) and *Callisia fragrans* (L. Woodson) on influenza virus (H1N1). **Yazmin Sánchez Roque**, Reiner Rincón Rosales, Rocío Meza Gordillo, Víctor Manuel Ruíz Valdiviezo, Teresa del Rosario Ayora Talavera, Guadalupe Ayora Talavera. Instituto Tecnológico de Tuxtla-Gutiérrez

BT-108

Zn2+ preconditioning triggers mechanisms that increase the cadmium accumulation capacity in photosynthetic *Euglena gracilis*. **Rosina Sánchez-Thomas** and Dr. Jorge Donato García-García. Cardiology National Institute "Ignacio Chavez"

BT-109

Micropropagation and induction of organogenesis *in vitro* of *Heliocarpus appendiculatus* Turcz and *Trema micrantha* L. Blume. **Nathaly del C Sánchez Villegas**, Ángel Sol Sánchez, Roberto de la Rosa Santamaría, Obdulia Baltazar Bernal, Elizabeta Hernández Domínguez, Francisco Javier Gabin Román. Colegio de Postgraduados, Campus Tabasco

BT-110

A novel model for the study of heavy metal stress in plants. **Lenin Sánchez-Calderón**, Ricardo Ortiz-Luevano, Leonardo Castanedo-Ibarra,Renato Rivera-Menchaca, Miguel Alvarado Rodriguez,Claudia González-Salvatierra,Ramírez-Pimmentel JG. Doctorado en Ciencias Básicas, Universidad Autónoma de Zacatecas

BT-111

Isolation and characterization of a thermostable lipase from a cDNA library of the white rot fungus *Bjerkandera adusta*. **María del Rayo Sánchez-Carbente**, Catalina Morales, Senorina Castañeda Gómez and Jorge Luis Folch Mallol. Laboratorio de Biología Molecular de Hongos, Centro de Investigación en Biotecnología, UAEM

BT-112

qPCR to assess the robustness of a manufacturing process to remove adventitious viruses from biopharmaceuticals. **Adán Sánchez-Ponce**, Alejandro Canales-Aguirre, Ana Laura Márquez-Aguirre. Biotecnología Médica y Farmacéutica. CIATEJ

BT-113
How the intensity of an electric field can modify the physiology of *Aspergillus niger* growing in solid state culture? **Victor Sánchez-Vazquez,** Marco Reza-Valdés & Mariano Gutiérrez-Rojas. Departamento de Biotecnología. Universidad Autónoma Metropolitana-Iztapalapa

BT-114
Timely detection of Mycobacterium using DNA microarrays. **José Luis Santillán Torres**, Lorena Chávez González, Simón Guzmán León, Jorge Ramírez Salcedo. Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, UNAM

BT-115
Establishment of *in vitro* shoot and root cultures of *Argemone mexicana L.* **José Germán Serrano-Gamboa,** Miriam Monforte-González y Felipe A. Vázquez-Flota, UBBMP, CICY

BT-116
Enzymatic synthesis of polyphenylene disulfide-like compounds from aromatic dithiols. **Estefanía Sierra Ibarra**, Marcela Ayala Aceves. Instituto de Biotecnología - UNAM

BT-117
Avocado snakin (*PaSn*)cDNA cloning into pCAMBIA-*PaSn* vector for enhancing fungal resistance in strawberry plants using *Agrobacterium* mediated transformation. **Sarai Suárez García,** Luis María Suárez Rodríguez, Alejandra Hernández García, Joel Edmundo López Meza, Alejandra Ochoa Zarzosa, Rodolfo López Gómez y Rafael Salgado Garciglia. IIQB. Universidad Michoacana

BT-118
Assessment of cross-reactivity and neutralizing capacity of single chain antibodies directed against Mexican scorpion venoms. **Selene Jocelyn Uribe Romero**, Timoteo Olamendi Portugal, Baltazar Becerril, Lidia Riaño Umbarila. Instituto de Biotecnología, UNAM

BT-119
Bifunctional gene for trehalose synthesis induces changes in photosynthesis in wheat plants under salinity. **Elisa M Valenzuela-Soto**, Fabiola A Cabral-Torres, Ciria G Figueroa-Soto, Juan Pablo Valenzuela-Avendaño. Centro de Investigación en Alimentación y Desarrollo AC

BT-120
Purification and identification of peptides with antimicrobial activity present in the venom of *Scolopendra viridis*. **Lucero Valladares Cisneros** y María del Carmen Gutiérrez Villafuerte. Centro de Investigación en Biotecnología, UAEM

BT-121
Heterologous expression of intrepicalcin, isolated from the venom of scorpion *Vaejovis intrepidus*. **Leonel Vargas Jaimes,** Verónica Quintero-Hernández, Hector H Valdivia y Lourival D Possani. Facultad de Ciencias, UAEM

BT-122
Study of genes involved in the stress response in *Saccharomyces cerevisiae* and their effect during Agave juice fermentation. **Naurú Idalia Vargas Maya**, Gloria Angélica González Hernández, Araceli López Andrade, Adriana García Tapia and Juan Carlos Torres Guzmán. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

BT-123
Fungal endophytes of *taxus globosa* schltdl., potential producers of secondary metabolites for biological control of *Phytophthora capsici* AND *Pythium* sp., plant pathogens of major agricultural crops. **Romalda Vásquez Gutiérrez,** Felipe de Jesús Palma Cruz, Lucia Martínez Martínez, Claudia López Sánchez. Instituto Tecnológico de Oaxaca

BT-124
Growth kinetics and effect of pH on the emulsifying activity of the bioemulsifier produced by *Acinetobacter bouvetii* using hexadecane as organic phase. **José Luis Vázquez Vázquez**, Gabriela Jaqueline Rojas Castillo, Mariano Gutiérrez Rojas. UAM-I

BT-125
Overproduce of the leucine aminopeptidase yspII of *Schizosaccharomyces pombe* in the *Pichia pastoris* yeast methylotrophic. **José Luis Cerriteño-Sánchez**, Gerardo Santos-López, Nora Hilda Rosas-Murrieta, Irma Herrera-Camacho. ICUAP-BUAP

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OR-18
ROS generation in cell transformation induced by metal mixture and OLA1 expression. **Elia Martínez-Baeza**, Emilio Rojas-del Castillo and Mahara Valverde-Ramírez. Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, UNAM

OR-19
Selection of a thermotolerant yeast and evaluation of the oxidative stress and antioxidant response. **Jorge Arturo Mejía-Barajas**, Melchor Arellano-Plaza, Salvador Manzo-Avalos, Rafael Salgado-Garciglia, Christian Cortés-Rojo, Mónica Clemente-Guerrero, Rocío Montoya-Pérez, Alfredo Saavedra-Molina. IIQB. Universidad Michoacana de San Nicolás de Hidalgo

OR-20
The hormone prolactin is a novel survival factor for the retinal pigment epithelium. **Rodrigo Meléndez García**, David Arredondo Zamarripa, Norman Adán, Edith Arnold, German Baeza Cruz, Carmen Clapp, and Stéphanie Thebault. Instituto de Neurobiología, Campus UNAM-Juriquilla

OR-21
Mitochondrial DNA oxidation and OGG expression change (8-oxoguanine DNA glycosilase) during heart failure development. **Luisa I Orta Gaytan**, Jorge E Vela, Irais Rivera and Noemí García. Escuela de Medicina, Tecnológico de Monterrey

OR-22
Does Bcl-3 activate p50 during oxidative conditioning hormesis response? **Gibran Pedraza-Vázquez**, José Luis Ventura-Gallegos, Roberto Lazzarini-Lechuga, Luis Enrique Gómez-Quiroz, Mina KonigsbeGR-Fainstein, Armando Luna-López. Instituto Nacional de Geriatría. UAM-I

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Dynamics of the intracellular H_2O_2 levels in the root of *Arabidopsis thaliana* in response to a specific NADPH inhibitor. Rocío del Carmen Pérez, Jesús S. López Bucio, Alejandra Hernández-Barrera, Rosana Sánchez**, Eric Johnson,** Hen-ming Wu, Alice Cheung, Federico Sánchez, Carmen Quinto and Luis Cárdenas. Instituto de Biotecnología, UNAM

OR-24
Evaluación de la producción de especies reactivas del oxígeno en mitocondrias aisladas de hepatocitos de conejos con una dieta moderada en grasa. **Mario Alejandro Pérez-Medina**, C Marissa Calderón-Torres, Santiago C Sigrist Flores, J Rafael Jiménez Flores. Morfofisiología, FES-Iztacala, UNAM

OR-25
Hepatic injury response against a pro-inflammatory stress in rats from different ages. **Posadas Rodríguez Pedro**, Pedraza Vázquez Gibran,Bello Monroy Oscar, Konigsberg Fainstein Mina, Gómez-Quiroz Luis Enrique, Luna López Armando. Investigación Básica. Instituto Nacional de Geriatría. UAM-I

OR-26
ROS detoxification analysis in the non-conventional yeast *Debaryomyces hansenii*. **Mónica Ramírez-Hernández**, Viviana Escobar-Sánchez, Angeles Cancino-Rodezno, Víctor Valdés-López, Luisa Alba-Lois and Claudia Segal-Kischinevzky. Facultad de Ciencias, UNAM

OR-27
Tetraspanin PLS-1 in the regulation of NADPH oxidases in *Neurospora crassa.* **Angélica Mariana Robledo-Briones**, María del Sol Hernández-Galván, Juan Carlos Santos-Rodríguez and Jesús Aguirre. Instituto de Fisiología Celular, UNAM

OR-28
Oxidative stress by nicotine in rat gingiva. **Yadira Romero Serrano**, Diana Barrera Oviedo. Farmacología, Facultad de Medicina, UNAM

OR-29
Dynamic of reactive oxygen species in root hair cells and pollen tubes are essential for polar growth. **Ana-María Velarde-Buendía**, Alejandra Hernández-Barrera, Karina Jiménez Durán, Rosana Sánchez, Eric Johnson, Federico Sánchez, Carmen Quinto, Hen-ming Wu, Alice Cheung and Luis Cárdenas. Instituto de Biotecnología, UNAM

OR-30
The release of cytochrome C is altered in the liver of rats with metabolic syndrome: role of cardiolipin. **Angélica Ruiz-Ramírez**, Miguel A Barrios Maya and Mohammed El Hafidi Bentlakder. Cardiovascular Biomedicine, National Institute of Cardiology Ignacio Chávez

OR-31
Participation of Fe-S Proteins Aco1p, Lip5p, Sdh2p and Rip1p in Ethanol toxicity in *Saccharomyces cerevisiae*. **Luis Alberto Sánchez Briones**, Rocío Pérez-Gallardo, Mauricio Gómez Gallardo, Alma Díaz-Pérez, Christian Cortes-Rojo and Jesús Campos-García. IIQB, Universidad Michoacana

OR-32
Oxidative DNA damage and antioxidant enzyme activity in two vampire bats species *(Desmodusrotundus y Diphyllaecaudata)*. **Toledo Pérez Rafael,** Carlos Cantellano De Gante, Conde-Pérezprina Juan Cristóbal, Armando Luna López, Miguel Ángel León Galván, Mina Konigsberg Fainstein. Departamento de Ciencias Básicas y de la Salud. UAM-I

OR-33
Oxidative stress in liver and brain in rats fed with a diet supplemented with 30% sucrose. **Héctor Valente-Godínez**, Olivia Vázquez-Martínez, Mildred Chagoya Sánchez, Isabel Méndez, Mauricio Díaz-Muñoz y Vania Carmona-Alcocer. Instituto de Neurobiología, UNAM

GENETICS

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Association of variants in *NR3C* and *SLC6A4* genes and Major Depression in Mexican Mestizos. **Luz Berenice López-Hernández**, Omar Medrano-Espinosa, Cristina Rodríguez-Hernández, Martha Georgina Ochoa-Madrigal, Agustín Coronel-Pérez, Guillermina Ávila-Ramírez, Javier Cano-Martínez and Silvia García. CMN 20 de Noviembre, ISSSTE

G-25
Pax8 produces aberrant transcripts in cervical tumors and derived cell lines. **Eduardo López-Urrutia,** Cesar Cortés-González, Veronica García-Castillo, Osvaldo Bautista-Isidro Abraham Pedroza-Torres Verónica Fragoso-Ontiveros Jaime Coronel and Carlos Pérez-Plasencia. Unidad de Biomedicina. FES Iztacala-UNAM

G-26
Association between the BDNF val66met polymorphism and BMI in Mexican children. **José Darío Martínez-Ezquerro**, Mario Enrique Rendón-Macías, Gerardo Zamora, Jacobo Serrano-Meneses, Yessica Arellanos, Beatriz Rosales-Rodríguez, Deyanira Escalante-Bautista, Raúl Sánchez González, Maricela Rodríguez Cruz, Mardia López Alarcón, Elith Yazmin Valencia Villalvazo, Cecilia Zampedri, and Haydeé Rosas-Vargas. Centro Médico Nacional Siglo XXI, IMSS

G-27
Association of the R577X variant in the ACTN3 gene with obesity in mexican mestizos. **Luis Ángel Montes-Almanza**, Froylan Arturo García-Martínez, Luz Berenice López-Hernández, Mirza Romero-Valdovinos, Angélica Alfosina Olivo-Díaz, José Gilberto Franco-Sánchez, Andrea Pegueros-Pérez, Ariadna Del Villar-Morales, Benjamín Gómez-Díaz. FES Zaragoza UNAM

G-28

Molecular identification and phylogenetic analysis of mosses of semiarid area from Durango. **Benjamin Nava Reyes**, Jorge Sáenz Mata, Jaime Sánchez Salas, Gisela Muro Pérez, Verónica Ávila Rodríguez, Jorge Arturo Alba Ávila. Biología Molecular. Universidad Juárez del Estado de Durango

G-29

An altered hydrotropic response (*ahr2*) mutant of Arabidopsis is tolerant to drought stress. **Laura Noriega Calixto**, María Eugenia Campos Torres, Delfeena Eapen and Gladys I Cassab López. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

G-30

Generation of membrane transporters´s deletion mutants of *Ustilago maydis* by the *BsaI* cloning system. **Dario Rafael Olicón Hernández**, Michael Feldbrügge, Silke Jankowski, Ana Niurka Hernández Lauzardo, Guadalupe Guerra Sánchez. ENCB-IPN

G-31

Gallibacterium anatis as model organism to study the CRISPR/Cas system in *Pasteurellaceae*. **María de Lourdes Pérez Hernández**, Candelario Vázquez-Cruz, Erasmo Negrete Abascal, Patricia Sánchez Alonso. Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla

G-32

C. glabrata strains that express both types of mating information are more sensitive to oxidative stress. **Karina Asyade Robledo Márquez**, Irene Castaño Navarro. IPICyT

G-33

Study of *KCNJ11* and *ABCC8* alelic variants from K_{ATP} channels in type 2 diabetic patients treated with sulphonylureas, biguanides or both. **Nidia Samara Rodríguez-Rivera**, María de los Ángeles Granados Silvestre, Marta A. Menjívar Iraheta, María Guadalupe, Ortiz López, Juan A. Molina Guarneros. Laboratorio de Inmunofarmacología, Farmacología, Facultad de Medicina, UNAM

G-34

Functional analysis of *pyr*4 gene encoding orotidine-5'-monophosphate decarboxylase and its usefulness as a selectable marker in *Trichodermaatroviride*. **Erick Rojas Espinosa,** Fidel Landeros Jaime, José Antonio Cervantes Chávez, Edgardo Ulises Esquivel Naranjo. Universidad Autónoma de Querétaro.

G-35

Escargot gene involvement in the establishment of gustatory neurons that respond to volatilized nicotine in *Drosophila melanogaster*. **Fernando Rosales Bravo**, Enrique Reynaud Garza, Verónica Narváez Padilla. Morelos State Autonomous University

G-36

Analysis of serotonin 2A receptor gene in depression associated to Parkinson's disease in Mexican population. **Elizabeth Ruiz Sánchez**, Elisa Alonso Vilatela, Petra Yescas Gómez, Hayde Noemy León Echeverria, Yéssica Myr Alcántara Flores, Diana Alvarado Crespo, Mayela Rodríguez Violante, Ángel Alberto Ruiz Chow, Pedro Montes del Carmen, Amin Cervantes Arriega y Patricia Rojas Castañeda. Neurotoxicología. Instituto Nacional de Neurología y Neurocirugía

G-37

Expression and subcellular distribution of β-dystroglycan in prostate cancer. **Ariana María Sandoval Duarte,** Juan de Dios Gómez López, Bulmaro Cisneros Vega. CINVESTAV-IPN

G-38

ERCC1 genotypes in Mexican lung cancer patients. **Cuauhtémoc Sandoval, Daniel A**. Medoza Posada, Arnoldo Aquino-Galvez, Bettina Sommer Cervantes, Georgina González-Avila. Instituto Nacional de Enfermedades Respiratorias "Ismael Cosio Villegas"

G-39

Recopilación de Encuestas y Muestras Biológicas Humanas de Estudiantes y Trabajadores del Campus Chontalpa de la Universidad Juárez Autónoma de Tabasco (UJAT), como primera etapa para identificar la asociación entre la infección por Dengue y el genotipo del Tabasqueño. **Karla Alejandra Silva Pérez**, Miguel Ricardez Santiago, Hernán Emiliano Ramírez Gamas, Valeria Guadalupe Balan Basto, Moisés

Renato Carmona Guzmán, Melquisedec S. Esquivel Sentíes. Universidad Juárez Autónoma de Tabasco, Campus Chontalpa

G-40

Establishment of endogenous control genes in *Sporothrix schenckii* for data normalization in Real-Time PCR. **José Elias Trujillo Esquivel**, José Asunción Martínez Álvarez, Patricia Ponce Noyola and Héctor Manuel Mora Montes. Biología, DCNyE. Universidad de Guanajuato

G-41

Functional characterization of three mannosyltransferases of *Saccharomyces cerevisiae* by complementation in *Candida albicans*. **Nahúm Valente Hernández,** and Héctor M Mora-Montes. Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato

G-42

A DNA damage dependent mechanism regulates the return of *Bacillus subtilis* spores to vegetative growth. **Luz Idalia Valenzuela-García**, Susana Campos-Castillo and Mario Pedraza Reyes. Department of Biology. University of Guanajuato

G-43

Evaluating the functionality of Gpn1carboxy-terminal domain and nuclear export signal in *Saccharomyces cerevisiae*. **Guerrero-Serrano Gehenna**, De las Peñas Alejandro, Castaño Irene, Calera Mónica R, Sánchez-Olea Roberto. Instituto de Física, UASLP

MEDICINE, HEALTH AND NUTRITION

M-48

Looking for new antimalarials. Virtual screening into the glycolytic enzyme phosphoglyceratemutase 1 from *Plasmodium falciparum*. **Lluvia Iveth Ríos-Soto**, Miriam Leticia Aguirre-Raudry, María Irene Betancourt-Conde, Alejandra Guadalupe Vazquez-Raygoza, Claudia Avitia-Domínguez, Mónica Andrea Valdez-Solana, Erick Sierra-Campos, Alfredo Téllez-Valencia. Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango

M-49

Regulation of mitochondrial permeability transition by Sirt3-catalyzed cyclophilin D deacetylation and its relevance for ventricular dysfunction in heart and isolated cardiomyocytes. **Sheryl Rizzo**, José A. Morales, Christian Silva-Platas, Elena C. González- Castillo, Luis Vega-Sevilla, Enrique Guerrero- Beltrán, Noemí García, and Gerardo García-Rivas. Escuela de Medicina. Tecnológico de Monterrey.

M-50

Hypothyroidism decreases the immunolocalization of Farnesoid receptor (FXR) inpancreatic isletand liver cells of rabbits. **Julia Rodríguez-Castelán**, Arturo Herrera-Flores, Francisco Castelán, Estela Cuevas. Maestría en Ciencias Biológicas, Universidad Autónoma de Tlaxcala

M-51

Cardiovascular risk factors in childhood influence the number of Colony-Forming Units generated *in vitro* by circulating endothelial progenitor cells in peripheral blood. **Rocío Rodríguez-Valentín**, Cidronio Albavera-Hernández, Alfonso Carreón-Rodríguez, Idanelli Barrios Jacobo, Eduardo Lazcano-Ponce, Eduardo Salazar-Martínez. Instituto Nacional de Salud Pública, Morelos

M-52

Preliminary analysis of pro-inflammatory and metabolic changes in school children with different proportions of body fat. **Cecilia Rosel Pech**, Ernestina Polo Oteyza, Mónica Ancira Moreno, Noemi Meraz Cruz, Yunuén Pruneda Padilla, Marcela Vela Amieva, Isabel Ibarra González, Felipe Vadillo-Ortega. Posgrado en Ciencias Biológicas UNAM

M-53

The physiological role of the T-type Calcium Channels in cell lines bone cancer (Osteosarcoma). **Jacaranda Rosendo-Pineda**, Nidia Beltrán, Juan Carlos Gómora & Heriberto Manuel Rivera. Laboratorio de Tecnología de Proteínas de Membrana, Facultad de Medicina. UAEM

M-54

Improvement of transduction with Ad-GFP vector mediated by siRNA- IFN-α in hepatic cells. **Sobrevilla-Navarro AA**, Sandoval-Rodríguez AS, García-Bañuelos JJ, Hernández-Ortega LD, Armendáriz-Borunda J, and Salazar-Montes AM. Institute of Molecular Biology in Medicine and Gene Therapy CUCS, University of Guadalajara

M-55

Intramuscular gene therapy using MMP8 gene modify profibrogenic gene expression in experimental liver fibrosis. **Jesús García-Bañuelos**, Eden Oceguera-Contreras, Daniela Gordillo-Bastidas, Ana Sandoval-Rodríguez, Blanca Bastidas-Ramírez, José Macias-Barragan, Belinda Gomez-Meda, Juan Armendaríz-Borunda. Institute of Molecular Biology and Gene Therapy.

M-56

Antidiabetic drug design. Computational approaches for optimization of a new Protein Tyrosine Phosphatase 1B inhibitor. **Marie Jazmín Sarabia Sánchez**, Alicia Hernández Campos, Pedro Josué Trejo Soto, Artemisa Luévano de la Cruz, Claudia Avitia Domínguez, José Manuel Salas Pacheco, Alfredo Téllez Valencia. Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango

M-57

Sorcin interacts with the mitochondrial calcium uniporter and inhibits calcium transport in cardiac mitochondria. **Christian Silva-Platas** and Gerardo García-Rivas. Escuela de Medicina. Tecnológico de Monterrey

M-58

Differences in the expression O-glycosylated glycol proteins in Cervical Cancer Cell Lines with and without HPV detected by *Amaranthus leucocarpus* Lectin. **Yael Ruíz Pichardo**, Victoria Jiménez Castillo, Jesús Cruz Santiago, Yobana Pérez Cervera, Francisco Urrea, Carlos Josué Solórzano Mata. Facultad de Odontología, Universidad Autónoma Benito Juárez de Oaxaca

M-59

Antimalarial drug discovery. Molecular docking in the dimer interface of triosephosphate isomerase from *Plasmodium falciparum*. **Alfredo Téllez-Valencia,** Claudia Avitia-Domínguez, Marie Jazmín Sarabia-Sánchez, Artemisa Luévano-De la Cruz, Iván Alejandro Favela-Candia, Mónica Andrea Valdez-Solana, Erick Sierra-Campos. Medicina y Nutrición, Universidad Juárez del Estado de Durango

M-60

Effects of histone deacetylase inhibitors on the development of bleomycin-induced pulmonary fibrosis. **María Fernanda Toscano Márquez**, Iliana Herrera Fuentes, Miguel Angel Negreros Amaya, Marco Antonio Espina Ordóñez, José G Cisneros Lira. Biología Celular, Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas"

M-61

Biological Studies of Metforminium Decavanadate, $(H_2Met)_3(V_{10}O_{28})\cdot 8H_2O$. **Samuel Treviño Mora**, Victor Enrique Sarmiento Ortega, Violeta Aburto Luna, Alfonso Daniel Díaz Fonseca, Patricia Aguilar Alonso, Diana Moroni González, Enrique González Vergara, and Eduardo Miguel Brambila Colombres. Investigaciones Químico Clínicas. BUAP

M-62

Circadian study of metabotropic glutamate receptors type 5 in the liver. **Isaías Turrubiate**, Mauricio Díaz-Muñoz, Isabel Méndez. Instituto de Neurobiología, Campus Juriquilla, UNAM

M-63

Hypoglycemic effect of watercress (*Nasturtium officinale*) extracts on hyperglycemic rats. **Mercedes Victoria Urquiza Martínez**, Sergio Gutiérrez Castellanos, Víctor Manuel Farías Rodríguez, Héctor Urquiza Marín and Bertha Fenton Navarro. Laboratorio de Glicobiología, UMSNH

M-64
T cells glycosylation pathway regulated by STAT-6 in a model of STAT-6$^{-/-}$*knockout* mice. **Janette Arias Escobedo**, Cecilia García Olivares, Ana Cristina Olivas Bejarano, Anna Kristyna Franco Flores, Blanca Ortiz Quintero, Jaime Chávez Alderete, Ricardo Lascurain Ledesma, Edgar Zenteno Galindo, Francisco Javier Urrea Ramírez. Depto. de Bioquímica, Instituto Nacional de Enfermedades Respiratorias.

M-65
Isolation and Characterization of Aptamers for Human Papilloma Virus Proteins Using Superficial Plasmon Resonance. **Diana Gabriela Valencia Reséndiz,** María Luisa Benítez Hess, Luis Marat Álvarez Salas. CINVESTAV-IPN

M-66
Role of TMPRSS4 in idiopathic pulmonary fibrosis. **Ana Mayela Valero Jiménez**, José G Cisneros Lira, Remedios Ramírez Rangel, Miguel O Gaxiola Gaxiola, Annie Pardo Cemo, Moisés Selman Lama. Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas"

M-67
Maternal Separation and Post Weaning Social Isolation Differentially Affect Stress and Metabolic Vulnerability in Adult Rats. **Javier Vargas León, Mariana Junco** y Naima Lajud. Laboratorio de Neurobiología del Desarrollo, División de Neurociencias. Centro de Investigación Biomédica de Michoacán – Instituto Mexicano del Seguro Social.

M-68
Searching potential inhibitors of acetohydroxyacid synthase from methicillin resistant *Staphylococcus aureus* through virtual screening. **Alejandra Guadalupe Vazquez-Raygoza,** María Irene Betancourt-Conde, Claudia Isela Avitia-Domínguez, Jorge Arturo Cisneros-Martinez, Miriam Leticia Aguirre-Raudry, Lluvia Iveth Ríos-Soto, Alfredo Téllez-Valencia. Universidad Juárez del Estado de Durango

M-69
Comparación del efecto neuroprotector de la silimarina y la silibina en un modelo murino de la enfermedad de Parkinson. **Víctor J. Zaldívar-Machorro**, Jesús Pérez-H. Claudia Meza López y Olguín y Anahí Chavarría Krauser. Medicina Experimental, Facultad de Medicina, UNAM

M-70
Comparison of the hypoglycemic activity in two varieties of *Averrhoa carambola* tested in streptozotocin-induced type 2 diabetic rats. **Cynthia Guadalupe Temores Ramírez,** Juan Florencio Gómez Leyva, John Paul Délano Frier, Blanca C. Ramírez Hernández, Javier E. García de Alba Verduzco, Julia Zañudo Hernández. Departamento de Ecología, División de Ciencias Biológicas y Ambientales. CUCBA.

M-71
Determination of plasmid profiles to a collection of *Escherichia coli* strains isolated from urinary tract infection (UPEC). **Dayanira Martínez-Cruz,** Ygnacio Martínez-Laguna, Patricia Lozano Zarain, Margarita María de la Paz Arenas-Hernández. Facultad de Ciencias Químicas, BUAP

MICROBIOLOGY AND PARASITOLOGY

MP-46
Description of hydrocarbonoclastic activity and putative function of the genes *CYP52* of *Yarrowia lipolytica*. **Jossue Mizael Ortiz-Alvarez**, Griselda Ma. Chávez-Camarillo, María de Lourdes Villa-Tanaca, César Hugo Hernández-Rodríguez. Escuela Nacional de Ciencias Biológicas, IPN

MP-47
Isolation, characterization, and molecula ridentification of PGPR from halophilic grass *Distichliss picata* (L.) Poaceae. **Rubén Palacio Rodríguez**, Jorge Sáenz Mata, María Leslie Delgado García, Osvaldo García Saucedo, Jaime Sánchez Salas, Gisela Muro Pérez. Universidad Juárez del Estado de Durango

MP-48

Identification of receptor ryanodine (IP3) in *Entamoeba histolytica.* **Isela Pérez Galarza,** Andrés Monsalvo Villagómez, Diana Jiménez López, Guadalupe Salazar-Enríquez, Ana Laura Cano Martínez, Alejandra Arreola Martínez, Omar González Maldonado, Andrés Salas Casas. Universidad Politécnica de Pachuca

MP-49

Purification and kinetic analysis of cytosolic and mitochondrial Thioredoxin glutathione reductase extracted from *Taenia solium* cysticerci. **Agustin Plancarte Crespo**, Gabriela Nava Balderas. Depto. de Microbiología y Parasitología, Facultad de Medicina, UNAM

MP-50

Evaluation of the non-catalytic binding function of Ts26GST a glutathione transferase isoform of *Taenia solium*. **Agustín Plancarte**, José Rodrigo Romero, Gabriela Nava, Horacio Reyes-Vivas, Martha Hernández. Facultad de Medicina, UNAM

MP-51

Homologue sequences to Iron Regulatory Protein in *Giardia duodenalis* genome. **Laura Yuliana, Plata-Guzmán,** Liliana, Soto-Castro, Milagro de Jesús, Luque-Ramírez, Jeanett, Chávez-Ontiveros, Héctor Samuel, López-Moreno, Rossana, Arroyo, Cuauhtémoc, Reyes-Moreno, Jorge, Milán-Carrillo, José Antonio, Garzón-Tiznado, Claudia del Rosario, León-Sicairos. Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa

MP-52

Physiological and molecular analysis of *Sclerotium cepivorum* Berk mutant not forming sclerotia. **Sandra Elizabeth González Hernández,** Cesar Arturo Ojeda Gutiérrez, Edgar Reyes Navarrete, Alberto Flores Martínez, Patricia Ponce Noyola. Universidad de Guanajuato

MP-53

Identification of a casein kinase 2(TvCK2) in *Trichomonas vaginalis*. **Laura Itzel Quintas-Granados**, María Elizbeth Álvarez-Sánchez. Autonomous University of Mexico City (UACM)

MP-54

Searching for molecular targets using the *K1* toxin from *Saccharomyces cerevisiae*. **Reyes-González Daniela,** Molina Vera C, Álvarez Hidalgo B, Campos Guillen J, Caballero Pérez J& Carlos Saldaña. Microbiología Básica y Aplicada. Campus Aeropuerto. Universidad Autónoma de Querétaro

MP-55

LEA proteins are involved on desiccation resistance and other abiotic stresses in *Azotobacter vinelandii*. **Selma Julieta Rodríguez Salazar**, Guadalupe Espín Ocampo, Soledad Moreno. Instituto de Biotecnología UNAM

MP-56

Genomic analysis of cyclic-di-GMP-related genes and their functional in *Azospirillum brasilense* Sp245 strain. **Araceli Romero-Pérez**, Alberto Ramírez-Mata, Adriana Gamboa-Pérez, José J. Aguilar-Piedras, M Luisa Xiqui-Vázquez, and Beatriz E Baca. Instituto de Ciencias. BUAP

MP-57

Analysis of *Avibacterium paragallinarum* operon related to quorum sensing and virulence. Esmeralda Rugerio López, Patricia Sánchez Alonso, Erasmo Negrete Abascal, Guillermo Horta Valerdi, Candelario Vázquez Cruz. Instituto de Ciencias, BUAP

MP-58

A SPCA1 in the Golgi apparatus of *Entamoeba histolytica.* Mario Alberto Rodríguez Rodríguez, Aarón Martínez Higuera, Hilda Lizbeth Pérez Herrera, Diana Jiménez-López, **Andrés Salas Casas**. Instituto de Ciencias de la salud. Universidad Autónoma del Estado de Hidalgo

MP-59

Genotypification of subtypes *Streptococcus mutans* of producing cavity in Querétaro, México. **Saldaña C,** Campos Guillen J, Camacho-Calderón N, Adame-Camarena G, Tavares De La Cruz A, & Morales-Tlalpan V. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro

MP-60

Analysis of the effect of benznidazole on the antioxidant metabolism of *Trypanosoma cruzi*. **Aketzalli Silva Carmona**, Zabdi González, Chávez Emma Saavedra. Instituto Nacional de Cardiología "Ignacio Chávez

MP-61

Comparative study of the secretable proteolytic activity higher than 160kDa in three groups of *Escherichia coli.* **Marycruz Trujillo Murillo**, Patricia Sánchez Alonso, Erasmo Negrete Abascal, Salustio Nájera Hernández, Esmeralda Escobar Muciño, Candelario Vázquez Cruz. Centro de Investigaciones en Ciencias Microbiológicas. Instituto de Ciencias. BUAP

MP-62

Homologue sequences to Iron Regulatory Protein in *Entamoeba histolytic a* genome. **Leticia Michelli Valle-García,** Liliana Soto-Castro, Laura Yuliana Plata-Guzmán, José G Acuña-Ochoa, José Antonio Cruz-Cárdenas, Jesús Christian Grimaldi-Olivas, Vianca Lizeth Ibarra-García, Mario León-Barraza, Jesús Rodrigo Payán-Benitez, Rossana Arroyo, José Antonio, Garzón-Tiznado, Claudia del Rosario León-Sicairos. Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa

MP-63

Assessment of anticoccidial activity on *Eimeria sp.* oocysts from the yeast *Meyerozyma guilliermondii* isolated from chickens. **Jorge A. Valle-Hernández**, Rosa E Quiroz-Castañeda, Mayra . Cobaxin-Cárdenas, Ma. Laura Ortiz-Hernández, Raunel Tinoco-Valencia, Leobardo Serrano-Carreón, Edgar Dantán-González. Centro de Investigación en Biotecnología, UAEM

MP-64

Spo0M and its role in the formation of the septum, a new role for a regulator of sporulation of *Bacillus subtilis*. **Luz Adriana Vega Cabrera,** María Luisa Tabche, José Luis Rodríguez, Enrique Merino, Liliana Pardo. Instituto de Biotecnología de la UNAM

MP-65

Transient disulfide reduction is required for EFF-1 mediated cell-cell fusion. **Jorge Verdín** and Benjamin Podbilewicz. Department of Biology, Technion - Israel Institute of Technology.

MP-66

In silico analyses of a transcription factor MTF1 in *Trichomonas vaginalis.* **José Luis Villalpando-Aguilar**, María Elizbeth Álvarez Sánchez. Autonomous University of Mexico

MP-67

Ammonia-oligotrophic and/or diazotrophic heavy metal-resistant *Serratia* spp. Isolated from pioneer plants and main tailings from Zacatecas. **Lily Xochilt Zelaya-Molina**, Zeltzin Magaña-García, César Hugo Hernández-Rodríguez. Microbiología, ENCB - IPN

MP-68

A Taenia crassiceps metacesto de factor enhances ovarian follicle atresia and oocyte degeneration in female mice. **Nadia Zepeda,** Sandra Solano, Natalia Copitin, Ana María Fernández, Patricia Tato, José Luis Molinari. Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

NEUROSCIENCE AND NEUROBIOLOGY

N-17

The administration of S-allyl cysteine reduces the neurological deficit and mortality in the middle cerebral artery occlusion model in the rat. **Luis Jardón-Aguillón**, Alma Ortiz-Plata, Perla D. Maldonado. Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suarez"

N-18

STIM1 and ORAI1 importance in Alzheimer's. **Diana Jiménez-López**, Mario Hernández Alejandro, Carlos Vázquez Calzada, Guadalupe Salazar Enríquez, Isela Pérez-Galarza, Andrés Monsalvo-Villagómez, Alejandra Arreola Martínez, Omar González Maldonado, Andrés Salas Casas, Ana Laura Cano Martínez. Ciencias Básicas e Ingeniería. Universidad Autónoma del Estado de Hidalgo

N-19

ALPHA $GABA_A$ receptor subunit is expressed in white matter astrocytes from the cerebellum. **Emmanuel Labrada Moncada**, Ataúlfo Martínez-Torres, Daniel Reyes-Haro. Neurobiología Celular y Molecular. Instituto de Neurobiología, UNAM. Campus Juriquilla

N-20

Searching for negative regulators of microglia activation: an alternative for Alzheimer's disease treatment? **Elisa Medrano Jiménez**, Jaime Totoriello García, Enrique Jiménez Ferrer, Alejandro ZamilpaAlvarez, Gustavo Pedraza and Leonor Pérez Martínez. Instituto de Biotecnología, UNAM

N-21

Effect on acetylcholine by ionic and oxidizing environments involved in Alzheimer's disease. **Armando Méndez-Garrido**, Maricarmen Hernández-Rodríguez, Martha Cecilia Rosales Hernández, Daniel Ramírez-Rosales. Instituto Politécnico Nacional

N-22

Role of *Nurr1* gene reduction and prenatal stress ondepression-likebehavior. **Pedro Montes del Carmen**, Elizabeth Ruiz Sánchez, Lourdes Paola Ruiz Flores, Patricia Rojas Castañeda. Laboratorio de Neurotoxicología, Instituto Nacional de Neurología y Neurocirugía

N-23

The ketone body β-hydroxy butyrate (BHB) reduces the production of reactive oxygen species and prevents neuronal death induced by glucose deprivation *in vivo* and *in vitro* models. **Teresa Montiel**, Alberto Julio-Amilpas, Eva Soto Tinoco, Cristian Gerónimo Olvera, Susana Flores and Lourdes Massieu. División de Neurociencias, Instituto de Fisiología Celular UNAM

N-24

Cytochrome P450 2J3 regulation in a LPS-induced model of neuroinflammation in astrocytes. **Navarro-Mabarak C**, Camacho-Carranza R, Espinosa-Aguirre JJ. Instituto de Investigaciones Biomédicas, UNAM

N-25

Neurological recovery in rats treated with a combination therapy after chronic spinal cord injury. **Roxana Haydee Rodríguez-Barrera**, Adrián Flores-Romero, Edna Elisa García-Vences, Liliana Blancas-Espinoza, Vinnitsa Buzoianu Anguiano, Ana María Fernández-Presas, Mina Konigsberg Fainstein, José Juan Antonio Ibarra Arias. Universidad Anáhuac México Norte

N-26

Differential expression of Dystrophins during murine brain development. **Griselda Rodríguez-Martínez**, José Romo-Yañez, Anayansi Molina-Hernández and Cecilia Montañez. CINVESTAV-IPN

N-27

Early life stress increases cytokine expression in the hippocampus and stimulates the release of cytokines in the circulation of ratpups. **Angélica Roque-Galicia**, Naima Lajud-Ávila, Alejandra Ochoa-Zarzosa, Luz Torner. Centro Multidisciplinario de Estudios en Biotecnología, FMVZ-UMSNH

N-28

Increased mitocondrial Complex IV activity by copper pre-treatment reduces MPP+-induced mitocondrial striatal damage. **Moisés Rubio-Osornio**, Marisol Orozco-Ibarra y Jorge Guevara Fonseca. Instituto Nacional de Neurología y Neurocirugía, Dr. Manuel Velasco Suárez

N-29

Epicatechin administration reduces circling behavior and dopamine depletion MPP+-inducted in rat. **Moisés Rubio-Osornio**, Sergio Montes López, Francisca Pérez-Severiano y Jorge Guevara Fonseca. Instituto Nacional de Neurología y Neurocirugía, Dr. Manuel Velasco Suárez

N-30

Alterated levels of histamine and H_1 receptor expression during central nervous system development in embryos from diabetic rats. **Solis González Karina**, De Nova Ocampo Mónica Ascención and Molina Hernández Anayansi. Instituto Nacional de Perinatología

N-31
Metabotropic glutamate receptors in the vestibular system of Gallus domesticus. **Ricardo Varela**, Eduardo Monjaraz, Alejandro Moyaho, Jorge Cebada and Amira Flores. Instituto de Fisiología, BUAP
N-32
Effects of sparteine on epileptiform activity induced by Pentylenetetrazole. **Fridha Viridiana Villalpando Vargas** y Laura Guadalupe Medina Ceja. Laboratorio de Neurofisiología y Neuroquímica, Departamento de Biología Celular y Molecular, CUCBA, U. de G.

GENETIC REGULATION & EPIGENETIC

GR-67
Function of TrxG during *Arabidopsis* root development. **Emilio Petrone-Mendoza**, Rosario Vega-León, Adriana Garay-Arroyo, Berenice García-Ponce de León, Elena Álvarez-Buylla and Ma. de la Paz Sánchez. Instituto de Ecología, UNAM
GR-68
Characterization of transcripcional regulation of human paraoxonase 1 in human hepatomacells. **Néstor Ponce-Ruiz**, Aurora Elizabeth Rojas-García, María de Lourdes Robledo-Marenco, Briscia Socorro Barrón-Vivanco, Yael Yvette Bernal-Hernández, Manuel Iván Girón Pérez, Irma Martha Medina-Díaz. Unidad Académica de Agricultura. Universidad Autónoma de Nayarit
GR-69
Regulation of *WRKY* genes in 35S::*AtGRDP1* lines under ABA treatments. **Jocelin Itzel Ramírez-Alonso**, Aída Araceli Rodríguez-Hernández, Carlos Vladimir Muro-Medina and Juan Francisco Jímenez-Bremont. División de Biología Molecular. IPICyT
GR-70
The expression of acidic ribosomal proteins in *Saccharomyces cerevisiae* during heat shock**. Juan Ismael Rea Hernández** and Samuel ZinkerRuzal. CINVESTAV-IPN
GR-71
Study of epigenetic changes in CCl_4 – induced cirrhosis modeland the hepatoprotective effect of IFC-305. **Jesús Rafael Rodríguez-Aguilera**, Carlos Alberto Guerrero-Hernández, Rosario Pérez-Molina R, Carla Elizabeth Cadena-del-Castillo, Lidia Martínez-Pérez, Félix Recillas-Targa and Victoria Chagoya de Sánchez. Instituto de Fisiología Celular, UNAM
GR-72
Functional analysis of Bdp1, a subunit of transcription factor TFIIIB, in *Leishmania major*. **Fiordaliso C Román-Carraro**, Luis E Florencio-Martínez, Rebeca Manning-Cela and Santiago Martínez-Calvillo. Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, UNAM
GR-73
Sucrose: sucrose 1-fructosyltransferase(1-SST) gene isolation and a method for quantifying gene expression. **Ismael de Jesús Román Guzmán**, Joaquin Alejandro Qui Zapata, Ernesto Tapia Camposy Patricia Dupré. CIATEJ
GR-74
Analysis of legume-miRNAs present in *Medicagotruncatula* in response to water deficit. **Paulette Sofía Romero Pérez**, Alejandra Alicia Covarrubias Robles, José Luis Reyes Taboada. Biología Molecular de Plantas, Instituto de Biotecnología, UNAM
GR-75
NF-kappaB inducing kinase (NIK) overexpression increases spheroid formation in breast cancer. **Mary JoseRozete Navarro**, Daniel Damián Hernández De Castilla, Karla Itzel Vázquez Santillán and Vilma Araceli Maldonado Lagunas. Instituto Nacional de Medicina Genómica

GR-76

Polymorphisms in ABCA1, HNF1α or PPARγ genes and their participation in dyslipidemias. **Kristel M Salgado Balderas**, Mayra J Álvarez Bahena, José Santos Ángeles Chimal, Jesús Santa Olalla Tapia. Facultad de Medicina. UAEM

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The response to injury of the filamentous fungus *Trichoderma atroviride* is regulated by small RNAs. **José Manuel Villalobos-Escobedo**, Nohemí Carreras-Villaseñor, Joel Rodríguez-Medina, Cei Abreu-Goodger and Alfredo Herrera-Estrella. LANGEBIO – CINVESTAV, IPN Irapuato

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“Méxıco, País de Asilo”

Fernando Serrano Migallón Facultad de Derecho, UNAM
Colegio de México

Cerebral Microcircuits

José Bargas. Instituto de Fisiología Celular. Universidad Nacional Autónoma de México. Mexico City, Mexico.

The cellular-molecular paradigm wants to understand how cells work: metabolism, signaling, genomics, proteomics, cell cycle, etc. A more systemic paradigm wants to understand how organs and organisms work: homeostasis, growth, adaptation, disease, etc. Network Theory has been applied to both paradigms in trying to understand complexity at the cellular and systemic levels. Here, we take an intermediate approach and try to understand how small modules of interconnected cells from the striatum interact by using Network Theory. One goal is to obtain quantitative parameters that could distinguish control from disease states in brain tissue. We used calcium imaging techniques to obtain multirecording of various neurons with single cell resolution (between 50 to 300 neurons). Our results show that cerebral microcircuits have the property of “Criticality”, that is, their connections are constantly changing having transitions between network states, the distribution of their functional connections could be fitted with a potency law: $P(K) = Ak^{-\gamma}$ with an average $\gamma \sim -1.6$. This exponent increased under pathological states suggesting a loss in criticality. In spite of criticality control circuits appeared robust with a mean path length < 2. Most connections were functional, that is, they were not monosynaptic but polysynaptic occupying an area of about 700 X 700 µm. These connections were based on physical synapses since blockers of synaptic receptors abolished network activity. Circuit topology showed that robustness was achieved by the activity of “hub” cells that interconnect different clusters of neurons over relatively long distances. Electrophysiological recordings showed that “hub” cells were commonly long axon interneurons. Given that connectivity was mostly functional we asked whether the striatal microcircuit depended on cortical connections. A section of cortical afferents totally abolished functional connectivity ($\gamma \sim -3$) leaving non-connected isolated clusters of neurons. Next, we evaluated the action of depleting the modulator dopamine (experimental Parkinsonism). The result was a loss of criticality ($\gamma \sim -3$), the reduction of clustering and the formation of a dominant group with few active “hubs”. We also evaluated clustering as a function of connections and demonstrated that microcircuit activity had a highly hierarchical architecture in control conditions. However, hierarchy is greatly lost in pathological states, for example, under experimentally L-DOPA induced dyskinesia. In conclusion, parameters yielded by Network analyses allow the possibility of quantitative tissue pathophysiology in the near future.

Fibrosis: Just a scar or an aberrant remodeling process?
The paradigm of Idiopathic Pulmonary Fibrosis

Annie Pardo

Pulmonary fibrosis is the final result of a large and heterogeneous number of diseases that affect the lung parenchyma. The most aggressive of them is idiopathic pulmonary fibrosis (IPF), a chronic, progressive and usually lethal lung disorder of unknown etiology. IPF is characterized by alveolar epithelial cell injury and activation, followed by migration/proliferation and activation of mesenchymal cells with the formation of fibroblasts/myofibroblasts foci, and finally by the exaggerated accumulation of extracellular matrix (ECM), mainly fibrillar collagens, with the subsequent destruction of the lung architecture.

It has long been proposed that matrix metalloproteinases (MMPs) play an important role in the pathogenesis of pulmonary fibrosis but the mechanisms are unclear. Since the disease is characterized by the exaggerated deposition of ECM proteins, during a long time it was considered that these enzymes should be down-regulated. Surprisingly however, the analysis of global expression pattern by oligonucleotide microarrays and immunohistological approaches showed that several MMPs (e.g. MMP7, MMP1, MMP2, and MMP19) are significantly increased in IPF lungs.

The family of MMPs is constituted by 23 different endopeptidases encoded by 24 genes including duplicated MMP-23 genes. The majority of MMPs are secreted enzymes, although some are expressed as cell surface enzymes and are known as membrane type MMPs (MT-MMPs). Additionally, recent studies have shown that certain MMPs, can also be found inside the cell and may act on intracellular proteins.

However, the role of these enzymes in the pathogenesis of IPF is uncertain. In part, the difficulty to identify MMP-associated mechanisms derives from their broad substrate specificity that include matrix proteins but also a number of non-ECM substrates that include chemokines, growth factors and others.

In this presentation I will focus on three MMPs: MMP7- MMP-1 and MMP-19 which play different and critical role in the aberrant fibrotic lung response.

The control of transcription during early development

Michael Eisen, Ph.D.
Investigator, Howard Hughes Medical Institute
Professor of Genetics, Genomics and Development
Department of Molecular and Cell Biology
University of California, Berkeley

The long-term goal of my lab's research is to understand how animal genomes encode spatial and temporal patterns of gene expression by applying integrated experimental and computational approaches to our primary model system, the early *Drosophila melanogaster* embryo. We are particularly interested in understanding how transcriptional enhancers work to regulate spatial patterns of transcription in the first few hours after fertilization. My talk will focus on recent studies from the lab aimed at elucidating why some sequences function as enhancers in the early embryo, and how enhancer activity is established. I will describe our discovery that the vast majority of early-acting enhancers contain a short, 7-basepair sequence that is bound by the maternally deposited transcription factor Zelda, and how our studies suggest that Zelda acts as a new kind of pioneer transcription factor whose role is to bind to sequences destined to become enhancers, and ensure they are accessible to be bound by patterning factors as development progresses.

“Dynamics of Endocytosis”

Tomas Kirchhausen
Boston Children´s Hospital

How Transcription Circuits Evolve and Produce Novelty

Alexander Johnson

Transcription circuits in cells are often viewed as optimized solutions, sculpted by the power of natural selection. But can this view account for the basic logic of transcription circuits? Why, for example, are some genes controlled positively, others negatively and some by both mechanisms? Why is cooperative binding of regulatory proteins to DNA so common? Why do many circuits seem bewilderingly complex? To rationalize the structure of modern circuits, we have been examining the evolutionary processes that produce them. We have reconstructed the evolutionary history of several transcriptional circuits across a large portion of the fungal lineage (the ascomycetes which include Saccharomyces cerevisiae and Candida albicans) nominally representing 300 million years of diversification. Although the DNA-binding specificity of transcription regulators are often preserved over these evolutionary times, the connections between regulators and the genes they control change rapidly. The ease of these wiring changes results from several basic features of transcription regulation, including regulatory protein modularity, cooperative binding, and the low information content of cis-regulatory sequences. Some wiring changes provide novel phenotypes, while others seem to preserve ancestral circuit output but alter the structure of the circuit through which that output is achieved. We found that, over evolutionary time scales, circuits can move through different wiring solutions without disrupting the output and that this process can lead to profound circuit diversification across species. We conclude that many aspects of modern transcription circuits are more easily explained through a knowledge of the evolutionary pathways of least resistance than through “first-principle” logic.

"Unfolded Protein Response in Health and Disease"

Peter Walter
Howard Hughes Medical Institute/
Biochemistry & Biophysics Dept
University of California, San Francisco

The unfolded protein response is an intracellular signaling pathway that adjusts the abundance and protein folding capacity of the endoplasmic reticulum according to need. The most conversed branch of the pathway mediated by the ER-resident transmembrane kinase/endoribonuclease Ire1, was first discovered in Saccharomyces cerevisiae. It mediates signal transduction via a non-conventional mRNA splicing mechanism that was since found conserved in all metazoan cells investigated. In response to accumulation of unfolded proteins in the ER, mRNA splicing results in the production of a transcription factor (Hac1 in yeast and XBP1 in metazoan cells) that up-regulates UPR targets genes, which in turn enhance protein folding in the ER to reestablish homeostasis. If homeostasis cannot be restored, cells commit to apoptosis. The UPR therefore makes life death decisions for the cell, which connects it to numerous human diseases, including inherited protein folding diseases, neurodegeneration, diabetes, and cancer. In addition to the highly specific endonucleolytic activity of Ire1 for HAC1/XBP1 mRNA, a more pleiotropic activity of Ire1, first discovered in Drosophila and then found in mammalian cells, initiates degradation of a set of ER-bound mRNAs, thereby reducing the load of proteins entering the ER lumen by a process termed RIDD for regulated Ire1-dependent mRNA decay. By contrast to other cells studied, Schizosaccharomyces pombe encodes no Hac1 homolog, displays no transcriptional upregulation of UPR target genes, and uses RIDD as the sole mechanism to balance protein folding capacity with protein folding load. A single mRNA encoding the major ER chaperone BiP escapes decay after Ire1 cleavage in its 3'UTR. Truncated BiP mRNA is stabile despite its lack of a poly-A tail and is actively translated. Mechanistic aspects and evolutionary considerations will be discussed.
Medical Institute

"Brucei-fying" triosephosphate isomerase from *Trypanosoma cruzi,* or vice versa.

Ruy Perez Montfort, Selma Díaz Mazariegos, Mónica Rodríguez Bolaños, Nallely Cabrera González, Marietta Tuena de Gómez Puyou and Armando Gómez Puyou.

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The human pathogens *Trypanosoma brucei* (Tb) and *T. cruzi* (Tc) have triosephosphate isomerases (TIMs), with sequences of 251 and 250 amino acids respectively, that are 73% identical between each other and have very similar biochemical and physicochemical properties. Yet, they also have important differences in their behavior in the presence of certain reagents that affect their physicochemical properties. Two of those differences are their susceptibility to inactivation by sulfhydryl reagents like methyl methane thiosulfonate (MMTS) and the reactivation after denaturation with guanidine hydrochloride (GdnHCl). Although TbTIM is susceptible to inactivation by MMTS, TcTIM is nearly 100 times more sensitive to this thiol reagent. And also, after being unfolded in the presence of GdnHCl, TbTIM reactivates to only 60 % of its initial activity after 40 minutes, but TcTIM recovers 90% of its original activity within the first 10 minutes. These very different behaviors of these two similar enzymes have intrigued us for many years, and we have been particularly interested in exploring which of the 65 amino acids, that are different in both sequences are responsible for these dissimilarities. After attempting multiple site directed mutagenesis experiments of one or more residues in the sequence, which we believed were involved in generating those differences in behavior, we consistently got either bad or inconclusive results. Thus, we designed a systematic method of converting the sequence of one enzyme into the other, that is: "brucei-fying" TcTIM, or vice versa. To approach this we took advantage of the "TIM-barrel" architecture of the three-dimensional structure of the protein dividing it into eight regions that roughly consist of one beta sheet, one loop and one alpha helix. Using gene synthesis technology and other methods of molecular biology we created chimeric TcTIMs that had an increasing number of regions of TbTIM. On these chimeras we tested the susceptibility to inactivation by MMTS or their behavior in reactivation after unfolding in the presence of GdnHCl. The results were unexpected and surprising, and we were able to identify different discrete combinations of regions of the enzyme as responsible for these different behaviors and physicochemical properties. Once the responsible regions were identified, the systematic "brucei-fying" of TcTIM, or vice versa, was developed further to create a method of "additive" site directed mutagenesis of the amino acids in those regions that still differed between both sequences. This led us to identify the amino acids that are responsible for the differences in susceptibility to inactivation by MMTS, or that are responsible for the different speed and the different extent of reactivation of TbTIM and TcTIM, after denaturation with GdnHCl.

Drug Discovery by Protein Structure Modeling. Proteins from Parasites as Target.

Arturo Rojo Domínguez.

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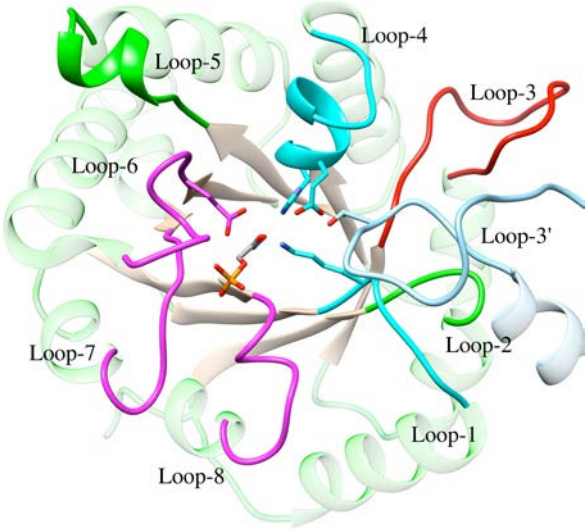
During the research life of a person or group, a gap sometimes develops between the concepts and data they collect, as a reflection of their interest in basic science, and its relevance to solve a specific problem in the short or medium term. In this talk it will be depicted how Dr. Armando Gómez-Puyou induced the bridge of this gap in the original research of several colleagues, to cross from the biochemical, thermodynamic and computational vision of macromolecules to face medical challenges. In particular, to encourage us to undertake those questions devoted to one of his passions, to face diseases suffered by underprivileged people by proposing new therapeutic treatments. In these cases, the investment recovering is frequently not guaranteed to pharmaceutical companies, so there is scarse or null private research, therefore he reminded us the social responsibility of public universities. Drug research comprises a large process of several steps, beginning in a first stage with target selection of an enzyme or biomolecule, automatic compound screening among a generally huge set of substances or chemical groups libraries, cautious selection of the best hits based in the peculiarities of the biological system, and *in vitro* tests of inhibitory activity of the selected substances, to verify their capacity or to discard them. The second stage comprises the so called ADMET, which stands for *in vivo* drug absorption and distribution in an organism, followed by its breakage by metabolism, excretion and tests of toxicity of the compound itself and its metabolized fragments. Drug discovery relates with the first stage, and some study cases will be presented in the search for inhibitors of superoxide dismutase from *Taenia solium*, and for other target enzymes from different parasites, including triose phosphate isomerase, one of the targets more investigated during the productive life of Dr. Gómez-Puyou.

Reflections on the Catalytic Power of the TIM Barrel

John P. Richard, *Department of Chemistry, University at Buffalo, Buffalo, NY, 14260, USA.*

The TIM barrel is a highly conserved protein fold, first characterized by examination of the X-ray crystal structure for triosephosphate isomerase (Figure). By one estimate the TIM barrel accounts for 10% of all known protein domains. This fold consists of eight alternating β–strands and α-helices $(\beta\alpha)_8$, where the eight parallel β–strands form the protein core and are covered by the α-helices at the protein exterior. The secondary structural elements are connected by a total of sixteen flexible loops, with the eight front loops and eight back loops being attached to the N-terminal and C-terminal ends of the β–strands, respectively.



A view from the "top" of the TIM barrel, which shows the convergence of the catalytic side chains at the active site of triosephosphate isomerase. The basic side chain from Glu165 (loop 6) abstracts the acidic carbon proton, the acidic side chain from His95 (loop 4) shuttles a proton between the enediolate oxygens, and the acidic side chains of Asn10 and Lys12 (loop 1) stabilize negative charge at these oxygen atoms.

The active site of triosephosphate isomerase and other TIM barrel enzymes is constructed from the back loops. The features of the TIM barrel which enable catalysis of a broad range of enzymatic reaction will be illustrated for TIM-catalyzed isomerization of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate. Results from studies to probe the role of the individual front loops of TIM on catalysis of the reaction of the whole substrate GAP and the substrate piece [1-^{13}C]-glycolaldehyde ([1-^{13}C]-GA) and HPO_3^{2-} (HP_i) will be presented. These include: (a) The effect of replacement mutations at loop 6 and 7. (b) Mutagenesis studies to probe the role of hydrophobic clamping residues at loop 6 and loop 8 in catalysis. (c) Mutagenesis studies to probe the role of hydrogen bonds between loop 6 and loop 7 in catalysis. These results support the proposal that dianions activate TIM for catalysis of isomerization by the utilization of binding energy to draw the enzyme conformational equilibrium from the inactive open form to the higher energy, catalytically active, closed enzyme.

The unfolding story of a TIM barrel.

D. Alejandro Fernández Velasco, Mariana Schulte-Sasse, Luis A. Becerril Sesínand Sergio RomeroRomero.Laboratorio de Fisicoquímica e Ingeniería de Proteínas. Departamento de Bioquímica. Facultad de Medicina. Universidad Nacional Autónoma de México 04510 México D.F. Tel. 55 56232259fdaniel@unam.mx

One of the more versatile an common scaffolds in nature is the $(\beta/\alpha)_8$or TIM barrel fold, named after Triosephosphateisomerase (TIM), an oligomeric enzyme at the crossroad of carbohydrate metabolism.The catalytic mechanism of TIM is well established; the catalytic efficiency of the enzyme from several organisms isin the diffusion-control limit; however the molecular origins of the catalytic rate acceleration produced by the enzyme are an active field of research. TIM is dimeric in eukariotes and bacteria and tetrameric in some Archea; the scaffold of the monomer, as well as their relative orientation in the oligomer is conserved in all forms of life.

The architecture and catalytic mechanism of TIM seem to be conserved in evolution; however there are subtle but important differences between TIM from different species; these were used by Prof. Armando Gómez Puyou to pioneer the useof TIM from parasites as candidates for drug target. Here we explore the diversity of the folding thermodynamics of TIM. We will show examples of this variety in the solvent-induced of eukaryotic TIMsand, notwithstanding their irreversibility, in their temperature-inducedunfolding. Noticeably, the temperature-induced unfolding of several bacterial TIMs is reversible; a detailed characterization obtained from their study will be presented.In order to address the historical evolution observed in the folding of this protein, as well as the origins of the conservation in its structure and function, results on the reconstruction of ancestral TIMs will also be given. Finally,the initial characterization of an idealized*de novo* TIM barrel will be presented.

Supported by PAPIIT IN 219913 and Facultad de Medicina UNAM

A protosilencer with unique properties modulates subtelomeric gene expression in *Candida glabrata*

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Adherence to epithelial host cells is thought to be a crucial first step in the pathogenicity of the opportunistic fungal pathogen *Candida glabrata*. This adherence is mediated by several members of a large family of cell wall proteins encoded by the *EPA* (Epithelial Adhesin) genes present in the *C. glabrata* genome. The number of *EPA* genes varies from 17 to 23 depending on the particular strain. The majority of the *EPA* genes are localized close to several telomeres in *C. glabrata*, the subtelomeric regions. The subtelomeric location of these genes results in a negative regulation of transcription through chromatin-based subtelomeric silencing. In cultures of the reference strain BG2, adherence to epithelial cells is mainly mediated by Epa1, the only member of the *EPA* family that is expressed in vitro. *EPA1*, *EPA2* and *EPA3* form a cluster that is localized at the subtelomeric region of the right telomere of chromosome E (E_{-R} telomere). *EPA2* and *EPA3* are subject to subtelomeric silencing that propagates from this telomere in a process that depends on the Sir2, 3, 4, Rap1 and Rif1 proteins, but surprisingly not on the yKu70 and yKu80 proteins. We identified a protosilencer with unique properties localized between *EPA3* and the telomere E_{-R}. This protosilencer called Sil2126, is responsible for the yKu70/yKu80-independent silencing of this telomere. In addition, the protosilencer element is telomere specific and can silence a reporter gene when placed 31.9 kb away from this telomere, but not when it is removed from the telomere context or when it is placed near other telomeres. Sil2126 is required but not sufficient to confer yKu70/yKu80-independence at other telomeres, perhaps because there is a requirement for other sequences that are only present at telomere E_{-R}. One possible such sequence is a novel cis-acting element, called the negative element (NE) that negatively regulates transcription of the *EPA1* gen cooperating with the silencing machinery. We have generated a set of strains containing a reporter-protosilencer system in combination with deletions of the protosilencer and or the NE to analyze whether the activity of the protosilencer requires the presence of the NE.
Together the data we have generated suggests that there are significant differences in the silencing mechanism at different subtelomeric regions, and underscores the importance of cis-elements for repressive chromatin formation and spreading in *C. glabrata*.

"The role of TrxG and PcG during Arabidopsis root development"

Sánchez MP
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Paramutation: trangenerational epigenetic inheritance and development

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Epigenetic regulation is essential for growth and development of eukaryotic organisms and is also responsible for the maintenance and reversal of nongenetic cellular memory that records developmental and environmental cues. Epigenetic alterations during development can lead to cancer, neurological disorders, heart disease, and other degenerative conditions. Paramutation is the epigenetic transfer of information from one homologous sequence to another to establish a state of gene expression that is heritable through mitosis and meiosis for generations. Originally described in maize, additional paramutation examples have been studied in plants and animals. The maize *b1* locus encodes a transcription factor that promotes the biosynthesis of purple anthocyanin pigments. Two alleles are involved in *b1* paramutation. The paramutable, darkly pigmented and highly expressed *BI* allele, and the paramutagenic, lightly pigmented and extremely stable *B´* allele. When *B´* and *BI* are crossed together, paramutation always occurs: BI is always changed into *B'*. *b1* paramutation is mediated by seven unique noncoding tandem repeats. Recent results indicate that paramutation involves a number of genes implicated in RNA-based transcriptional gene silencing mechanism. However, not all aspects of paramutation can be explained by known mechanisms of RNA-directed DNA methylation (RdDM) mediated gene silencing. Our research group is trying to discover the molecular basis of paramutation and to understand its biological role during plant development.

Chromatin structure modulates differential gene expression of the chicken α-globin locus

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Genomic loci composed of more than one gene are frequently subjected to differential gene expression, with the chicken α-globin domain being a clear example. In the present study we aim to understand the globin switching mechanisms responsible for the epigenetic silencing of the embryonic π gene and the transcriptional activation of the adult α^D and α^A genes at the genomic domain level. In early stages, we describe a physical contact, through the chromosome conformation capture (3D), between the embryonic π gene and the distal 3' enhancer that is lost later during development. We show that such a level of regulation is achieved through the establishment of a DNA hypermethylation sub-domain that includes the embryonic gene and the adjacent genomic sequences. The multifunctional CCCTCC-binding factor (CTCF), which is located upstream of the α^D gene promoter, delimits this sub-domain and creates a transition between the inactive sub-domain and the active sub-domain, which includes the adult α^D gene. In avian-transformed erythroblast HD3 cells that are induced to differentiate, we found active DNA demethylation of the adult α^D promoter, coincident with the incorporation of 5-hydroxymethylcytosine (5hmC) and concomitant with adult gene transcriptional activation. These results suggest that autonomous silencing of the embryonic π gene is needed to facilitate an optimal topological conformation of the domain. This model proposes that CTCF is contributing to a specific chromatin configuration that is necessary for differential α-globin gene expression during development.

The Human Genome, fourteen years latter

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Three main encyclopedias are supported by the human genome: biology, history and medicine. How have we improved our understanding of the information in those three fields? The Human Biological System has opened new pathways full of surprises. The extraordinary technological advancement has allowed the parallel approach for thousands or millions of informational molecules in each experiment.

In order to analyze such a huge number of data, we need to use sophisticated bioinformatics approaches and develop Biology Systems.

Understanding biology includes latest publication of first findings of large projects: ENCODE and the Human Proteome. These projects unlocked questions about the magnitude and significance of structural variants and new types of non-coding RNAs.

Genomics, as a history book, grows from major projects of the Hap Map and 1000 genomes, likewise different regional projects that have increased the Databases of Human Genetic Diversity (dbSNP contains more than 40 million single nucleotide polymorphisms). Having this massive information, now it is possible to analyze the history of human evolution from his origins in Africa to the most contemporary civilizations, including DNA sequencing of more ancient fossils.

Medical application of the human genome is becoming outstanding, after a decade of apparent “slow down”, thanks to knowledge about specific genes associated to thousands of diseases (GWAS studies), and great sensitivity and complexity of gene molecular methods today.

Dynamics of the Human Genome

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Some years ago, variations among human genomes was believed to be concentrated in single nucleotide substitutions, microindels, and variation in the number of subunits of minisatellites and microsatellites. In 2004 two seminal papers indicated the presence of structural variation in the genome of healthy persons. The term structural variation refers to large variation events in the genome, including deletions, insertions, inversions and translocations of genetic material. Actually, in number of nucleotides, structural variation seems to be larger than variation due to single nucleotides. The molecular basis of structural variation can be explained by the occurrence of genomic rearrangements in the human genome.

Our group has developed a research project to demonstrate the occurrence of genomic rearrangements, at relatively high frequency, in the genome of human somatic cells. For this purpose we decided to study DNA inversions in the genome of blood cells. We predicted potential sites for inversion rearrangements by locating all the sites in the genome containing identical repeated sequences with an inverse orientation. Following a PCR-based experimental strategy, previously developed in our group to detect genomic rearrangements in bacteria, we showed that inversion rearrangements are indeed frequent in the genome of blood cells. Most interesting was the finding that such rearrangements are more frequent in adults than in newborns.

To better understand human genome rearrangements we decided to focus in sequenced human genomes. We were not satisfied with the algorithms that have been used to compare genomes. Accordingly, we decided to develop a novel experimental approach. This approach has been called COIN-VGH (COntext dependent Individualization of Nucleotides and Virtual Genomic Hybridization. With this algorithm we re-analyzed the haploid region (non-pseudoautosomal region of the X chromosome) of the genomes of Craig Venter and of James Watson. We found more precise localization of single nucleotide substitutions than those presented in the original studies.

We are now optimizing the COIN-VGH algorithm to analyze complete genomes obtained by new generation techniques. To validate the experimental approach we sequenced the genomes of a TRIO (father, mother and child). We found that the novel algorithm can define single nucleotide differences with great precision and without rendering false positive variation. Most important, this algorithm is particularly suited to found *de novo* variation on the human genome. The implications of these results will be discussed.

Patterns of Genetic Diversity in Latin America: Insights from Human Population Genomics

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Indigenous populations from the American continent have been largely underrepresented in large-scale genomic studies, yet they are bearers of a unique history from one of the regions of the world where proportionally more novel variation remains to be discovered. I will discuss recent efforts to characterize the genetic profile of Native Americans throughout the continent and focus on regional approaches aimed at resolving finer scale population structure patterns in Mexico, South America, and the Caribbean. By generating genome-wide SNP data for hundreds of individuals from both indigenous and recently admixed cosmopolitan populations and developing novel methodologies to investigate ancestry patterns at the sub-continental level, we reconstruct the pre- and post-colonial history of each region. We trace back the origin of ancestral components of admixed Latin Americans to their closest source among Native American, European, and African populations. Our work demonstrates that dense population genomic data coupled with novel methods of admixture deconvolution afford the possibility of reconstructing human population genetic history with far greater resolution than previously thought.

The impact of genomics in the study and clinical management of cancer.

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There are more than 100 different types of cancer. Every one of them develops because of the accumulation of alterations in the cellular genome (mutations) throughout time, resulting in uncontrolled cell growth and division of the cells from a particular organ or tissue. Cancer cells can also invade the surrounding tissues and can even get into the circulatory system in order to invade distant organs, in a process called metastasis. The firsts evidences suggesting that cancer arises as a consequence of the accumulation of mutations came from the late XIX Century and were obtained thanks to the observation of alterations in cell division in several tumors. In the last decades, high-throughput genomic analyses have led to the identification of an important number of genetic alterations associated with the development of several human tumors. These technologies have allowed us to obtain the whole genome sequence of a tumor and compare it against the normal DNA of a patient. Different international collaborative efforts are gathering huge amounts of genomic information from a variety of human tumors, including whole genome sequences, expression and epigenetic data as well as clinical information, facilitating the identification of biological alterations that might have clinical relevance. In this way, in the next decade, the genomic characterization of tumors will become a standard tool for the management of cancer patients, and it will have a profound impact in the way we prevent, detect and treat cancer, thanks to the development and application of targeted treatments, which will be more effective and less toxic for cancer patients.

Energy/redox metabolism, gene-environment interactions, and dopaminergic cell death associated with Parkinson's disease.

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Parkinson's disease (PD) is a multi-factorial disorder with a complex etiology including genetic risk factors, exposures to environmental agents and aging. While environmental toxicants are unlikely the single cause of PD, understanding the cellular dysfunction induced by these agents will reveal the molecular mechanisms contributing to neurodegeneration. In the brain, energy metabolism and redox homeostasis are tightly linked. However, the exact contribution of impaired energy metabolism to cellular dysfunction (oxidative stress, protein aggregation and cell death) is unclear. Using an integrated nuclear magnetic resonance (NMR) and mass spectrometry (MS) metabolomics platform we have determined, for the first, the alterations in energy/redox metabolism in dopaminergic cells exposed to mitochondrial toxins (6-OHDA and 1-methyl-4-phenylpyridinium [MPP+]) and environmental agents (pesticides paraquat, dieldrin and rotenone) known to increase the risk to develop PD. Differential alterations in glucose metabolism (glycolysis, pentose phosphate pathway [PPP] and the tricarboxylic acid [TCA] cycle) were shown to precede dopaminergic apoptotic cell death. Importantly, we revealed that a dysfunction in cellular bioenergetics was a significant contributor to environmental toxicity. For example, we demonstrated that the redox cycling pesticide (paraquat) had the ability to "hijack" the PPP to increase the generation of NADPH and the generation of oxidative damage, which was paralleled by an impairment of NADPH-dependent antioxidant systems (glutathione peroxidase, thioredoxins, catalase and peroxiredoxins). Paraquat toxicity was also associated with an increased in GLUT-like mediated glucose transport. Accordingly, inhibition of glucose uptake/metabolism and the PPP, as well as the presence of free radical scavengers (ascorbic acid) were shown to prevent paraquat toxicity. Further studies demonstrated that the metabolic master regulator adenosine monophosphate-activated protein kinase (AMPK) and autophagy, a survival mechanism activated upon energy stress, exerted a protective effect against paraquat toxicity. In addition, we also found that overexpression of the PD-related gene SNCA (α-synuclein) and its mutant form Ala53Thr potentiated paraquat toxicity, and that this synergistic effect was also regulated by glucose metabolism. All together, these results ascertain the transcendental role of cellular energy metabolism dysfunction in dopaminergic cell death induced by gene-environment interactions linked to PD progression.

Nrf2 redox signaling confers long term protection in post-ischemic hearts

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Oxidative stress is considered one of the main effectors of myocardial dysfunction in ischemia and reperfusion injury. However, a paradigm in cardioprotection is that reactive oxygen species (ROS) may also exert beneficial effects depending on their amount, compartmentalization and timing of generation. In the cardioprotective response exerted by postconditioning (PostC), ROS and kinase signaling are activated, and also mechanisms that attenuate oxidative stress.

It is widely known that the nuclear factor E2-related factor 2 (Nrf2) increases gene transcription of antioxidant enzymes under stress conditions. Nrf2 transcriptional activity depends on dissociation from its repressor, the Kelch-like ECH associated protein 1 (Keap1), which sequesters Nrf2 in the cytoplasm, orchestrating its ubiquitination and degradation. The mechanism by which Nrf2 is stabilized and translocated to the nucleus is controversial. In one hand, it has been proposed that chemopreventive agents and ROS react with specific thiol groups, inducing the dissociation of both proteins and leading ultimately to Nrf2 nuclear accumulation. On the other, experimental evidence suggest that Nrf2 phosphorylation at serine-40 may release Nrf2 from Keap1.

Therefore we evaluate the hypothesis that the novel pathway PKC/Nrf2 participates in the long-term protective mechanisms induced by PostC application by maintaining the antioxidant defense system in ischemic/reperfused hearts.

Hepatocyte Growth Factor/c-Met as a master regulator of redox cellular status and oxidative stress

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The hepatocyte growth factor (HGF) and its receptor, the tyrosine kinase protein c-Met, have been gaining relevance as a central modulators of the repair process in epithelial tissues. In the last years, our group has been characterizing the mechanism of this effect finding that the major trigger in the survival and repair processes is the modulation of the reactive oxygen species (ROS).
Mechanistically, c-Met regulates the NADPH oxidase in a biphasic manner, first increasing the activation of this enzyme complex, and then decreasing it by transcriptional repression. The early increment in ROS was directed to activate the transcription factor Nrf2, which in addition induced the expression of survival and antioxidants proteins conferring a protective effect in hepatocytes. At 12 and 24h of HGF treatment the expression of the NADPH oxidase components were diminished leading to an abrogation of the cytotoxic effectors such as TGFβ.
In order to figure out the physiological relevance of HGF/c-Met as regulator of oxidative stress and redox cellular status, we were focused to address the effect of c-Met in the oxidative stress in the context of liver fibrosis, hepatocellular carcinoma and in drug-induced liver injury. In all experimental models of liver disease we found a prominent participation of c-Met as a regulator of ROS content. In fact, the genetic elimination of c-Met signaling induced oxidative stress and down regulated many genes related to stress response.
Met-KO mice subjected to a carcinogenic stimulus developed tumors earlier than Met WT mice. Even more, the fibrogenic process was augmented in the MetKO animals and was associated with changes in the expression of genes associated to oxidative stress, judged by microarrays experiments.
Finally, HGF demonstrated to be a good protective factor against antituberculosis drug-induced hepatotoxicity decreasing the ROS generated by the biotransformation of these drug in the liver, such effect was also corroborated in lungs.
Our data leave clear that HGF/c-Met regulates ROS content by the intervention in ROS generating systems and the expression of antioxidant proteins presenting relevance in liver diseases.
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Redox state as an inductor and modulator of cellular senescence

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Cellular senescence (CS) is usually characterized by a proliferation arrest after a maximal round of replications (Replicative Senescence, RS) and is associated with aging and multiple age-related diseases. CS can be achieved by multiple pathways. Most of them involve the activation of negative cell cycle regulators as well as a shift to an oxidative status. However, the exact participation of these events in senescence establishment and maintenance is not completely understood. Thus we investigated the content of three final cell cycle regulators, as well as the redox state in some critical points during the pre-senescent and the full senescent states. Our results highlight the existence of a critical pre- phase in senescent phenotype establishment, in which cell proliferation stops with the participation of the cell cycle inhibitors, and a second maintenance stage where the exacerbated pro-oxidant state inside the cell induces the physiological decline characteristic in senescent cells.
As mentioned before, there are many stimuli that can prematurely induce CS, such as oxidative stress (Stress-Induced Premature Senescence, SIPS) and the recently described Proteasome Inhibition Induced Premature Senescence, (PIIPS). This kind of senescence exhibits the same hallmarks that SIPS and RS, including the proliferation arrest, SA-beta galactosidase activity and overexpression of some cellular cycle down regulators. Senescent cells secrete cytokines, growth factors, chemokines and matrix metalloproteinases. This pro- inflammatory secretion is known as Senescence Associated Secretory Phenotype (SASP) and it has been related with local inflammation which leads to cellular transformation and neurodegenerative diseases. SASP has been well characterized in RS and SIPS but not in PIIPS. Hence, we also determined SASP components in PIIPS and compared them to RS and SIPS in order to determine if SASP components are different depending on the stimuli used to induce senescence.

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Evolution of genomes by copy and paste: phylogenomics reveals non-vertical evolutionary relationships in fungi

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Genomes are shaped in many ways during the course of evolution. Whereas point mutations can alter the function of already existing functional genomic elements, gene duplications or genetic transfers from other organisms immediately generate novel genetic material upon which selection can act. Phylogenomics provides us with the tools to study such events and interrogate their possible impacts. In this talk I will provide an overview of several of our findings related to the genome evolution of fungal organisms, for which hundreds of genomes are now available. Contrary to general expectations for eukaryotes and for a group of organisms with a cell wall and no phagocitosis, we found that horizontal gene transfer is common in fungi. This process has mediated the acquisition of important phenotypic innovations and has often involved the transfer of entire gene clusters between phylogenetically distant lineages. Gene duplication in the form of family expansions, or duplication of entire genomes has also played a major role in the evolution. Finally, hybridization between different species is emerging as a common evolutionary mechanism that readily generates new species and innovative phenotypes through the combination of entire genetic complements. Altogether the emerging picture is that fungi present highly plastic genomes prone to rapid adaptations through the duplication of existing genetic material or the lateral acquisition of genes.

Ecological metagenomics of cycads and their bacterial symbionts: metabolic insights into a million-year old association

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Cycads (Cycadales) are the most ancient group of extant land plants (Permian) with specialized root structures that house nitrogen-fixing bacterial symbionts, and the only gymnosperms known to fix nitrogen. We hypothesize that cycads have survived changing habitats by re-sampling root symbiont bacterial diversity, and by acquiring their metabolites or their enzymatic machinery to produce adaptive secondary compounds uniquely known to cycads. We established a hybrid field-lab system based on sampling coralloid roots of endangered Mexican *Dioon* to identify phylogenetic and functional communities. An axenic-culture-based Sanger-16S and genomic characterization of communities associated with *D. caputoi* and *D. merolae*, two cycad species with contrasting distribution, identified known and new symbiotic cyanobacterial taxa and suggested the presence of non-cyanobacterial symbionts for the first time in the literature. A 'dynamic community' experiment with an emphasis in function, increased sample resolution and depth to characterize coralloid root communities associated with *D. merolae,* sampled from two contrasting environments. We generated metagenomes and 16S-Illumina iTAGs from root bacterial community collections and plant and microbial cultures as reference, from the two environments. Here we describe ecological (who, how communities change) and metabolic patterns (functional traits of symbionts) of cycad root communities. Phylogenomic and ecological analyses inform bacterial taxonomy and provide an unprecedented ecological and functional metabolic understanding of the evolutionary process in the cycad-bacteria relationship, and of root symbionts in general. Genome-scale metabolic models of micro-communities will lead to insights into natural products and other metabolites mediating bacterial interactions within the plant. In the light of rapid climate and landscape change, surviving cycad lineages and their symbionts are a beacon for understanding the impact of changing environments in species' survival.

Experimental evolution of genome architecture and complexity in RNA viruses

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Experimental evolution has led to several breakthroughs in evolutionary biology: confirmation of hypotheses on adaptive trajectories, the role of population size and chance events, the benefits of sex, evolvability, and evidences for coevolution. One aspect of evolution that has received scant attention, however, is the evolution of genome complexity: the dimensions and organization of the genome. There are clear differences in genome architecture over organisms; it is also clear that this complexity can be altered on relatively short time scales. Although some theoretical and *in silico* studies have addressed this topic, scant attention has been given to *in vivo* experiments testing these ideas.

To study the evolution of genome architecture we are working with *Tobacco etch potyvirus* (TEV), a plant RNA virus that has been developed as a model system for experimental evolution. We are exploring the evolutionary dynamics of both decreasing an increasing genome size and complexity by duplications of existing genes, complementation of viral genes by *in trans* expression, incorporation of new genes, changes in gene order, and segmentation of the viral genome. At the one side, TEV can stably incorporate new genes that provide additional functions (e.g., an additional suppressor of RNA silencing or a DNA repair enzyme). At the other side, essential viral functions can be removed from its genome if provided *in trans* by the host. Together, these studies show that genome architecture is not as rigid and constrained as previously thought.

Evolution of the transcription circuit underlying biofilm formation in *Candida* species

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It is becoming clear that changes in transcription circuits underlie the evolution of many phenotypic traits, including virulence in human pathogens. The ability of microorganisms to form surface-associated communities, also called biofilms, is essential for the colonization of the human host. Recent characterization of the transcription circuit that regulates biofilm formation in the human fungal pathogen *Candida albicans* identified six core transcription factors that bind and regulate over a thousand target genes. Despite its complexity, several lines of evidence suggest that this circuit evolved relatively recently. To understand how such a complex transcription circuit came about, we characterized biofilm formation and the underlying transcription circuits in several related species of *Candida*. As early evidence suggested, only closely related species to *C. albicans* are able to form a structured biofilm with a basal layer of yeast cells and an upper layer of hyphae. In agreement, the biofilm transcription circuit seems to be considerably different in species that form simpler biofilms. This is the case of *Candida parapsilosis* where only two of the six core transcription factors play a role in monolayer biofilm formation. However, even in the species that are able to form structured biofilms the transcription circuits have undergone considerable change. The role of the six core transcription factors in biofilm formation is not fully conserved. For example, Rob1 is essential for biofilm formation in *C. albicans* binding to the promoter of over 90 genes whereas in *Candida tropicalis* this transcription factor is not involved in biofilm formation and it seems to have lost its DNA binding domain. Despite this important difference in the transcription circuit, *C. tropicalis* is able to form structured biofilms. Transcription profiling and genome-wide protein-DNA interactions revealed that most of the changes in the biofilm circuits occurred in the target genes, possibly due to modifications in cis-regulatory elements. For some core transcription factors as many as 70% of the target genes have changed when comparing species that have diverged about 55 million years ago and still form structured biofilms. Apart from emphasizing the evolutionary plasticity of transcriptional circuits, our results shed light into how complex transcription circuits evolve and specifically how biofilm formation emerged in this group of important human pathogens.

TGF-beta and GPCR Signals Converge to Regulate the Stability of Ski and SnoN Transcriptional Cofactors in Hepatocytes

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Transforming growth factor-β (TGF-β)-induced antimitotic signals are highly regulated during cell proliferation in normal and pathological conditions, such as liver regeneration and cancer. Upregulation of the transcriptional cofactors Ski and SnoN during liver regeneration may favor hepatocyte proliferation by inhibiting TGF-β signals. We have found a novel mechanism that regulates Ski and SnoN protein stability through TGF-β and G protein- coupled receptors (GPCR) signaling. Ski and SnoN proteins are distributed between the nucleus and cytoplasm in normal hepatocytes and the molecular mechanisms controlling Ski and SnoN protein stability involve the participation of actin-cytoskeleton dynamics and vesicle trafficking. Cytoplasmic Ski is partially associated with actin and localized in cholesterol-rich vesicles. Ski protein stability is decreased by TGF-β/Smads, GPCR/Rho signals and actin polymerization, whereas GPCR/cAMP signals and actin depolymerization promote Ski protein stability.

We conclude that TGF-β and GPCR signals differentially regulate Ski and SnoN protein stability and sorting in hepatocytes, and we suggest that this regulation may occur in hepatocytes during liver regeneration.

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RhoGEFs: integral components of signaling modules

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Signal transduction cascades critical for tumor angiogenesis and metastasis are essential for cancer progression. Rho GTPases delineate the spatio-temporaleventslinked to dynamic adjustments in the shape of growing capillaries and the mobilization of cells from the bone marrow during an angiogenic response. Thus, RhoGEFs, the group of guanine nucleotide exchange factors that activate Rho GTPasesin response to angiogenic factors secreted by growing tumors, putatively integrate signaling modules essential to mount anangiogenic response in cancer. We hypothesized that RhoGEFsintegrate angiogenic signaling cascades via their interaction with kinases and receptors that control the status of Rho GTPases which, as molecular switches, are regulated in time and space during an angiogenic response.We assessed the contribution of the bone marrow to the growth of LLC carcinoma in a murine preclinical model, assessed the expression of RhoGEFs in endothelial cells and Tie2-positive bone marrow-derived cells and initiated a systematic characterization of the angiogenic potential of multiple RhoGEFs. In parallel, we focused our attention on the signaling properties and angiogenic potential of G protein coupled receptors such as TEM5/GPR124, an orphan GPCR identified by its expression in tumoral endothelium, and CXCR4, a chemotactic GPCRs that activatesP-Rex1, a RacGEFthatinteracts with kinases such as mTOR and PKA and participates in CXCR4-dependentangiogenicresponses.

Our results indicate that Tie2-positive cells from the bone marrow contributed to tumoral growth. These cells, as well as microvascular endothelial cells, express multiple RhoGEFs. Again of function approach used to assess the signal transduction potential of twelve of the most abundant endothelial RhoGEFs, including RGS-RhoGEFs, Intersectins and PLEKHG5 revealed that these RhoGEFs,expressed as catalytic domains targeted to the plasma membrane,behaved as constitutively active variants and had drastic effects on the shape of endothelial cells and showed paracrine effects on non-transfected cells.TEM5, a potentiallyangiogenic G protein coupled receptor, inducedfilopodial projectionsin endothelial cells. This morphological effect was linked to dynamic adjustments of the actin cytoskeleton and correlated with the ability of TEM5 to modulate the agonistic properties of cancer derived factors that controlled the activation of Rho GTPases and the PI3K/AKT/mTORC2 signaling pathway. These responses were potentially attributable to the interaction of TEM5 carboxyl-terminus with RhoGEFs such as ITSN2L, a GEF for Cdc42, and Elmo1, an adaptor of an atypical GEF known to activate Rac.Regardingthe mechanisms of regulation ofP-Rex1, a multidomainRhoGEF specific for Rac1, our results indicate that this RacGEF integrates angiogenic signals regulated by its interaction with PKA, which phosphorylates this RacGEF at S436, within the domain previously characterized as relevant for its interaction with mTOR. In addition, members of the group of 14-3-3phospho-serine/threonine-dependent adaptorsare potential regulators of P-Rex1. In conclusion, microvascular endothelial cells and Tie-2 positive bone marrow cells are activated by angiogenic stimuli, contribute to tumor growth, and express a complex array of RhoGEFs that might contribute to its positive effect on cancer progression. MultipleRhoGEFs, some of them in the signaling cascades of GPCRs,control endothelial cell shape, showing their potential participation in tumor angiogenesis and supporting their possible role as drug targets in cancer.

Modulation of Toll-like receptor 4 (TLR4) signaling in mast cells. Claudia González Espinosa, Departamento de Farmacobiología, Cinvestav. Calzada de los Tenorios No. 235, Colonia Granjas Coapa, CP14330, Tlalpan, D.F. cgonzal@cinvestav.mx

Mast cells (MC) are important players on allergic responses and other inflammatory reactions. Its activation through the high affinity IgE receptor (FcεRI) and the Toll-like receptor 4 (TLR4) lead to the secretion of preformed mediators and de novo synthesized cytokines that promote local and systemic inflammation. Recent research has shown that activation of MC through TLR4 receptors with bacterial lipopolisaccharide (LPS) leads to the secretion of tumor necrosis factor (TNF) and other pro-inflammatory cytokines that contribute to the survival to bacterial infections. Utilizing bone marrow-derived MC, inflammatory reactions in murine models and MC-deficient mice that allow specific MC reconstitution, some of the key molecular events leading to cytokine secretion after LPS stimulation of MC have been characterized and selected signaling pathways able to inhibit or potentiate TLR4-mediated cytokine production in this cell type were have been described. TLR4 receptors in MC are coupled to MyD88-dependent signaling pathways, leading to the activation of IKK and nuclear translocation of NFκB. Besides those reactions coupled to cytokine gene transcription, TLR4 triggering provokes the phosphorylation of SNAP-23, a SNARE protein involved in granule exocytosis. Also, TLR4- dependent cytokine secretion requires calcium movilization, MAPK activation and TTx-resistant SNARE proteins. Distinct mediators modify the secretion profile of LPS-activated MC, such as monomeric IgE, opiates, adrenaline and acetylcholine, affecting the cytokines produced in vitro and the inflammatory response in vivo. The blockage of TRAF6 ubiquitin ligase activity, which leads to the inhibition of MAPK and IKK activation, has been identified as one of the most important steps on the negative control of TLR4 receptor signaling cascades. Particularly, in the inhibitory effects of opiates, association of TRAF6 with β-arrestin is observed, indicating that molecules involved in the desensitization of GPCRs are able to modulate TLR4-mediated responses. Our data indicate that cytokine production in MC after stimuli of innate immunity is controlled by mediators produced in adaptive immune responses, stress and other pathophysiological conditions.

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Modulation of G protein-coupled receptors by receptor tyrosine kinases. J. Adolfo García-Sáinz. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Apartado Postal 70-248; México D.F. 04510. Tel (52-55) 5622 5613. Email: agarcia@ifc.unam.mx.

G protein-coupled receptors (GPCRs) are one of the most abundant classes of membrane proteins, representing 3-5% of the proteins coded in known genomes. The total number of GPCRs in the human genome is currently estimated in ≈ 800 different proteins, of which at least 40% are orphan receptors. Their importance cannot be overestimated since they mediate mayor functions or our organism, are key players in the pathogenesis of many diseases and are targets of 30-40% of currently prescribed drugs and a much larger percentage of illegal drugs.

Regulation of receptor function is a key element in the survival of cells and organisms and involves many long-term and short-term processes. Long-term regulation mainly represents the dynamic steady-state of receptor´s abundance, controled by the rates of transcription, mRNA processing and stability and by protein turnover. Short-term regulation is tightly regulated by post-transcriptional modifications, such as phosphorylation, palmitoylation and ubiquitination/ SUMOylation and by receptor vesicular trafficking (internalization, recycling).

GPCR signaling is rapid (although there are long-term events, involving modulation of transcription) and transient. This implies fast turn-on and turn-off rates. The turn-off rate is frequently named desensitization and is associated to receptor phosphorylation. There has been a conceptual change regarding desensitization since it is currently gaining acceptance that it is not an end of signaling but rather a switch (to G protein-independent events). Receptors are in constant cycles of desensitization/ resensitization.

Receptor desensitization can be induced by agonist stimulation (homologous) and by activation of unrelated receptors or pathways (heterologous). Work in our laboratory and others during the last 10 years has shown that another family of receptors, the receptor tyrosine kinases, modulate the function of GPCRs. Examples will be presented on the major pathways, currently identified, in heterologous desensitization and the molecular entities involved. This complex modulation of receptor function, are likely involved in the normal function of our organism and also in pathogenesis of morbid entities, exposing possible targets for pharmacological intervention.

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Genomic approach of a monogenic disease: hemophilia as a model

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As with monogenic diseases, hemophilia A and B have a direct relationship between factor VIII *(F8)* and factor IX *(F9)* gene mutations, respectively, and their causative effect on protein deficiency, either in function or reduced antigen level in plasma. These aspects are related but do not totally explain a more complex clinical phenotype such as the age of initial symptom onset, bleeding tendency, inhibitor development, arthropathy tendency, etc. The complex relationship between clinical behavior and genetics of hemophilia has challenged the approach of diagnosis and research methods, expanding the scope of analysis to other related genes and considering a multiple level approach, including genetic, cellular and functional testing.

Hemorrhage phenotype attenuation in hemophilia by prothrombotic genes

We have studied hemophilia A and B families with some affected members who show a striking attenuation of bleeding symptoms. We studied the clinical manifestations, clotting activity levels and prothombotic markers: Factor V G1691A (Leiden) and Factor II 20210A, MTHFR C677T and MTHFR A1298C. The effect of Factor V G1691A and Factor II 20210A was demonstrated in the attenuation of hemorrhagic symptoms in hemophilia patients. The attenuation of hemophilia phenotype was mainly observed in the delay of bleeding symptom onset and secondly in a lower frequency of bleeding episodes. On the basis of the feasible molecular analysis by routine PCR of prothrombotic genes and their frequency in Mexican population (1-5%), screening is recommended in those hemophilia patients with non-congruent clinical behavior in regard to severity by clotting activity.

Structure-function relationship of *F9* gene mutations at the subcellular level

To search for mechanisms that cause mutations in hemophilia and its impact on the relationship between the structure-function of mutant proteins, selected *F9* mutations were inserted by directed-site mutagenesis into an expression vector and transfected to a cell line, to study the interaction of mutant FIX proteins with other intracellular components involved in their posttranslational processing and secretion. In the study of *F9* mutations causing a severe phenotype, two mutations originated by recurrent mutagenesis at non-CpG were identified. They affect the structure-function of FIX by changes of a cysteine position in the second epidermal growth factor (EGF2) domain of *F9* gene (C111S and C111Y mutations). The disruption of the disulfide bond in the mutants has an important effect on the native folding of FIX due to their accumulation in the intracellular space in regard to wild type FIX. C111Y showed a higher impact than C111S on its transport through ER with a predominant degradation at proteasomes.

Calibrated Automated Thrombography as functional approach in hemophilia

Functional approaches can provide prognostic parameters in relevant clinical issues of hemophilia, such as the inhibitors development in patients with severe hemophilia A. Thrombin generation assay (TGA) by the standardized method of Calibrated Automated Thrombography (CAT), describes the overall clotting capacity of patients' plasma *in vitro*. The fundamental premise of the method is based on thrombin as a central molecule of coagulation whose increase or decrease reflects any alteration from the hemostasis equilibrium caused by hemorrhagic or thrombotic factors. Different parameters of the TGA, as the endogenous thrombin potential (ETP), have shown correlation with clinical behavior in hemophilia patients. The change in the ETP showed a relationship between inhibitor type and clinical treatment response. TGA permitted an individual evaluation of treatment response and showed usefulness such as objective criterion of responsiveness for a better selection of therapeutic resources.

Conclusions

The various approaches presented here, emphasize the importance of a comprehensive overview in hemophilia, considering multiple interactions among genes, metabolic pathways and different approaches including molecular data, gene expression assays, biochemical analysis and clinical aspects.

Hemoglobinopathies

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The hemoglobinopathies have been widely studied because its relevance to the knowledge of biochemical and genetic topics.

In present lecturer we will focus on three main aspects: **1.** The contribution to human variation by the knowledge of more than 1700 mutations at the hemoglobin genes (*HBA1, HBA2, HBB, HBD* and *HBG1* and *HBG2*), many of theirs of relevance to understand the genotype-phenotype relation. We will give some examples of mutations found in northwestern Mexico. **2.** The analysis of several elements participating in the regulation of the expression of Fetal hemoglobin in normal and pathologic conditions. **3.** The role of the study of polymorphisms at the globin genes in human migrations and in evolution.

1.- Hemoglobinopathies are broadly classified as: A. Those with a structural defect in one of the globin subunits. B. Those with a quantitative defect in the production of one of the globin subunits, or thalassemia syndromes. The main example of the first category is HbS (β6, Glu>Val) known as the first example of molecular disease, a mutation highly prevalent in Africa, but it is observed worldwide. We have found variants with high oxygen affinity, (Hb Tarrant, α126, Asp>Asn) and with polymerization properties (Hb Colima, β49, Ser>Cys). In regard to thalassemias, there are described about 500 mutations, many of them linking to hemolytic anemia. In our laboratory we had identified 20 mutations at the *HBB* gene and seven at the *HBA* gene.

2.- Under normal conditions, HbF ($\alpha_2\gamma_2$) predominates during fetal life and in the first months after birth, but the levels decrease in the first year of life reaching values close to 1.0%. It has been shown that individual variations in the levels of HbF are genetically influenced through complex interactions of two types; a) *in cis* (located in the beta-globin gene locus family at 11p15.5, and b) *in trans* (elements located on other chromosomes as 2p16 (BCL11A), 6q23 (HBS1L-MYB) and 19p13.3 elements (KLF1).

3.- At the β globin cluster there are many SNPs, some of them are constructed as haplotypes which have been useful to determine human migrations, evidencing genetic variability among populations,

to know the evolutionary history of human being as well as to elucidate the origin of several mutations responsible of hemoglobinopathies.

FRAGILE X SYNDROME AND CANCER

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ABSTRACT

Fragile X syndrome (FXS) is the most frequent form of inherited intellectual disability. It is characterized by cognitive, physical and behavioral features that overlap with some common neurological and psychiatric abnormalities, including autism spectrum disorders. FXS is caused by a triplet expansion that inhibits expression of the FMR1 gene, whose product (FMRP) regulates the translation of an important number of proteins, including those involved in receptor signaling and spine morphology in the brain.

Interestingly, accumulated evidence demonstrate that FMRP expression can be linked to cancer. A decreased risk of cancer has been reported in patients with FXS while a patient-case with FXS showed an unusual decrease of tumour brain invasiveness. On the other hand, *FMR1* mRNA, encoding FMRP, is overexpressed in hepatocellular carcinoma cells.

The Wnt-β-catenin signalling pathway plays a crucial role in the regulation, differentiation, proliferation and cellular death processes; consequently, alterations in this pathway are involved in numerous abnormalities of development, growth and homeostasis in animal organisms. Wnt proteins include a numerous family of secretion glycoproteins which join to Frizzled receptors and Low Density Lipoprotein Receptor-related Protein, in order to stabilize the critical β-catenin protein, and to initiate an intricate signaling cascade, which is related to multiple nucleo-cytoplasmatic processes. Alterations in the canonical Wnt-β-catenin signaling pathway have been associated with variations in a number of proteins participating in this route, or with activation / inactivation of oncogenes and tumor suppressor genes, which explain different processes of tumorigenesis and human diseases. Evidence showing the relation between the Wnt-β-catenin signaling pathway with different neoplasic processes is presented.

Application of genomic medicine to chronic-degenerative disorders.

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Medical practice and research have entered the genome era. The evolution from medical genetics to genomic medicine has been gradual and reflects a continuum but there has been a marked transition in the few years since the completion of the first human reference genome sequencing. The application of genomic medicine involves diagnosis, prognosis, prevention and/or treatment of disease and disorders of the mind and body, using approaches informed by knowledge of the entire diploid genome and the molecules it encodes.

The cyclical nature of genome investigation is driven by a constant flow of knowledge between medical practice and research (both “bench to bedside” and “bedside to bench”).

Genomic medicine represents a powerful armamentarium to tackle down most of chronic diseases which have not, so far, defeated. Thus, this new and powerful bio-technologic set of weapons enable us to make use of molecular diagnostic to detect silent diseases otherwise undetectable by conventional analysis. Moreover, elucidation of the complete and final draft of the human genome code has allowed, scientists the designs of specific farmaco-genomic treatments for patients on basis of their individual genetic code. Regarding new medical treatments, gene therapy is here as a true hope for treatment of many chronic diseases. Two thousand and seventy six FDA-approved clinical protocols are currently undergoing around the world, and sooner than later we´ll be witnessing the results.

In this lecture, knowledge will be provided on our efforts to tackle down fibrotic diseases, i.e., cirrhosis and renal fibrosis.

Protective immune response against rotavirus infection

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Rotavirus is the primary etiologic agent of severe gastroenteritis in human infants and neonates of different species, mainly in developing countries. Due to the important role that the rotavirus infection has in childhood diarrheal diseases, there is considerable interest in developing therapeutic strategies that are effective to prevent or at least control the severity of the diarrhea caused by this virus. Recently two live attenuated vaccines against rotavirus have been developed; Rotarix and RotaTeq. However, although the vaccines have shown up to 90 % protection efficacy against acute pathology in developed and some developing countries, these types of vaccines always have the potential to induce intestinal intussusception and it is uncertain whether they can protect against new rotavirus strains.

In this way, it is important to design non-infectious vaccines of new generation, focused on a heterotypic immune response. VP6 constitute the middle layer protein of the rotavirus virion, it is the most conserved protein and is target of heterotypic T and B cell responses. We have found in a mouse model of rotavirus infection, that intranasal inoculation of a 14 mer VP6 peptide, that contains a Th cell epitope, is sufficient to induce a high level of protection against the intestinal infection. Similar results were found when VP6 cross-linked to a monoclonal antibody anti- DEC 205 (present in skin dendritic cells) was inoculated subcutaneously. The possible mechanisms of protection and the implication of this model in future new generation vaccines will be discussed.

Epidemiology and evolution of Influenza A virus, application of next-generation sequencing

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Influenza A virus is one of the most important pathogens of humans, responsible for 250,000 to 500,000 deaths annually and potentially millions during major pandemics. They are enveloped viruses with a genome composed of 8 segments of single-stranded RNA of negative polarity. Based on the antigenicity of their hemagglutinin and neuraminidase, influenza A viruses are classified into 16 HA subtypes and 9 NA subtypes, although recently new subtypes circulating in bats were described. Influenza A viruses infect many species of wild and domestic birds, with wild aquatic birds being though to represent natural reservoir of all influenza types. Apart of birds, influenza A viruses infect a large number of mammals, including humans. Influenza viruses are antigenically variable pathogens with a high evolutionary rate that gives them the capacity to evade the immune system and keep circulating in human populations. The two main evolutionary mechanisms that allow influenza viruses to constantly evolve and re-infect their hosts are antigenic drift and genetic shift. Antigenic drift occurs as a result of progressive accumulation of mutations that become fixed in the viral genome. These mutations can confer minor changes in the viral proteins that may be advantageous for viral fitness, including the capacity to escape immune system recognition. During antigenic shift, an influenza A virus strain may acquire the HA segment, and possibly the NA segment as well, from an influenza virus of a different subtype, resulting in a viral strain with novel antigenic proteins. We used next generation Illumina sequencing technology to determine whole genome sequences of human, porcine and avian influenza A viruses, for which evolutionary analyses were done. The high coverage also allowed us to analyze nucleotide polymorphisms present in unprecedented detail

MOLECULAR PATHOGENESIS OF HUMAN PAPILLOMAVIRUS ONCOPROTEINS

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The main etiological agent associated to cervical cancer is the human papillomavirus (HPV), being the high-risk types 16 and 18 the most frequently present in this cancer. The HPV is a DNA virus that belongs to the newly named *Papillomaviridae* family. The mechanisms by which HPVs induce cell transformation is through the oncogenes E6, E7 and E5 that codify for oncoproteins that have developed strategies to alter the cell cycle and apoptosis through targeting different cellular pathways, favoring viral persistence and promoting cellular transformation.

The E7 is a small 98 amino acids oncoprotein, with a NH-terminal domain that contains the CKII phosphorylation sites and the pRb binding site involved in the disruption of the E2F/pRb1 complex. The E7 oncoprotein shows different molecular weights and cellular localizations (cytoplasmic and nucleus), and phosphorylation may explain some of these variations. Recently, our group identified three isoforms of E7 from HPV16 present in CasKi cells, E7a1 (17.5 kDa and pI 4.68), E7a (17 kDa and pI 6.18) and E7b (16 kDa and pI 6.96), and by immunofluorescence showed that E7 is present in ER, Golgi and nucleus. This suggests that during the processing and transit of E7 through the different cell compartments, the protein could be post-translationally modify.

In contrast, the E5 oncoprotein is capable of increase the half-life of growth factor receptors, which suggest that E5 has a key role in viral life cycle and important in the early development of the neoplasia. Recently, we showed that E5 synergizes with EGF-receptor signaling pathway to down-regulate the $p27^{Kip1}$ protein levels and increase the S-phase allowing the cells to enter faster into the cell cycle (possibly a mechanism of E5 oncogenic activity) and preparing the suitable conditions for HPV establishment in the cell and the expression of the mayor oncoproteins E6 and E7.

The adenoviral oncoproteins: Multiple roles in virus-cell interactions

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The adenoviral genome encodes three groups of oncogenes that are responsible for establishing conditions in the infected cell that are conducive to efficient viral replication and progeny production. The products of these oncogenes are multifunctional proteins that inhibit anti-viral cellular defenses and simultaneously induce the almost exclusive expression of viral genes. The E1A proteins activate transcription of S-phase genes and inhibit the antiviral response. In turn, the proteins encoded in the E1B and E4 genes are responsible for the selective expression of viral late mRNA, regulation of tumor suppressors and inhibition of apoptosis. Together, these viral early proteins cooperate to induce cell transformation or the efficient production of viral macromolecules and progeny.

The study of adenoviral oncogenes has shown that their products are implicated in the reorganization of the infected-cell nucleus as they participate in the formation of virus-induced structures that constitute nuclear microenvironments that colonize the cell nucleus. These so called viral replication centers (RC) are the sites where viral DNA is replicated and transcribed, and where postranscriptional processing of viral late mRNAs that are selectively exported to the cytoplasm initiates. Interestingly, formation of viral RC recruit and co-opt cellular defense mechanisms that include components of the DNA damage response (MRN, BRCA1, ATR); tumor supressors (PML, p53); anti-viral response (STAT1); and a growing list of cellular proteins that would normally interfere with viral replication. Hence, viral RC may serve as hubs to direct viral replication and simultaneously control cellular defense mechanisms.

Using adenovirus-infected normal human cells we have found that formation of RC depends on concerted interactions between the early adenoviral proteins and cellular proteins that are co-opted in these sites. The presence of the E1B and E4 proteins in RC correlates with the efficient replication and expression of the viral genome; concomitantly these viral early proteins are seemingly also required to recruit cellular factors that are inhibited during viral replication. These findings suggest that the assembly of RC and the reorganization of the cell nucleus underlie the molecular mechanisms that the E1B and E4 adenoviral proteins regulate to take control of the infected cell during viral replication.

Protein synthesis inside mitochondria: the story of subunit 1 of the cytochrome *c* oxidase.

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Cytochrome *c* oxidase (C*c*O) is the last electron acceptor of the mitochondrial respiratory chain. Subunit 1 (Cox1), the largest subunit of the enzyme, is encoded in the mitochondrial DNA by the *COX1* gene. The protein is synthesized within mitochondrial ribosomes and inserted into the inner membrane to assemble with other subunits. In the yeast *Saccharomyces cerevisiae* synthesis of Cox1 is highly regulated, and is coordinated with assembly into the membrane (1). Our lab. is interested in understanding the mechanisms for regulation of Cox1 synthesis and assembly. Mss51 and Pet309 are translational activators that act on the *COX1* mRNA 5'-untranslated region to allow ribosomes to initiate translation (2,3). Today the mechanisms for translational activation are not well understood. We have demonstrated that Pet309 physically interacts with the *COX1* mRNA and with the mitochondrial ribosomes, and have studied different mutants of the protein to identify regions that are necessary for Pet309 functions. We have also demonstrated that a sub-population of Mss51 interacts with the mitochondrial ribosomes, and that this protein regulates the interaction of Pet309 with the *COX1* mRNA.
In addition, newly made Cox1 interacts with Mss51 and with other proteins to form high molecular weight complexes, which form intermediates of the C*c*O assembly. In the lab. we have described that the Cox1 carboxyl terminal end negatively regulates Cox1 synthesis (4), and have observed that it is the structure, rather than specific amino acids of the Cox1 C-terminal end that are necessary for this regulation. Along the talk we are going to review what is the current knowledge on the mechanisms that couple the mitochondrial Cox1 synthesis and assembly into the inner membrane.

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Dissecting the peripheral arm of the mitocondrial ATP synthase of chlorophycean algae.

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Mitochondrial F_1Fo-ATP synthase (complex V) makes ATP using the electrochemical proton gradient generated by the respiratory chain. It is an oligomeric complex embedded in the inner mitochondrial membrane that works like a rotary motor. *Chlamydomonas reinhardtii* and *Polytomella* sp., two members of the chlorophycean lineage of unicellular green algae, have a highly-stable dimeric mitochondrial F_1Fo-ATP synthase, with an estimated molecular mass of 1600 kDa. The chlorophycean enzyme contains the eight conserved polypeptides present in the vast majority of eukaryotes that represent the main components of the proton-driven rotary motor and the catalytic sector of the enzyme: subunits alpha, beta, gamma, delta, epsilon, a (ATP6), c (ATP9), and OSCP. Nevertheless, and in sharp contrast with other mitochondrial F_1Fo-ATP synthases like the one from beef heart, the algal enzyme seems to lack several classic components: the subunits of the peripheral stalk b, d, f, A6L, and F6, the subunits responsible for dimer formation e and g, and the regulatory polypeptide IF_1. Instead, the algal enzyme contains nine subunits with molecular masses ranging from 8 to 60 kDa named ASA1 to ASA9 (for ATP Synthase Associated proteins). These polypeptides have no clear orthologs in the databases and seem to be unique to chlorophycean algae. The nine ASA subunits build up a highly-robust peripheral stalk with a unique architecture, as observed on single-particle electron microscopy (EM) images.

Our group has found of interest to gain further insights on the close-neighbor relationships between the ASA subunits and their interactions with some of the classical subunits. We have therefore explored with some detail the topological disposition of the components of the algal mitochondrial ATP synthase using different experimental approaches: detection of subunit-subunit interactions based on cross-linking experiments, the yeast two hybrid system or reconstitution with recombinant subunits; generation of sub-complexes after partial dissociation of the dimeric ATP synthase; inference of subunit stoichiometry based on labelling of cysteine residues and modelling of the overall structural features of the complex from small-angle X ray scattering data and EM image reconstruction. Based on the results obtained from these diverse experimental strategies, we suggest a refined model for the topological disposition of the 17 polypeptides that constitute the algal mitochondrial ATP synthase.

Unveiling the ζsubunit of the α-proteobacterial F_1F_O-ATPase nanomotor: a novel natural inhibitor of rotation different to bacterial ε and mitochondrial IF_1.

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The F_1F_O-ATP synthase is a reversible nanomotor, found in energy-transducing membranes of bacteria, chloroplasts, and mitochondria that fuels life with ATP, coupling a transmembraneprotonmotive force to the chemical synthesis of ATP from ADP and Pi.Its basic structure and mechanism has been conserved through the biological kingdom, however the specific challenges that every ATP synthase should confront in each particular cell had promoted particular adaptations to obtain an optimal operation.Such is the case of the diverse mechanisms for regulating the ATP hydrolysis activity of the enzyme; this activity represents a powerful mechanism to generate a $\Delta\mu H^+$, but it can also lead to a hazardous loss of ATP under conditions of low oxygen or low light. Through biologic evolution,
some regulatory subunits have been integrated to the core structure of the F_1F_O complex to inhibit rotation of the nanomotor in the F_1F_O-ATPase turnover sense this favouring the ATP synthase turnover.Namely, theonly known F_1-ATPase regulatory subunits arethe rotaryε subunit of bacteria(1) and the accessory inhibitor IF_1 protein of mitochondria(2).

Despite its evolutive relevance, the regulatory mechanism of the F_1F_O α- proteobacteria, a class phylogenetically related with the ancestor of mitochondria, was unknown until our group discovered a novel inhibitory subunit in the ATP synthase of the α-proteobacterium*Paracoccusdenitrificans* denominated ζ(3). Remarkably, this subunit is not related in sequence neither in tertiary structure to the canonical F_1-ATPase inhibitors from bacteria (ε) or mitochondria (IF_1).In order to resolve this completely novel regulatory mechanism F_1F_Onanomotor, the characterization of the *P. denitrificans*F_1-ATPase ζ subunit (*Pd*ζ) was carried out by combination of kinetic, biochemical, cross-linking, bioinformatic, proteomic, and structural approaches showing that ζ blocks rotation through its N-terminal domain with a novel control mechanism and structure conserved along the α-protobacterial class(4,5)

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Energy associated with the binding of nucleotides in the isolated α and β subunits and their role in $(\alpha\beta)_3$ and $\alpha_3\beta_3\gamma$ subcomplexes.

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Nucleotide-induced conformational changes of non-catalytic α and catalytic β subunits with rotatory axis γ play a crucial role in the rotary mechanism of F1-ATP synthase. The interfaces formed in the complex affects conformational changes between subunits. To gain insights into the energetic bases that govern the recognition of nucleotide by isolated α and β subunits and complexes, the binding to Mg(II)-free and Mg(II)-bound adenosine nucleotides was characterized using high-precision isothermal titration calorimetry. A model that considers simultaneously the interactions of subunits with MgATP and ATP or MgADP and ADP, and in which ATP is able to bind two Mg(II) atoms sequentially, was used to determine the formation parameters of the α and β subunits. The two subunits exhibit nucleotide-binding thermodynamic signatures similar to each other, characterized by enthalpically-driven affinities in the μM range. Nevertheless, contrary to the catalytic subunit that recognizes MgATP and MgADP with comparable strength, the noncatalytic subunit much prefers the triphosphate nucleotide. Besides, the α-subunit depends more crucially on Mg(II) for stabilizing the interaction with ATP, while both subunits are rather metal-independent for ADP recognition. These binding behaviors are discussed in terms of the properties that the two subunits exhibit in the whole enzyme.

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Genomic history of domestication of common bean

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Modern civilization depends on few of the world's estimated 300,000 plant species for its nourishment, including common bean (*Phaseolus vulgaris*), the most important grain legume for human consumption. These crop plants were mostly derived by several thousand years of human selection, which transformed wild ancestors into high-yielding domesticated descendants. We report the reference genome (549.6 Mb) of a Mesoamerican bean, containing
28,520 protein-coding genes that together with twelve re-sequenced wild and domesticated genomes allowed the identification of DNA tracts targeted by artificial selection. This information along with a transcriptional atlas revealed hundreds of non-coding RNAs selected for during domestication, as well as genes likely defining organ identity. Our data provide powerful genomic resources for bean breeding programs faced with an ever-expanding human population and unpredictable environmental challenges.

Architecture and evolution of a minute plant genome

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It has been argued that the evolution of plant genome size is principally unidirectional and increasing owing to the varied action of wholegenome duplications (WGDs) and mobile element proliferation1. However, extreme genome size reductions have been reported in the angiosperm family tree. Here we report the sequence of the 82- megabase genome of the carnivorous bladderwort plant Utricularia gibba. Despite its tiny size, the U. gibba genome accommodates a typical number of genes for a plant, with the main difference from other plant genomes arising from a drastic reduction in non-genic DNA. Unexpectedly, we identified at least three rounds of WGDin U. gibba since common ancestry with tomato (Solanum) and grape (Vitis). The compressed architecture of the U. gibba genome indicates that a small fraction of intergenic DNA, with few or no active retrotransposons, is sufficient to regulate and integrate all the processes required for the development and reproduction of a complex organism. we infer from our analyses of U. gibba coding sequence that natural selection preserved a core set of gene functions, most of which have returned to single copy along with considerable genomic fractionation after three WGDs. Relaxed selection pressure or unnecessary functions probably led to gene losses, whereas in other cases, gene family expansions may have been promoted by selection. The U. gibba genome architecture demonstrates that angiosperms can evolve diverse gene landscapes while overall genome size contracts..

Common bean transcriptomics: analysis of global regulators for the rhizobia symbiosis and the abiotic stress responses

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Common bean (Phaseolus vulgaris) is the most important legume for human consumption in the world. It establishes symbiosis with rhizobial soil bacteria that reside in the specialized organs known as nodules symbiotic nitrogen fixation takes place.

The common bean genome sequence was recently disclosed (Schmutz et al. 2014; www.phytozome.net). Our group has contributed to develop common bean transcriptomics and used this approach to analyze global gene expression in the rhizobia symbiosis and in the plant responses to abiotic stresses such as nutrient deficiency. Combining the *P. vulgaris* genome sequence and predicted gene calls with the Illumina-RNAseq platform we have measured the gene expression patterns from seven tissues (24 RNA samples) of *P. vulgaris* cv. Negro Jamapa (O'Rourke et al. 2014). RNA samples included those collected from young and mature effective nodules as well as ineffective nodules incapable of SNF; we identified nodule specific genes and genes that are differentially expressed in effective vs. ineffective nodules.

The common bean transcriptome revealed more than 3,000 transcripts annotated as Transcription Factors (TF). These are essential global regulators for different cellular processes, including nodule development and response to abiotic stress. Current projects form our group focus in the identification and functional analysis of different TF families from common bean.

MicroRNAs (miRNAs) are recognized as central regulators of gene expression in plants controlling different fundamental processes. The miRNAs' role in post-transcriptional regulation is mediated by the almost perfect complementarity with their target mRNAs thereby causing its degradation or its translation inhibition. We used the recently released genome of common bean combined with high- throughput sequencing data from 6 small-RNA libraries obtained from 5 plant organs (Peláez et al. 2012), and degradome sequencing to identify a high confidence genome-proof common bean miRNA set and the associated targets.

Abstract for Congress of Sociedad Mexicana de Bioquímica 2014

Genome-wide perspectives on evolution and molecular adaptation in polar bear

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The development of high-throughput sequencing technology has opened doors to conservation and population genomic studies of wildlife and other non-model species. We have used a next-generation sequencing strategy to detect more than 13 million genome-wide variants in the polar bear and its closest relatives, the brown bear and American black bear. While their nuclear genomes reflect a species tree consistent with expectation, showing polar and brown bears to be sister species, the mitochondrial genome and 5–10% of the nuclear genome of the enigmatic brown bears native to Alaska's Alexander Archipelago (ABC brown bears) is more closely related to polar bears than to other brown bears, indicating the ABC brown bears are descendants of ancient admixture between the two species. Coalescent analyses suggest relatively ancient splits among polar, brown and black bears providing sufficiently independent evolutionary histories to leave imprints in the polar bear nuclear genome associated with ecological adaptation to the Arctic environment. However, paleodemographic estimates reveal that the polar bear has experienced considerable fluctuations in its effective population size with a prolonged and dramatic decline during the last few hundred thousand years. Taking such demographic factors into account has suggested a much more recent divergence date estimate leaving the exact split time between the two species unresolved. Nevertheless, the polar bear is a prime example of adaptive evolution in response to the extremes of life in the high Arctic extending from evident morphological features to more subtle physiological traits, and open questions remain whether candidate genes and positive selection played a significant impact in the evolution of the polar bear. We have examined the bear genomes and based on multiple different approaches identified candidate genes possibly involved in adaptation to life in the Arctic environment. As such, polar and brown bears provide an excellent system for investigating the evolutionary impact of climatic fluctuation for wildlife historical biogeography and species divergence.

NEURONAL DAMAGE AND EPILEPTOGENESIS INDUCED BY MONOSODIUM GLUTAMATE NEONATAL TREATMENT

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The existing dynamic balance between neuronal excitation and inhibition is altered drastically in response to neuronal damage, becoming a trigger for the onset of seizures, which coupled to initial damage, could lead to epileptogenesis. Between general inducing mechanisms of neuronal damage, the excitotoxicity is defined as the process that produces neuronal death by over-excitation, mainly mediated by glutamate, through both sensitive and non-sensitive N-methyl-D-aspartate (NMDA) ionotropic receptors. In our group, the systemic administration of monosodium glutamate (MSG, 4 mg/g body weight, at days 1, 3, 5 and 7, subcutaneously administered) in newborn rats has been used to study the neurochemical changes, histological and functional produced by the excitotoxic damage. Then, we have demonstrated that neonatal MSG treatment has short-and long-term effects, which are associated with the cognitive deficit and with the increased seizure susceptibility, observed after treatment. In this regard, we have recently showed that animals 60 days old, neonatally treated with MSG are more susceptible to seizures induced by 4-aminopyridine, bicuculline-methyl-iodide and pentylenetetrazol, but apparently resistant to those induced by NMDA. Furthermore, it also has been observed that the neuronal excitation threshold after the MSG treatment decreases drastically. The relevance of these results will be discussed with relationship to possible therapeutic strategies that could be followed after an excitotoxic neuronal damage.

“Mast cells and their involvement in brain damage and epileptogenesis processes”

Luisa Rocha
México

"Ferritin a stress oxidative marker in the neurodegenerative processes"

Alberto Lazarowski
Facultad de Farmacia y Bioquímica
Universidad de Buenos Aires, Argentina

"Are early metabolic alterations in the hippocampus of the mouse model APPswe/PS1d9 alzheimer's disease the cause of disease?"

Antoni Camins Espuny
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Molecular epidemiology of infectious diseases

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BACKGROUND. Molecular epidemiology of infectious diseases combines classic epidemiology with genotyping of the infectious pathogens. This symposium focuses on the molecular epidemiology of two globally relevant infections which are especially prevalent in Mexico and other developing countries: tuberculosis (TB) and human papillomaviruses (HPVs) associated to cervical cancer (CC).
TUBERCULOSIS. One third of the human population has latent TB infection by Mycobacterium tuberculosis Complex (MTC) bacilli. Each year around 10 million new cases of pulmonary TB (PTB) and one million deaths by this disease are registered in the world. *Mycobacterium tuberculosis* is the most prevalent MTC species. The primary TB infection is usually “latent” (i.e., MTC bacilli remain in the lungs in a latent state) but they may become “active” and propagate to induce tissue destruction. MTC bacilli have a double-stranded circular DNA chromosome whose sequence is around 4.4 million base-pairs long. Several molecular methods exploiting differential genomic characteristics allowing the identification of species, lineages, families and even individual MTC strains, are used in molecular epidemiology studies of TB.
HPV INFECTIONS. CC is the second cause of death by cancer in women around the world. It is due to the neoplastic transformation of epithelial cells located in the uterine cervix, which is caused by persistent infection of certain HPV types. Icosaedral HPV virions encapsidate circular double-stranded DNA genome molecules ~8,000 base-pairs long encoding six early (E) non structural genes and two late (L) structural genes. More than 160 HPV genotypes have been identified, among which HPV16 and HPV18 are the most prevalent in CC. Advances in the knowledge of HPV molecular biology and pathogenesis have led to the development of screening tests based on viral genotyping and to develop effective prophylactic vaccines.
CONCLUSIONS. Knowledge of MTC and HPV biology and genomics have contributed to a better understanding of the global and regional determinants of their epidemiology and to improve the strategies to control and prevent TB and CC.

Genetic diversity and transmission of the *Mycobacterium tuberculosis* Complex in San Luis Potosí

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BACKGROUND. The discovery of insertion elements and other repeated sequences in the genomes of the *Mycobacterium tuberculosis* Complex (MTC) species has led to genotyping pulmonary tuberculosis (PTB) isolates and to identify *M. tuberculosis* lineages shown to be associated to their geographical origin. MTC genotyping has led to new insights and TB epidemiology concepts that had been accepted for decades. Among these is the discovery of the highly virulent Beijing strains which tend to develop multidrug resistance and are spreading throughout the world.
RESULTS. We have analyzed the molecular epidemiology of TB for the first time on the complete territory of a Mexican state by spoligotyping and MIRU-VNTR genotyping. In San Luis Potosí 324 isolates (97.6%) had *M. tuberculosis* and eight (2.4%) had *M. bovis* spoligotypes; *M. tuberculosis* Lineage 4 predominated (93.8%), followed by Lineages 1 (4.3%) and 2 (1.9%). Spoligotype diversity and transmission-mutation indexes were 0.91 and 0.11, respectively. Twenty-five MIRU-VNTR genotypes were resolved among the 31 isolates from the seven epidemiologically linked spoligoclusters identified. Six *M. tuberculosis* isolate pairs were involved in recent transmission chains, only three of which had proven contacts. Two transmission chains were caused by Beijing isolates.
CONCLUSIONS. Our results confirm the genetic diversity of circulating MTC strains in San Luis Potosí that are part of the wider TB epidemic in Latin America. They also indicate that PTB epidemiology in San Luis Potosí is mostly due to endogenous reactivation, not recent transmission. Beijing strains appear to be emerging since they are involved in two of the six transmission chains identified.
PERSPECTIVES. Molecular epidemiologic surveillance should be performed in San Luis Potosí and all over the country. It may be implemented by a two phase investigation system in which the first interview to search for contacts is followed by a second interview of the TB patients whose MTC isolates are grouped in spoligoclusters. This strategy could increase the detection of cases with nonconventional epidemiologic links to prevent secondary infections. On the other hand, since in San Luis Potosí endogenous reactivation predominates over recent transmission, the DOTS program should be complemented with detection and treatment of latent PTB cases to prevent endogenous reactivation.

Experimental pulmonary tuberculosis, epidemiological contributions

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BACKGROUND. The host response against Mycobacterium tuberculosis show a wide spectrum of clinical manifestations in those patients who fail to control the infection. The course of the infection and its epidemiological consequences depend upon a complex interplay of host, environmental and bacterial factors. Experimental animal models have helped to define the influence of bacterial genetic diversity on virulence and on the immune response that is induced. For this purpose, experimental animals such as mice, guinea pigs and rabbits have been infected with selected clinical isolates obtained from outbreaks or from clinical epidemiology settings.
RESULTS. Mouse models have contributed substantially to defining the variability in virulence and immune response in relation to mycobacterial genetic diversity. Low dose aerosol infection in C57Bl mice or high dose intratracheal infection in BALB/c mice have demonstrated wide variability in virulence and immune responses induced by different bacterial genotypes, and each genotype has different phenotypes, with high and low virulence variants.
CONCLUSIONS. In general, these studies have shown that high prevalent strains from big epidemiologic clusters are more virulent than low prevalent sporadic clinical isolates, and highly virulent strains induce non-protective immune responses with some correlation with clinical–epidemiological data.
PERSPECTIVES. In the future selected strains from these types of studies should be analyzed with molecular technologies, such as mycobacterial genome sequencing or massive RNA sequencing to define the transcriptome using the lungs from infected mice. These kind of studies in correlation with wide epidemiologic studies will contribute to define the genes and factors from the bacteria and the host that contribute to the immune response and efficient control or progression of this significant infectious disease.

High prevalence and anomalous association with neoplastic lesions suggests an outbreak of human papilloma virus type 33 cervical infection in San Luis Potosí, Mexico

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BACKGROUND. To determine the prevalence of high risk (HR) and low risk (LR) human papilloma virus (HPV) types and their association with cervical neoplastic lesions 700 women residing in the state of San Luis Potosí, with diagnoses of normal cytology, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and cervical cancer (CC) were subjected to colposcopy, cervical scraping and biopsy sampling in 2007-2010. HPVs were genotyped using nested multiplex PCR of the E6 gene.
RESULTS. HPV-DNA prevalences: overall 67.7%, HR-HPV 63.1%, LR-HPV 21.3%; highest (78.2%) in the 15-24 yr group; 52% of the infections were by a single HPV type and the rest by two to six types. Most prevalent HR types: HPV33 (33.1 %), HPV16 (16.6 %) and HPV18 = HPV51 (6.7 %); most prevalent LR types: HPV6/11 (8.3 %), HPV43 (7.9 %) and HPV66 (5.3%). HR-HPV prevalence by lesion grade: normal 29.6%, ASCUS 26.7%, LSIL 63.3%, HSIL 68.2%, CC 90.5%. HR-HPV prevalence trends in progressive neoplastic lesions: *increasing* (LSIL < HSIL < CC) for HPV16, HPV39, HPV18, HPV58, HPV31 and HPV35; *asymptotic* (LSIL < HSIL ≈ CC) for HPV51 and HPV68; *U-shaped* (LSIL < HSIL > CC) for HPV33.
CONCLUSIONS. Two thirds of the women have cervical HPV infections which predominate in the 15-24 yr group. Half of the infections are caused by one and the rest by multiple HPV types. HPV33 is the most prevalent type, followed by HPV16. HR-HPV prevalence increases with the severity of cervical neoplastic lesions. HPV33 prevalence is highest in LSIL and its *U-shaped* trend differs from the *growing*/*asymptotic* tendencies of the other HR-HPV types. An outbreak of HPV33 may be causing its anomalous prevalence and association with LSIL. Prophylactic vaccination of the women included in the study could have prevented 23% of the infections, and 25% of the HSIL and 62% of the CC cases.
PERSPECTIVES. Neoplastic progression occurs only in the cervical epithelium of women persistently infected by high-risk HPV types and is the major biomarker of cervical cancer risk. In the current HPV post-genomic post-vaccine age, HPV detection and genotyping will soon become the primary screening test and will be used to monitor the efficacy of vaccines and to detect outbreaks.

Nanobiotechnology: challenges and opportunities in an emerging discipline

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A review of the fundamental principles behind nanoscience will be offered. Then, the use of such concepts in biotechnology will be discussed, under the light of potencial novel applications in a well-established science. A number of specific examples will be described in detail.

Biological systems as targets for study of new properties induced by nanomaterials

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Theconvergence of nanotechnology and medicine has generated a new expectation in thefield of pharmaceuticaltherapy. Thesilvernanoparticles (AgNPs) are widelyused in medicine and chemistryindustryduetotheirantimicrobialproperties. Howeverthereis a lack of informationabouttheir new biophysicalproperties, functions and effects at differentlevels of biologicalorganization, and theirimpacton human health. Theaim of thecurrentpresentationis show therecentadvancesthatourlaboratory has beeninvestigate, relatedwiththeeffectsthatconfertheAgNPs at differentbiological targets, and theirpotentialtoxicor beneficial implications in the cardiovascular (CVS) and respiratory (RS) systems. Weobserved in coronary and aorticbloodvessels, thattheseNPsinduced dual effects, at lowconcentrations, inducedvasoconstriction; at highconcentrations, stimulatedvasodilationmediatedbytheactivation of endothelialnitric oxide synthase (eNOS), which produces lowconcentrations of nitric oxide (NO), animportantvasodilator and antihypertensiveagent. However in the RS; in trachea, weshownthatAgNPs, inducedtoxiceffects, whenmodifiedthecontractileaction in presence of theendogenouscontractilemolecule, acetylcholine (ACh), inducinghyper-reactivitymediatedbythe inducible nitric oxide synthase (iNOS), promotinglargeamounts of NO relatedwithallergicmechanisms. These data suggest a specific and selectivemechanism of actioninducedbyAgNPSdependingonthebiological target. Furtherstudies are neededtoelucidatethesignalingpathwaysresponsibletopromotetheirtoxicor beneficial effects in the CVS and RS.

Heterogeneous biocatalysis. Laccase as an example.

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Heterogeneous biocatalysis is the use of immobilized enzymes (or enzymatic extracts) on inert supports in order to improve their operational stability and to facilitate the removal of reaction product. Heterogeneous biocatalysisis an important process in many fields such as pharmaceuticals, dyes, environmental, energy, among others.
It is well known for some enzymes that catalytic activity and stability are increased when they are immobilized on nanostructured supports. Here, the heterogenous biocatalysis with oxido reductase enzymes, which carry out the catalysis by means of radical reactions, is reported. The reaction mechanisms, molecular channels, position of the active site, surface composition and flexibility of amino acid residues of the enzyme were studied in order to make a better immobilization. Laccase (multicopper oxidase) from the white-rot fungus *Coriolopsis gallica*, in which theactive-site is composed by four copper atoms and is divided into three sub-sites, T1 (1 Cu^{2+}), T2 (2 Cu^{2+}) and T3 (1 Cu^{2+}). According to electron paramagnetic resonance (EPR) studies, the T1 site take out electrons from the substrate molecule and then the electrons are transferred to the T2/T3 sites, in which the coppers reduce O_2 to H_2O.
It has been observed that the removal of electrons from the substrate tothe site T1 can be conducted bytunneling, so it seemsunnecessary to havethe enzyme active site exposed to perform the catalysis when laccase is immobilized on a nanostructured support. Furthermore, the laccase immobilization on nanostructured support confers operational and catalytic stabilization to the enzyme.

Virus-like nanoparticles as potential carriers of cytochrome P450 for chemotherapy pro-drug activation

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Theencapsulation of enzymesinside virus-likenanoparticles (VLPs) orotherproteincages has been a fastgrowingtopicbecause of itsimplications in biocatalysis as well as theirpotential as enzymaticdeliverysystems. Wereportforthefirst time theencapsulation of a CYP450 whichbelongto a family of enzymesmedicallyimportant. A variant of CYPBM3 from*Bacillusmegaterium,*that has beenrationallymutatedforenhancedperoxidaseactivity and forincreasedstabilityagainstthe suicide inactivationusingcombined QM/MM calculations [1], wasassayedforencapsulation. Thisstablevariantwaseffectivelyencapsulated in VLPsconstituted of coatproteinfromcowpeachloroticmottle virus (CCMV). ThecatalyticVLPs are abletotransformthechemotherapeutic pro-drug, tamoxifen, and theemerging pro-drugresveratrol [2]. Thechemicalnature of theproductswasidentified, confirming similar active productsthanthoseobtainedwith human CYP. TheenzymaticVLPsremainstableafterthecatalyticreaction. Theaim and innovation of thisworkistomake more efficientchemotherapydrugsactivatingthemmainly in the target tissueavoidingthedramaticsideeffects and reducingthe doses. Theencapsulation of this CYP450 in viral structureswillprovide a modelforthedesign of biocatalyticallynanoparticleswithpotential medical applications.

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POR QUÉ ES IMPORTANTE LA LAICIDAD EN BIOÉTICA

Roberto Blancarte
El Colegio de México/Colegio de Bioética

Esta presentación abordará la compleja relación entre ciencia, moral religiosa, ética ciudadana y regulación estatal en sociedades plurales y modernas como la mexicana. Para ello, en una primera parte se explicará la razón de ser del Estado laico, sus orígenes y desarrollo. En una segunda parte, se tocarán los diversos puntos nodales y razones centrales que conducen a la necesidad de una gestión laica tanto de la esfera pública como de la investigación científica, en este caso particular en el área de la bioética. Dos cuestiones especialmente relevantes serán la del lugar de las creencias religiosas en la esfera pública y la manera como se definen socialmente los derechos humanos y la moral publica.

De las Células Troncales a la Dignidad Humana Pasando por el Cigoto.

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En la literatura sobre genética médica o asuntos reproductivos en nuestra especie, se usa mucho el termino dignidad humana, como algo que debe protegerse a toda costa y sobre casi cualquier otra consideración. Si se revisa en el diccionario de la Real Academia Española, el significado de esta expresión, pues parece bien que es algo que merece nuestra protección. El asunto empieza a empantanarse, cuando nos percatamos que este concepto, dignidad humana, es cierto para cualquier miembro de nuestra especie sin importar la edad, incluyendo no sólo a bebes, niños, fetos, embriones y aun el cigoto mismo. La pregunta inevitable es ¿por qué el cigoto?, una sóla célula tiene dignidad, cuando ninguna de las característicasde lo que se define como dignidad es aplicable al cigoto. Un análisis casi detectivesconos llevó a la conclusión que el cigoto tiene dignidad porque todos somos hijos de Dios, un Dios creador como lo señala la tradición judío-cristiana. El principal impulsor de esta idea, parece ser el Vaticanocomo se desprende de una publicación de Lourdes Motta en 2008, sobre la clonación humana en las Naciones Unidas. En uno de los anexos se incluye una opinión de la Santa Sede que asegura que toda persona tiene dignidad por ser hijo o hija de Dios. Esta concepción ha funcionado en las Naciones Unidas por ejemplo, donde varias de sus
decisiones en relación a la fertilización asistida o la clonación, se basan en lo recién dicho. Para México, país laico, resulta contradictorio el tomar decisiones de política interna o externa en bases meramente religiosas.

Muerte encefálica y trasplante de órganos: ¿estamos bien?

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Moralmente, extraer un órgano de alguien, aun cuando sea para beneficiar a otro, se justifica sólo si dicho órgano ya no le es útil a la persona de donde proviene. El análisis de beneficio/riesgo debe ser el mismo para quien dona el órgano como para quien lo recibe. La forma más segura de determinar que un órgano ya no es útil para un sujeto es cuando éste dejó de existir como persona, cuando ha muerto. A esto se le conoce como la “regla del donador muerto” y constituye la justificación moral para el uso de órganos para trasplante.

En la sociedad encontramos diversas concepciones de muerte, la mayoría de origen religioso pero que en realidad responden más a conceptos culturales. Identificamos tres ideas básicas surgidas a través de la historia de la humanidad:

el concepto religioso-filosófico tradicional que utiliza la separación de alma y cuerpo; el concepto médico secular tradicional enfocado al cese de los fluidos vitales (sangre-aire, la función cardiopulmonar); y la posición moderna sobre la pérdida de la capacidad integradora del cuerpo dada por el sistema nervioso central (conocida como “muerte cerebral”). Una posición más reciente destaca la importancia de la conciencia humana para sus habilidades de pensar, razonar, sentir, experimentar e interactuar con otros, además del control de las funciones corporales (la llamada “muerte cerebral superior”).

La aceptación final de estas concepciones depende de autoproponernosuna “cláusula de conciencia” en donde lasociedad en general y los individuos en particular, al verse confrontados con eldiagnósticodemuertedeterminadoconcriterioscerebrales(¿inclusocerebralessuperiores?), tengan la posibilidad de decidir si éstos son aceptables o no; el principio de autonomía debe prevalecer.

Etica científica: la responsabilidad de los científicos y los límites de la ciencia

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La ciencia es una actividad creativa cuyo fin es la obtención de conocimientos sobre todos los aspectos de la naturaleza y sus mecanismos, mediante la observación y la experimentación, para lograr un análisis objetivo de la realidad. En esta definición está implícito que una conducta científica ética tiene como valor central la honestidad intelectual para planear, realizar y analizar la investigación de la manera más objetiva posible, buscando no confirmar la hipótesis planteada sino contestar a la pregunta que la hipótesis genera, sea en el sentido positivo o negativo. Un científico que no sigue esta regla fundamental, por la razón que sea, incurre en una conducta no solo equivocada sino claramente fraudulenta, ya que antepone la subjetividad a la objetividad. Aunque los fraudes han ocurrido desde la aparición de la ciencia moderna, cuando empezó a basarse en datos experimentales más que en teorías filosóficas, el crecimiento extraordinario de la ciencia en todo el mundo se ha acompañado de un incremento de las diversas modalidades de fraude científico. Estas modalidades del fraude propiamente dicho van desde el invento de resultados cuando ni siquiera se hicieron los experimentos, hasta la modificación de los resultados obtenidos para que concuerden con la hipótesis generada por el autor. Otro tipo de fraude es el de plagio de enfoques experimentales, de resultados o de interpretaciones, presentándolos como propios y originales a pesar de haber sido copiados de una investigación ya publicada.

En otros casos, el fraude consiste en mejorar los datos u observaciones mediante un manejo inescrupuloso de los análisis estadísticos o modificaciones de los datos para que parezcan más claros o más definitivos. Sin embargo, para juzgar qué tan fraudulentos son estos cambios es necesario considerar varios factores, como el contexto general del trabajo, qué tan grave es la alteración de los datos y, sobre todo, qué tan reproducible es el resultado cada vez que se repite el experimento. Es importante distinguir los verdaderos fraudes de los errores, que son inevitables en cualquier actividad humana, aun en la investigación científica. Sin embargo, en estos casos es precisamente la ética científica la que obliga al científico a reconocer el error en cuanto se percate de ello o alguien se lo haga ver, aunque esto signifique en ocasiones tener que retractar la publicación.

Un aspecto fundamental de la ética científica es su práctica durante la formación de nuevos investigadores, es decir durante la educación científica. Un tutor con conducta no ética generará en sus estudiantes actitudes científicas inaceptables, a veces sin que ellos se hagan conscientes de que tal conducta es fraudulenta.

MEDIATOR 25 regulates root architecture and intraspecific plant competition Via auxin signaling. José López-Bucio[1*], Javier Raya-González, Randy Ortiz-Castro, León Francisco Ruíz-Herrera, Edith Muñoz-Parra, Ramón Pelagio-Flores.
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Root system architecture is a major determinant of water and nutrient acquisition as well as stress tolerance in plants. The Mediator complex is a conserved multi-protein complex that acts as universal adaptor between transcription factors and the RNA polymerase II. In this report, we characterized possible roles of the MED8 and MED25 subunits of the plant Mediator complex in the regulation of root system architecture in Arabidopsis (*Arabidopsis thaliana*). We found that loss-of-function mutations in PFT1/MED25 increase primary and lateral root growth as well as lateral and adventitious root formation. In contrast, *PFT1/MED25* over-expression reduces these responses, suggesting that PFT1/MED25 is an important element of meristematic cell proliferation and cell size control in both lateral and primary roots. PFT1/MED25 negatively regulates auxin-transport and response gene expression in most parts of the plant, as evidenced by increased and decreased expression of auxin-related reporters *PIN1::PIN1::GFP*, *DR5:GFP*, *DR5:uidA* and *BA3:uidA* in *pft1-2* mutants and in *35S:PFT1* seedlings, respectively. No alterations in endogenous auxin levels could be found in *pft1-2* mutants or in *35S:PFT1* over-expressing seedlings. However, detailed analyses of *DR5:GFP* and *DR5:uidA* activity in wild-type, *pft1-2* and *35S:PFT1* seedlings in response to indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA), and the polar auxin-transport inhibitor 1-*N*-naphthylphthalamic acid (NPA), indicated that PFT1/MED25 principally regulates auxin transport and response. Using an agar-plate system, we compared the growth of WT and PFT1/MED25 mutants varying the density of individuals per plate. Mutant seedlings were unable to sense the number of individuals in the vicinity, indicating a mechanism for quorum-sensing perception in plants. These results provide compelling evidence for a role for PFT1/MED25 not only as an important transcriptional regulator of root system architecture but also in intraspecific competition through auxin-related mechanisms in Arabidopsis.

Phaseolus vulgaris TOR gene is required for rhizobial infection, nodule development and symbiotic nitrogen fixation

Target of rapamycin (TOR) is an atypical Ser/Thr protein kinase that belongs to thephosphoinositide 3-kinase (PI3K)-related kinase family and is structurally andfunctionally conserved among all eukaryotes. TOR has emerged as a centralcoordinator of nutrient, energy, and stress signaling networks (Wullschleger et al., 2006; Robaglia et al.,2012, Yuan et al., 2013). An *ArabidopsisTOR* mutant is embryo lethal and affects plant growth, implicating TOR in an essential role during plant growth, development and senescence. The role of TOR in symbiotic association is poorly understood. This prompted us to explore the role of TOR in the *P. vulgaris-Rhizobium* symbiosis. TOR transcripts were downregulated byRNAi approach in the *phaseolus* hairy roots. The *Rhizobium* inoculatedtransgenic roots show infection thread abortions within the epidermal cell. Nevertheless, some infection events resulted in development of structurally altered nodules. Ultrastructure of these nodules showslarge symbiosomeswith fewer bacteroids, degenerating bacteria and degrading infection pockets. The nitrogenase assay further confirmed the non-functionality of the nodules.Given our results we propose that TOR is involved in rhizobial infection and nodule development processes of*Phaseolus vulgaris*.

Zygotic Genome Activation in inbred and hybrid embryos of *Arabidopsis thaliana*

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Fertilization in plants and animals creates a diploid zygote by fusion of haploid egg and sperm. This amazing process requires male and female gametes to reconcile their highly differentiated genetic and epigenetic states, while establishing the template for the adult organism. In animals, it is well accepted that initial development of the zygote is primarily under control of maternally deposited RNAs and proteins, present in the egg before fertilization. Maternal control of early embryogenesis avoids errors in development while the newly diploid zygotic genome is consolidated. Depending on the animal species, the zygotic genome takes full control of development several hours to a day after fertilization.

Despite the importance of zygotic genome activation (ZGA) for embryogenesis and seed development, this process is less understood in plants than in animals. In Arabidopsis, two recent genome-wide transcriptional studies used hybrid embryos as the basis for profiling of maternal and paternal transcripts, and came to opposite conclusions, causing confusion regarding ZGA in plants. One study concluded that there was immediate transcriptional activation of virtually the entire genome (Nodine and Bartel, *Nature*, 2012), while a second study found that early embryogenesis was primarily under maternal control, with paternal transcription that increased gradually over the first few days of embryogenesis (Autran et al., *Cell*, 2011).

My laboratory took a functional genetic approach to test the activity of paternal alleles after fertilization. Using 49 *embryo defective* (*emb/+*) mutants of *Arabidopsis thaliana*, we tested whether wild type paternal alleles could rescue early embryo phenotypes caused by mutant maternal alleles. Our data demonstrates that in isogenic embryos, some genes show full activity as soon as they are required, while the majority show gradually increasing activity during early embryogenesis. We also tested gene activity in different hybrid embryo combinations, where we found surprisingly large variation in paternal gene activity, explaining the inconsistencies in previous transcriptional studies (Del Toro De León et al., *Nature*, in press). Our results show that genome-wide zygotic activation does not occur in Arabidopsis, resolving a debate in the literature that began almost 15 years ago. Instead, paternal allele activity varies widely between genes. Further, our observations on the effects of hybridization on gene activityin embryos have important implications for basic research on gene regulation, embryogenesis, and seed development, as well as for the study and use of heterosis (hybrid vigor) in crops.

Tracking the genetic pathway of pollen rejection in *Nicotiana*

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Plants avoid self-fertilization trough a genetic mechanism called self- incompatibility (SI), which allows plants to discriminate between self-pollen and pollen coming from relative plants, rejecting after recognition the self-pollen. The recognition reaction relies on the *S*-allele specific interaction between the male determinant (pollen expressed) and the female determinant (pistil expressed). However, genetic evidences indicate that modifiers genes (MG) are also essentials. In our group, we identified and cloned *NaStEP*, which is an essential MG to SI in *Nicotiana*. *NaStEP* codes a Kunitz-type proteinase inhibitor that seems to be specific to subtilisin-like proteases. NaStEP is taken up by both compatible and incompatible pollen tubes (PT). Its suppression in *Nicotiana spp*. causes SI breakdown. When NaStEP is suppressed, HT-B protein is degraded on the inside of both incompatible and compatible PT, which is contrary to what happens in SI *N. alata*, where HT-B is only degraded in crossbreeding compatible PT, indicating that NaStEP is a positive regulator of the HT-B stability in *Nicotiana* PT during the SI response. Because NaStEP is taken up by PT and is essential for SI, we hypothesized that its function is through protein-protein interactions with pollen proteins. To test it, we performed yeast two-hybrid assays and we found that NaStEP interacts with a specific pollen protein that is only expressed in *Nicotiana* SI species. NaSIPP has homology to mitochondria phosphate transporters. This interaction was corroborated by a BiFC approach using *Arabidopsis* hair roots. To get further information about NaSIPP localization in PT, we co-expressed NaSIPP fused to the fluorescent protein RedTomato along with the construct Mito-GFP, which targets GFP to mitochondria. Results indicated that an important NaSIPP fraction colocalized with mitochondria. In addition, when NaStEP was fused to GFP and NaSIPP to redTomato and we used them to co-expressed both constructs in PT, we found that both proteins colocalized in organelles which are probably mitochondria.

Results will be discussed under S-RNase compartmentalization pollen rejection model.

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Title: **Epigenetic regulation in the common bean (*Phaseolus vulgaris* L.) and its potential use for crop improvement.**

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Abstract.

Genetic studies along with biochemical and cell biological analyses in plant model systems have enabled researchers to understand how proteins are recruited to chromatin and how they regulate their target genes and to elucidate their functions. Accordingly, it has become evident that a majority of human genes that were suspected or known to play a role in disease had orthologs in the model plant *Arabidopsis thaliana* and many other plants (like the common bean, *Phaseolus vulgaris* L.). Also, it is now recognized that many disease and defense responsive mechanisms in Arabidopsis appear to be under epigenetic control, analogous to roles played by animal Polycomb Group/Trithorax Group (PcG/Trx) complexes in the regulation of senescence, disease and cancer, though it is not clear how plant pathogens manipulate, for example, host post-translational modifications (PTMs) and how they use these PTMs to solve their own biological requirements. While it has become clear in recent years that many stress responses involve epigenetic components, we are far from understanding the mechanisms and molecular interactions. Extending our knowledge is fundamental, not least for plant breeding and conservation biology.

Grain legumes, and particularly the common bean (*Phaseolus vulgaris* L.), are known to be recalcitrant towards *in vitro* regeneration. Consequently, genetic transformation is hard to achieve for this organism. Therefore, it is crucial the development of an efficient method for the creation and establishment of regeneration-competent callus and its transformation, as a first step towards an efficient plant regeneration system and genetic transformation in *P. vulgaris*.

In addition, the establishment of a chromatin state required for inducible defense against pathogens ('priming'), and how to manipulate the epigenetic processes involved in the interaction plant-microorganism are key points in order to improve future plant breeding and crop productivity.

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Epigenetic Control by Small RNAs duringMaize Somatic Embryogenesis

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Small RNAs (sRNAs) play important roles in response to developmental cues, biotic or abiotic stresses, during fertilization and embryogenesis. Maize somatic embryogenesis is induced, by placing the immature zygotic embryo under high auxin levels. Upon few weeks somatic embryos could be readily observed on the original explant. However, it takes more time to establish a highly proliferating culture of the induced somatic embryos conserving a high ability to regenerate healthy plants. Deep sequencing of sRNAs during maize somatic embryogenesis induction, establishment and maintenance revealed profound changes in specific microRNAs (miRNAs) and repeat-associated siRNAs (ra-siRNAs) mapping to particular transposon sequences.

Three essential components of the sRNAs biogenesis pathways and their regulatory function areArgonaute (AGO), Dicer-like (DCL) and RNA-dependent RNA polymerase (RDR) proteins.A phylogenetic analysis of reported ESTs in maize identified 5 RDR, 5 DCL and 19 AGO genes in maize showing putative homology relationship with the reported *Arabidopsis thaliana* proteins. The expression profile at transcript level of thesegenes drastically changes between the explant (immature embryo) and early-induced proliferative callus (four months of subculture). Consequently, the levels and size of ra-siRNAs mapping to specific transposons, mostly from Copia and Gypsy families, showed great fluctuations between the induction and establishment periods of somatic embryogenesis. The methylation status and expression levels of specific transposons were analysed to approach the epigenetic regulation in earlyestablished- and long term-embryogeniccalli.

Maize plants can be regenerated from somatic embryos in the presence of photoperiod when the hormones are removed from the culture medium.The abundance during plant regeneration of miRNAs highly expressed in maize embryogeniccalli, majorly respond to the hormone removal, while the photoperiod has little effect on their abundance. On the other hand, miRNA target gene expression seems to be strongly regulated by the photoperiod, and a correlation between miRNAs and their target levels can only be seen in the dark. The expression of specific miRNAs changes with the time of subculture, establishing a pattern in highly proliferating long term-subculturedembryogeniccallidifferential to the original explant. How is this related to plant regeneration efficiency? Early-established calli are usually inefficient for plant regeneration, whereas the long term-establishedones are preferred for plant regeneration.

Our findings expose an interesting and novel panorama of howsRNA-mediatedepigenetic regulation impacts the establishment, propagation and plant regeneration during maize somatic embryogenesis.

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miRNAs and fruit development

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More than 80% of our food comes fromflower and fruit parts. Therefore, understanding the genetic networks that regulate how these organs are formed is important. We are interested in studying the regulators of these networks with a special focus in pistil and fruit development. Transcription factors are one of the main regulators, and also miRNAs are involved. MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression in animals and plants, and are involved in the control of many developmental processes, including fruit development.We analyzed the expression of miRNAs in different tissues of 3 species of algae and 31 representative species across vasculature plants and this study demonstrated widespread conservationand divergence of microRNAs. Based on miRNA conservation, we performed a miRNA expression screenduring fruit development of the cactus species *Opuntiaficusindica*, or the so-called prickly pear cactus. Furthermore, we started to analyze the function of some miRNAs in tomato fruits. The latest results will be presented.

Epigenetic changes during in vitro culture of important plants

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ABSTRACT

In vitro plant cell and tissue culture techniques are the basis of many micropropagation and breeding programs for scientific research. Plant tissue culture (PTC) involves organogenesis and embryogenesis, and the outcome depends on the different conditions to which the tissue is exposed. PTC is a stressful environment – high relative humidity, low ventilation rate, high concentrations of plant growth regulators, and low light availability – for plants that need to rapidly change their molecular regulation in order to respond fast and efficiently during cell division and growth. New data has come out about a connection between plant morphogenesis and epigenetics. Epigenetics is a very sensitive regulatory mechanism, which in most of cases is affected by the environment. Although it is known that, under plant morphogenesis, the genome has little or no change, DNA methylation and histone modifications are very susceptible to those *in vitro* environmental conditions. *Agave spp*. and *Coffea spp*. are two successful cases of plants propagated *in vitro* that need to remodel their chromatin in order to generate new organs. In the present work, I will talk about how key genes related to organogenesis and embryogenesis are regulated by DNA methylation and histone modifications and how it will be important to identify factors that help to find climate-resistant plant and increase plant productivity.

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Order vs chaos: the regulation of vesicle traffic in *Neurospora crassa*

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In eukaryotic cells vesicle traffic involves budding, transport, tethering and fusion of vesicles with the acceptor membrane. Small RabGTPases in their GTP-boundactive state interact with the membrane of the vesicles and promote their association with tethering factors, before the subsequent fusion of the vesicles with a target membrane. Fungal hyphae contain at their apex the Spitzenkörper (Spk), a multi-vesicular structurewhere cargo-carrying vesicles concentrate before going on to fuse with the plasma membrane (PM). We have found that the exocyst, an octameric-tethering factor, is needed for the accumulation of vesicles at the Spk. To further characterize the regulatory mechanisms that ensure the directionality of the secretory vesicles that reach the Spk, we analyzed the *N. crassa*Rab-GTPase YPT-1 (Rab1), a homologue of the *Saccharomyces cerevisiae* Ypt1p*,* which regulates different secretory pathway steps. Fluorescently tagged YPT-1 was found at the Spk at the hyphal apex and at diverse punctate structuresthorough the hypha. Co-expression of differentially labeled YPT-1 and the predicted post-Golgi RabGTPases SEC-4 (Rab8) and YPT-3 (Rab11) showed that YPT-1 was confined to the microvesicular core of the Spk, while SEC-4 and YPT-3 occupied the Spk macrovesicular peripheral layer, suggesting that the orderly traffic of macrovesicles and microvesicles at the Spk is differentially regulated by distinct Rabs. The accumulation of YPT-1 and YPT-3 at the core and the outer layer of the Spk, respectively, was insensitive to brefeldin A, an ER to Golgi inhibitor, confirming previous observations that suggested the existence of non conventional secretory pathways involved in the biosynthesis of cell wall building vesicles.Partial colocalization of YPT-1 with the early Golgi markers USO-1 (p115) and VRG-4, and the late Golgi marker VPS-52 confirmed that the cytoplasmic YPT-1 punctate structures corresponded to both, early and late Golgi cisternae, consistent with the Golgi maturation model. In sum, these studies show the involvement of RabGTPases in regulation of vesicle traffic.

How do fungi transform dead plants into fruiting bodies?

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Fungi are the great recyclers in terrestrial ecosystems, as it is estimated that plant photosynthesis would deprive the carbon dioxide from the atmosphere in few years without their action. They are the only organisms able to efficiently breakdown lignocellulose, a highly recalcitrant material, whose degradation required the concerted action of many enzymes. Their ultimate goal is to produce spores ensuring their dispersal and reproduction. How fungi achieve the transformation of plant biomass into spores is not yet fully understood. We use the filamentous fungus *Podospora anserina* to understand the molecular mechanisms that participate in both the degradation of lignocellulose and the formation of the sexual fruiting body, the only mean of spore formation in this species. This fungus is well suited for such a task, because a high quality genome sequence and a very efficient gene inactivation system are available. Moreover, *P. anserina* is able to complete in ten days its lifecycle using woody materials as sole food source. Through a combination of forward and reverse genetic approaches, associated with genomics and cytology, we were able to identify enzymes and signaling pathways connecting biomass degradation and fruiting body formation. A common theme in both processes is the involved of reactive oxygen species generated by several enzymes, including
NADPH oxidases. In the talk, I will present an overview of these signaling pathways, including some of the partners that we recently identified.

A non-canonical function of the yeast high osmolarity glycerol (HOG) pathway

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Eukaryotic organisms, including yeast,contain evolutionary conserved signaling MAPK pathways that are utilized in many stressing environmental conditions. One of them is the HOG pathwaythat is dedicated to producecompatible solutes, such as glycerol, needed to cope with external high osmolarity. The HOG pathway is composed of two branches each containinga transmembrane protein (Sho1p and Sln1p) that act on downstream proteins. These two branches converge in the MAPKK Pbs2p. Scaffold and kinase activities of Pbs2p are required to activate the MAPK Hog1p by phosphorylation, which once active, goes into the nucleus to regulate transcription of several genes.The SHO1 branch is composed of proteins that are share with the pheromone-response and filamentous MAPK pathways. This branch includes Ste20p, Ste50p and the MAPKKK Ste11p. The SLN1 branch, on the other hand, is a phosphorelay system composed of the histidine-kinase Sln1p, the phosphotransfer protein Ypd1 and the response regulator Ssk1p. This last protein triggers autophosphorylation of the redundant MAPKKK Ssk2p and Ssk22p, subsequent phosphorylation of Pbs2p and activation and nuclear internalization of Hog1p.Additionally the HOG pathway is known to participate in some other non-osmotic stresses, including cold stress, heat stress, low pH, inhibition of sphingolipid synthesis, etc. In these cases the kinetics of Hog1p phosphorylation is different from that observed upon hyperosmotic stress. It is unknown how Hog1p is activated by these stresses, but in most cases, its activation requires only the SLN1 branch or the SHO1 branch, but not both. We have analyzed the participation of the HOG pathway in the response to the antibiotic tunicamycin (Tn), which causes endoplasmic reticulum stress in yeast. We determined that resistance to Tn requires Hog1p, the scaffold, but not the kinase activity of Pbs2p and the presence of the phosphorelay SLN1 system. When cells are treated with Tn, Hog1p is not phosphorylated and does not go into the nucleus, but its kinase activity is required to allow normal growth. A gain of function suppressor screening allowed us to determine that an ER protein required for dolichol synthesis (apolyprenyl alcohol required for protein glycosylation)is able to reverse the Tn sensitivity of the *hog1* mutant. These findings suggest that Hog1p may be required to directly or indirectlypromote protein glycosylation.

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ROS signaling and fungal development

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We proposed that once confronted with oxidative stress, microorganisms evolved mechanisms not only to cope with stress but to also use this ancestral form of stress to regulate cell differentiation. Our research is aimed at establishing the role of reactive oxygen species (ROS) as growth and cell differentiation signals and to understand the mechanisms that modulate ROS production, perception, detoxification and action in filamentous fungi. We have shown that the ROS-producing enzymes NADPH oxidases (NOX) are essential for sexual differentiation in *Aspergillus nidulans* and *Neurospora crassa* and for polar growth and cell-fusion in *N. crassa*. Currently we are studying NOX regulation and localization. Regarding ROS perception and detoxification, we found that response regulator (RR) SskA transmits oxidative stress signals to the stress MAPK SakA, which in turns physically interacts with ATF/CREB transcription factor AtfA in the nucleus. This defines a general stress-signalling pathway, which plays differential roles in oxidative stress responses during growth and development. We have shown that SakA phosphorylation is a conserved mechanism to regulate transitions between non-growing (spore) and growing (mycelia) states. We show that SrkA protein kinase is part of the SakA pathway and mediates its repressing functions during sexual development. In addition to SakA, we show that the transcription factors SrrA and NapA are differentially involved in ROS signaling and cell differentiation.

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A new proposal for bacterial evolution: What we have learned from the analysis of *Azotobactervinelandii* genome.

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Classical population genetics models are based in the fact that organisms in the present generation have a direct ancestor in the previous generation, so organisms have a common ancestor, and all lineages will at some point become extinct, due to genetic drift. However if organisms produce dormant forms as seeds that germinate in a period longer than the reproductive time of individuals, the premise of the presence of an ancestor in the previous generation of a contemporary organism, will not be true in all cases. It has been shown that the dynamics of populations of organisms producing dormant forms that germinate in a period that “jump” a number of generations that is considerably smaller than the number of individuals in that population (N), will be the same as populations that do not produce seeds.

Blath et al (1), described recently a population genetics model that consider the case where a population produces seeds that “jump” an unbounded number of generations, in such a way that the period of dormancy in average is much higher than the reproductive time of individuals. This model predicts that populations presenting stron-seed bank effects will not behave as described by classical population genetics models. Individuals in the present generation might not share a common ancestor, genetic variability is huge due to the existence of a genetic pool that is not subject to selection, and hence linages might not become extinct.

We have proposed that bacteria fulfill the postulates of the Blath et al, model, since they produce, spore or cysts that can last for much longer periods (tens of years) than their generation time (a couple of hours); besides, a considerable part of bacterial genomes is encoded as part of phage genomes. These considerations plus the existence of horizontal gene transfer (HGT) are the basis for the proposal of a novel model of bacterial evolution that will be discussed.

The analysis of *Azotobactervinelandii*genome gives strong support to this novel model, since we showed that this bacterium posses genes that are essential for its biology that are not derived from a common ancestor with *Pseudomonas*, its most closelyphylogenetically related bacterial genera.

Post-transcriptional and post-translational alginates regulation synthesis in *Azotobacter vinelandii.*

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Alginates are an important family of biopolymers with biotechnological interest.These polymers are linear polysaccharides, which are composed of variable amounts of (1–4)-β-D-mannuronic acid and its epimer, α-L-guluronic acid. The GacS/GacA two-component system is involved in the control of alginate production.A mutation in *gacA*abrogates alginate synthesis.In many γ-proteobacteriaGacS/GacA controls a post-transcriptional regulatory system that consists of a protein called RsmA or CsrA, and two or more small RNAs (sRNAs). Previously in *A. vinelandii* we identified and characterized two sRNAs,named *rsmZ1* and *rsmZ2*,mutations in *rsmZ1* and *rsmZ2* diminished the alginate production. We also showed that RsmA interacts with the *algD* mRNA, encoding GDP-mannose dehydrogenase, a key enzyme of the alginate biosynthetic pathway. Recently we identified seven new sRNAs of the Rsm family. In similar growth conditionsmutants in these sRNAs didn´t present alteration in the alginates production.Interestinglywe find that the regulatory regions of *rsmZ1* and *rsmZ2* are more complex than the regulatory regions of the *rsm*genes recently discovered. We are currently studying the differential expression of thesesRNAs*rsm*genes. Our efforts to construct amucoid strain carrying an *rsmA* mutation were unsuccessful; the isolated derivatives carrying the *rsmA* mutation also carried wild-type*rsmA* copies. *A. vinelandii* is a polyploid bacterium, and the isolation of mutants carrying wild-type copies of the mutant gene (merodiplioid) is a common phenomenon. However we are able to recover null mutants in non mucoid strains, this fact suggests that the alginate overproduction in *rsmA* mutants is probably toxic for the bacteria.

Moreover we found threeprotein histidine kinases (HPK)involved in alginate production. The HPK´s belong to two component systems that possibly control the c-di-GMP levels. In other bacteria the c-di-GMP controlspost-translationallyproteins that possess PilZ domains. The domain analysis of Alg44 alginates polymerization protein reveals a PilZ domain which could be related with the alginates synthesis regulation in *A. vinelandii*.

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Isolation and Characterization of Cr(VI)-Reducing Bacteria with Potential Application in Soil Bioremediation Strategies

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Hexavalent chromium is a dangerous mutagen and oxidizing agent, highly soluble and able to permeate biological membranes. The uncontrolled Cr(VI) industrial wastes and their improper disposal have resulted in an anthropogenic pollution of various environments including soils and aquifers. The selective pressure in these environments have led to developed of several strategies to resist high concentrations of Cr(VI) in microorganism exposed to this metal, mainly through chromate efflux and Cr(VI) reduction to Cr(III), which is highly insoluble, less toxic and unable to permeate biological membranes.The study of isolated Chromium-reducing microorganisms with high levels of resistance to Cr(VI)as well as their enzymes chromate reductases, have taken great interest in recent decades due to their potential application in bioremediation processes and wastewater treatmentsas a safe and cost-effective technology alternative to the expensive traditional physicochemical methods. The present study had two aims: the first was the evaluation ofthe efficiency of differentelectron donors to promote the Cr(VI) reduction in samples of polluted soil from a deposit of industrial wastes located in Guanajuato, México. The second aim was the isolation and characterization of bacteria Cr(VI) resistant and reducersfrom the aquifer located near the zone of the waste residues. The assays of soil biostimulation in batch suggest that this technology offers a potential solution to the serious pollution problem on this site;acetate, molasses and the mixture of molasses with lactate were all efficient to promote the microbial reduction of Cr(VI).We isolate fifteen different facultative anaerobe microorganisms belonging to the phyla proteobacteria, firmicutes and actinobacteria showed high levels of resistance, with a minimum inhibitory concentration of about 1195 mg/l de Cr(VI). One of them is closely related to *Klebsiella pneumonie* KCTC 2242, it is able to perform Cr(VI) and Fe(III) reduction under anaerobic conditions. The identification of this strain (ChroAq1), was performed initially by sequencing the DNA 16S, gyrA, rpoB and parC genes and recently we obtained the complete genome of ChroAq1 with Ilumina technology. The sequence genome analysis showed the presence of the gen *chrA*, which encode a chromate pump, considered the major chromate resistance determinant. This microorganism resists concentrations about 1.8mM of Cr(VI) in anaerobic conditions and
22mM in aerobic conditions. Complete reduction of 50µMCr(VI) to Cr(III) was achieved within 24h. The cell-free extract of *K. pneumoniae* ChroAq1 showed a chromate-reductase activity with NADH and NADPH as electron donors.In other hand, genes probably involved in the reduction processes were detected and its role in reduction processes is being studied.In conclusion, *K. pneumoniae* ChroAq1 shows a remarkable resistance and the capability to reduce Cr(VI) thereby greatly interest [illegible] ial applications.

"Biosynthesis of arseno-organic molecules in Actinobacteria"

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Here we report the first example of a biosynthetic pathway for arsenorganic metabolites, identified by means ofusing an evolution-inspired genome mining approach, termed EvoMining.Abiosynthetic gene cluster in the model strains *Streptomyces coelicolor*(Sco6837 – Sco6808) and *S. lividans* 66/1326 (Sli1077 – Sli1103) were predicted, and experimentally confirmed, to be involved in the biosynthesis of an arsino metabolite.Using a synthetic biology approach, the incorporation of arsenic into the key arsonopyruvate precursor via a homologous enzyme of the central metabolic AroAor 5-enolpiruvylshikimate-3-phosphate synthase (now termed as arsonoenolpyruvate synthase, Sli1096) was demonstrated using mass spectrometry analysis. Comparative metabolite profiling using HPLC and ICP-MS ofselected mutants constructed in *S. lividans*and *S. coelicolor*allowed us to identifyunprecedented arsenolipidmetabolites.RT-PCR expression analysis of key genes of this biosynthetic gene cluster, including Sli1096,showed a positive correlation between the presence of Arsenic, phosphate starvation and synthesis of arseoniolipids. This observation suggests that arsenolipids may act as part of a contingency mechanism for restoring membrane integrity during low phosphate conditions. Moreover, we mined bacterial genomes for the occurrence of otherarseno-organic metabolite biosynthetic gene clusters,findingat least 13 other *Streptomyces, Nocardiopsis*, *Kutzneria*and *Saccharomonospora*species with the potential to synthesize arsenorganic compounds beyondarsenolipids.

Dosage matters: Revealing the immediate fitness effects of experimental gene duplication

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Duplicate genes are widely distributed at high frequencies across eukaryotic genomes. It has been observed that certain types of genes are often retained in duplicate, while others are universally present as single-copy genes. What is the role of selection for gene dosage in determining such disparate duplication frequencies? Here, we experimentally duplicate over 900 essential genes in *Saccharomyces cerevisiae* using centromeric constructs bearing yeast genes controlled by their native promoter and terminator sequences. We measure the immediate phenotypic consequence of such genetic perturbation by quantifying the relative fitness of single-copy against double-copy strains under normal or stressful laboratory conditions using a high-resolution phenotyping method. Our results showed that 13% of the strains bearing an additional gene copy grow significantly slower than the wild-type, indicating that gene duplication of certain genes has an immediate deleterious fitness consequence. Gene duplications with a fitness disadvantage were enriched among genes that encode for proteins that participate in complexes and in important cellular processes, like structural organization and ribosome biogenesis. Experimental gene duplication also resulted in an immediate fitness advantage for a modest number of strains, and this fraction was increased to 12% when yeast was grown under osmotic stress. Intriguingly, such beneficial effects under stress correlated to genes with a high intrinsic disorder content. Our results suggests that natural selection of duplicated gene dosage influences the presence or absence of redundant gene copies in eukaryotic genomes, which may represent an initial step for the further diversification of gene function.

Transcriptomicanalyses of theapterygotaninsect*Thermobia domestica*: insightsintotheevolutionaryorigin of plantcellwalldigestionin insects

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Abstract

Plantcellwalls (PCW) are anessentialfoodsourceforinsectsthatthriveonwood, foliage, and detritus. Thedegradation of PCW involvestheaction of severalenzymescollectivelynamed, PCW degradingenzymes (PCWDE). Originally, theseenzymeswereconsidered exclusive to cellulolytic bacteria, fungi and protozoans, and thus, itwasassumedthatotherplant-feedinganimalssuch as insects, lackendogenous genes and enzymes to digest PCW. However, afteryears of debate, thecloning and characterization of genes encodingendogenous PCWDE in severalinsects (Isoptera, Blattaria, Orthoptera, Coleoptera), havefinallydemonstratedsymbiotic-independentdigestion of the PCW in insects. Insect PCWDE includecellulasesbelonging to GlycosylHydrolaseFamily (GHF) 5, GHF9, and GHF45; hemicellulases (GHF11 and GHF16); and pectinases (GHF28). Nevertheless, the irregular distribution of these genes in insects, has raisedquestionsabouttheirevolutionaryorigins. Someevidencesuggeststhat genes encodingendogenous PCWDE, such as GHF11 hemicellulases and GHF45 cellulases, havebeenacquiredthroughdifferentevents of horizontal gene transfer, butmost of theavailableevidencesuggeststhatthe genes enconding GHF9 cellulases, werepresent in thelastcommonancestor of thehexapods. Itisrecognizedthatwingless basal hexapods, alsoknown as Apterygota, representkeymodels to understandtheevolution of insects. Therefore, in order to clarifytheevolutionaryorigin of PCW digestion in insects, westudiedtheenzymesinvolved in thedigestiveprocess of PCW of the basal apteryogotaninsect*Thermobia domestica* (Zygentoma), byusingtranscriptomicanalysesfrommidgut and salivaryglandtissues. Wealsoconducted a comprehensiveanalysis of enzymeactivitiesinvolved in thedigestion of severalpolysaccharides and disaccharides in thegutextracts of *T. domestica.*Ourresultsindicatedthatseveraltranscripts of genes encoding PCWDE such as: GHF9 endoglucanases, GHF16 β-1,3-endoglucanases, GHF30 β-1,6-glucanases, and GHF5 β-endomannanases, wereexpressed in thesalivaryglands and midgut of *T. domestica*. Thehighestlevels of enzymaticactivitydetected, wereagainstcarboxymethylcellulose and licheninforpolysaccharidases, and againstlaminaribiose and cellobiosefordisaccharidases; and moderatelevels of activityweredetectedagainststarch, amylopectin, maltose, pectin and xylan. In general, theenzymaticprofile in thegutextractscorresponds to thetranscriptomicprofilefoundfor*T. domestica.* Theseresultsprovidekeyevidence to demonstratethatZygentoma, the more ancestral living insects, possesstheirownenzymes to digest PCW, therebyconfirmingthat at leastsome genes encodingenzymesinvolved in theprocess of PCW degradationwerepresent in thelastcommonancestor of Hexapoda.

The cellular crosstalks of metabolism by moonlighting activities

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Moonlighting proteins are a recently-defined class of multifunctional proteins in which a single polypeptide can perform more than one molecular function. While an increasing number of moonlighting proteins have been described from yeast to humans, the extent at which such proteins occur in living organisms remains largely unknown. Here, we introduce a systematic strategy to screen for moonlighting enzymes in yeast metabolism, based on the comparison of high-resolution phenotypic profiles of gene-knockout and catalytic-site mutations. We used our experimental strategy to test ten well-characterized enzymes of biosynthetic metabolism. I will present evidence of new multifunctional enzymes and genetic information about their moonlighting functions. Our results challenge our current view of the proteome and suggest that a pervasive moonlighting behavior may interconnect a wide variety of cellular processes.

Functional genomics characterization of Excretory/Secretory proteins from *T. solium* genome

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Background. The taeniasis/cisticercosis is a neglected zoonotic infection caused by *Taeniasolium*. This parasite disease is mainly found in poor and rural regions of Africa, Asia and Latin America where it has a large impact on public health. Excretory/secretory (ES) proteins play an important role in the host-parasite interface and are crucial for parasite survival. Prediction of ES proteins from genome data is a new approach to identify potential therapeutic targets for human parasite diseases. The *T. solium* genome was recently reported, providing a wealth of information from which novel ES proteins might be identified.
Results. Here we identified and annotated all the predicted ES proteins for *T. solium* genome through an integration of bioinformatics tools. The *T. solium* ES proteins represented 6.5% of the total proteins encoded in the genome. Gene Ontology, pathway mapping, protein domain and motif, enzyme code distribution, fold-recognition and antigenic index analyses were performed to functional genomics annotation of the secretome. The top 10 most statistically significantly enriched GO terms of the ES proteins showed an overrepresentation of biological activities that are strongly related to the typical functions of secreted proteins. Additionally, we developed a novel measurement to evaluate the antigenicity of ES proteins using the sequence length and the antigenic index. This measurement was formalized as the Antigenic Region Abundance (ARA) value. ARA values for ES proteins showed a similar value to that obtained for a set of experimentally confirmed diagnostic proteins and were very different to the calculated value for the non-ES proteins of the *T. solium* genome.
Conclusions.This comprehensive analysis of the *T. solium*secretome provides functional information for comparative evolutionary analyses and represents the first step to prioritize the ES proteins of therapeutic, diagnosis and immunological interest.

Characterization haem and hemoglobin binding membrane protein (*spbhp-37)* in *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae is a main causative agent of bacterial pneumoniae, meningitis and otitis media. This bacterium also can cause invasive diseases such as bacteremia and septicemia. It causes considerable morbidity and mortality throughout the world, especially among young infants, elderly and immunocompromised individuals. Hemoglobin (Hb) and haem can support the *S. pneumoniae* growth and viability as sole iron sources. Unfortunately, the uptake mechanism of Hb and haem has been poorly studied. In this work, we present the first efforts that attempted to explain the mechanism involved in iron acquisition of this pathogen. Membranes of *S. pneumoniae* were separated and proteins were purified by haem-affinity chromatography. This strategy allowed us to purify seven membrane proteins. An experiment of competence with haem and iron showed two potential haem- and Hb-binding proteins (*spbhp-22 y spbhp-37*). Their Hb-binding function was confirmed by overlay assay using Hb and Vitamin $B_{12;}$ their respective identities were obtained by mass spectrometry. The *spbhp-37* was identified as a lipoprotein. Interestingly, lipoproteins are required for virulence in some bacteria playing a variety of roles in host-pathogen interaction. Sequence analysis and molecular modeling realized with the program I-tasser indicated *spbhp-37* is anchored to the extracellular surface. Molecular docking of *spbhp-37* with both, Hb and haem, will perform using the software Moe to indicate the amino acid residues of *spbhp-37* that are involved in the binding of these iron sources. Maybe this protein maintains the levels of iron of this pathogen and it is necessary for infective process.

Prediction of Internal Ribosome Entry Sites (IRES) in *Saccharomyces cerevisiae* using computational strategies

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Internal Ribosome Entry Sites (IRES) work as a cap-independent translation initiation sites in eukaryotic cells. Current computational strategies fail to predict cellular IRES elements. We have designed and tested an IRES prediction system in the *Saccharomyces cerevisiae* genome.

Protein translation mediated by Internal Ribosome Entry Sites is an alternative mechanism used by cells when the canonical cap-dependent initiation mechanism is compromised. Frequently, under stress conditions, several eukaryotic initiation factors are scarce then, the aforementioned cap-dependent process is repressed. The list of conditions that provokes diminished protein production rates includes but is not limited to: nutrient limitation, viral infections, heat shock, hypoxia and endoplasmic reticulum stress. Nevertheless, synthesis of a subset of stress response proteins remains active or is increased. This phenomenon promotes cell survival against detrimental conditions. IRES mediated translation allows cells to adapt to adverse environmental conditions by selectively express proteins in response to stress.

Predicting IRES is a complex task because there is no primary sequence or structure conservation among them. Additionally, there are a number of other characteristics that are related to the presence of IRES, but none of them can be used as a single indicator of IRES existence in a particular untranslated region (UTR). Therefore, we propose analyzing combinations of several variables to allow IRES identification. Some of the variables used in our computational strategy are: dinucleotide frequencies, minimum folding energy, 5'-UTR length, intergenic region length, among others. Additionally, we used comparative genomics to take into account the conservation of some of these variables across species in genes translated by IRES. Our results suggest that IRES are conserved in some related genes in different organisms. Currently, there are two kinds of IRES reported: A-rich and highly structured. Our methodology was designed to predict both types, although we have only successfully used it to predict the foremost type.

As far as we know, our work represents the first accurate methodology for the prediction of cellular IRES. In the development of this strategy, we used data mining strategies combined with comparative genomics that resulted in a predictive accuracy over 96%. This system can guide experimental efforts to discover new IRES and therefore increase our knowledge of this complex, however important protein translation process.

Genome-wide open chromatinprofiling reveal new components of *Anopheles gambiae* innate immunity system

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Mosquitoes are vectors of infectious disease as a consequence of their anthropophilic blood feeding habits. *Anopheles gambiae* bite is considered the most deadly, since it does transmit *Plasmodium* parasites in Africa, causing over 600 thousand deths yearly. Mosquito innate immunity system is a key component of vector-parasite interactions, highly relevant for the transmission of malaria. This has led to an intense study of the immune reponse of mosquitoes to invading microorganisms. In particular transcriptomic studies have described the expression profiles of mosquito tissues in reponse to challenge with a diversity of microorganisms. However, only a few *cis*-regulatory elements, and their cognate regulatory factors, controlling transcription of innate immunity genes have been described. The lack of this type of mosquito immunity system components precludes the description, analysis and understanding of the full network of molecular interactions potentially involvedin susceptibility and refractoriness of mosquito tissues to the invassion by foreign microorganisms.

With the aim of identifying multiple *cis*-regulatory elements for mosquito innate immunity genes, we carried out a genome-wide chromatin structure profiling of*A. gambiae* immunecompetent cells. We applied chromatin fractionation followed by massive parallel sequencing and sequence library analysis with DFilter and MACS software for the detection of open chromatin peaks throughout *A. gambiae* genome. Detection was confirmed by qPCR for some peaks to validate our datasets. Thousands of *cis*-regulatory sites were detected and more than 500 of these mapped within 2 Kb of either end of immunity gene annotations. Oligo analysis of the full set of *cis*-regulatory sequences revealed the presence of multiple motifs matching the binding sites for transcription factors known to participate in transcriptional regulation of immunity genes in vertebrates and *Drosophila*, consistent with the immune function of these *A. gambiae* derived cells.

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Interactionofantimicrobial peptideswithmembrane models bymolecular dynamics simulations.

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A wide variety of organisms produce antimicrobial peptides (AMP) as part of their defense system against pathogens. Most AMP membrane attack and cause cell lysis. In recent years they have been considered as potential antibiotics, which may act mainly against bacteria and fungi. The pandinina2 is an amphipathic peptide, isolated from the venom of the African scorpion *Pandinus imperator*. This peptide exhibits activity against bacteria as well as a strong hemolytic activity. The pandinina2GVG,is an analogue of pandinina2, that maintains its antibacterial activity but decreases its hemolytic activity. To analyze the structure, dynamics and interaction of pandinina2and pandinina2GVG with membranes, molecular dynamics simulations with membrane models (POPC and POPG) were performed. The results indicate that the peptides are electrostatically attracted to the two types of membrane. When pandinina2 lies on the surface of the membrane begins to disordering at the extremes, but the central residues retain their secondary structure. Pandinina2GVG provides a greater number of interactions with the membranes and reach completely disordered. After 150ns of simulation, pandinina2 remains at the surface of the two types of membrane and few residues reach the hydrophobic core, the important residues for protein-lipid interactions are phenylalanine and tryptophan, which are at the extreme N-terminal. The pandinina2GVG-membrane systems were simulated by 150ns and also observed that residues from the N-terminus are important for interaction with lipid membranes, Pandinina2GVG moreover presents a greater affinity for the membrane POPG.

Changes in *Arabidopsis thaliana* root proteome in response to capsaicin

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Capsaicin (*N*-(vainillil-8-metil)-6*E*-nonamonoenamida) is analkamide whichis synthesized and accumulated in chili peppers(*Capsicum* spp.). It has been reported that the treatment with a variety concentrations of capsaicin affected primary root growth and lateral root formation.However, the molecular response of the influence of capsaicin in plants is unknown.In this study, we used a proteomic approach to investigatethe response of soluble proteins of*Arabidopsis thaliana* rootsto capsaicin.Two-dimensional gel electrophoresis (2-DE) coupled with ITMS analysis has been used to identify differentially accumulate proteins after capsaicin treatment. The results revealed that the abundance of 45 protein spots was significantly altered ($p \leq 0.05$) in response to capsaicin; about 48.9 % of this proteins were up-regulated, 35.6 % were down-regulated and 15.6 % were restricted to control (they are detected in control sample only). All 45 proteins were identified using ITMS. These capsaicin-responsive proteins are implicated in a variety of physiological processes, including energy metabolism (35.6 %), amino acid metabolism (15.6 %), defense response and hormone perception (20 %), detoxification (17.8 %) and protein degradation and processing (11.1 %). PCA analysis allowed the data separation according to the treatments(control and capsaicin), these results are also supported by clustering analysis. Our results indicate that *A. thaliana* has an extensive range of functional responses to capsaicin exposure and provide further insight into the molecular mechanism of the regulation of plant growth by capsaicin.

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Identification of a R2R3-MYB transcription factor gene (*CaMyb31*) that regulates the capsaicinoid biosynthetic pathway in chili pepper fruits (*Capsicum annuum* L.)

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Capsaicinoids are very important secondary metabolites that are restricted to the *Capsicum* genus and are biosynthesized through the acylation of the aromatic compound vanillylamine with a branched-chain fatty acid (Fujiwake et al., 1982a,b; Ochoa-Alejo and Gómez-Peralta, 1993; Sukrasno and Yeoman, 1993; Stewart et al., 2005; Mazourek et al., 2009). These compounds have several effects including analgesia, anticancer, antiinflammation, antioxidant and anti-obesity activities (Luo et al., 2010). Despite the importance of this pathway, it is surprising that little is known about the regulatory mechanisms for the accumulation of capsaicinoids in chili pepper fruits.

The generation of chili pepper cDNA libraries and comparative gene expression analysis of pungent (serrano 'Tampiqueño 74') and non-pungent fruits (pimiento 'California Wonder'), allowed us to identify a R2R3-MYB transcription factor gene (*CaMyb31*) possibly involved in the regulation of capsaicinoid biosynthesis. The expression of this gene showed a positive correlation to degree of pungency during the development of serrano 'Tampiqueño 74' chili pepper fruits, and showed a similar expression pattern to those of the structural biosynthetic genes *Kas*, *pAmt*, *Comt, Ca4H* and *AT3* by PCR and HPLC analyses.

The function of *CaMyb31* was investigated *in vivo* by using virus-induced gene silencing (VIGS). For this analysis, a *Tobacco rattle virus* (TRV) construct (pTRV2:*CaMyb31)* was generated. Immature fruits of plants infected with pTRV2:*CaMyb31* showed a statistically significant reduction of *CaMyb31* gene (82%) expression compared to immature fruits from non-infected control plants. Interestingly, *CaMyb31* silencing caused a significant reduction in the expression of structural genes of the capsaicinoid pathway [*Ca4H* (81.86%), *4CL* (80.66%), *C3H* (76.45%), *HCT* (49.08%), *Comt* (42.68%) and *pAmt* (71.04%) from the phenylpropanoid pathway, and *BCAT* (88.58%), *BCKDH* (48.54%), *Kas* (70.04%) and *Acl* (30.30%) from the branched-chain fatty acid pathway] and also in the levels of capsaicinoids in the fruits of the silenced plants [capsaicin (74.26%) and dihydrocapsaicin (73.42%)]. Our results are the first evidence indicating that MYB-like transcription factors are involved in the regulation of the capsaicinoid pathway.

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The biogenesis and control mechanisms of ribosomal activity in *Saccharomyces cerevisiae*.

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The universally conserved stalk is a lateral protuberance of the large ribosomal subunit that optimizes protein synthesis, regulating the activity of the soluble translation factors.Two heterodymers of the most acidic ribosomal proteins (pI 3.6-3.8), termed P1/P2 proteins, are anchored to the C-terminal domain of P0. In *S. cerevisiae* there are two types of each one of the P1 and P2 proteins (α ad β). Therefore, in yeast the pentameric structure of the stalk is[P2β-P1α]-P0-[P1β-P2α]. In the cytoplasm,P0 displaces and replaces its nuclear paralogue Mrt4 in the recently exported 60S subunits, but it was not known at which stage of ribosomal biogenesis the acidic heterodimers were assembled onto the 60S subunit. We have evidence showing that both phosphorylated and nonphosphorylated heterodimers are assembled only in active ribosomes but not in native 60S subunits, and they detach from the 60S subunit at the moment when the two ribosomal subunits dissociate from the mRNAat the end of a translation round. We alsodemonstrate thatmutation in P2 genes have opposite effects in growing yeast cultures; the D4 ($P2\alpha^-$) strain culture has the most accelerated growing-time than the other single mutant strains (D5-$P2\beta^-$, D6-$P1\beta^-$, D7-$P1\alpha^-$), and the control strain (W303), and reaches the stationary phase at $O.D._{600nm}$= ~3.0, one O.D. above thewild type.In contrast, the D5 strain has the lowest growing-time than the other mutant strains and the wild type. These effects are reversed whenthe corresponding missing gene is restored in each strain. Analysis of ribosome profiles from different growing phases of wild type strain revealed that the 60S and polysomal fractions decrease as the culture ages, and the ribosomes “stol” as monosomes in stationary phase at $O.D._{600nm}$ = 2.0.D4 ribosomal profiles show minimal differences through the *log* phase, and adopt a stationary pattern at $O.D._{600nm}$ = ~3.0 abruptly. D5 ribosomal profiles show short peaks through the *log* and *lag* phases of growth. All these data strongly suggest at least two novel control mechanisms of ribosome activity. First the association and dissociation of heterodimers onto the ribosome in specific stages that could function like “emergency brakes” which are reflected in translation efficiency. Second, cells are able to maintain the ribosomal population as stationary monosomes until sensing the favorable conditions to restart metabolism. It is also possible, that the abundance of 60S subunits in the stationary phase could act as a third unknown mechanism of control.

Altered Intracellular Ca^{2+} Signaling Exacerbates Mitochondrial Fragility in Ventricular Myocytes from Obese Rats.

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Obesity is a major risk factor for development of cardiovascular diseases, and heart dysfunction may occur as consequence of numerous mechanisms, including Ca^{2+} dysregulation and mitochondrial dysfunction, leading to a decrease in ATP synthesis, increased mitochondrial fragility and cell death by apoptosis.

High cytosolic Ca^{2+} and reactive oxygen species (ROS) promote opening of the mitochondrial permeability transition pore (MPTP), which is key in apoptosis induced by mitochondrial disruption. Previously, we reported that mitochondria from obese rats had enhanced ROS production and, oxygen consumption, as well as decreased oxidative phosphorylation. Furthermore, those mitochondria were more sensitive to MPTP opening. Therefore, the aim of this work was to characterize Ca^{2+} signaling in ventricular myocytes and determinate a possible relation between Ca^{2+} and mitochondrial fragility in hearts from obese rats.

We used obese Zucker rats (32 weeks old), which presented concentric hypertrophy and diastolic and systolic dysfunction. Concomitantly, they presented metabolic alterations as hyperglycemia, hyperlipidemia and increase leptin. We performed confocal imaging of cell volume and Ca^{2+} signaling in isolated myocytes, at basal conditions and upon β-adrenergic stimulation (β-AS).

In Zucker rats we found cellular hypertrophy, since cell volume increased by 27%. Diastolic Ca^{2+} sparks showed a small 26% (N.S) increase, while the systolic Ca^{2+} transient amplitude remained unchanged. Nevertheless, cytosolic Ca^{2+} removal decreased by 40%, suggesting a decreased SERCA2 activity. Furthermore, SR Ca^{2+} content also decreased by 9% (N.S). Nevertheless, systolic Ca^{2+} handling was preserved upon β-AS response.

We suggest that the mitochondrial fragility could be exacerbated by higher resting cytosolic Ca^{2+} due to lower SERCA2 removal activity. This would promote MPTP opening. Based on the metabolic alterations and heart dysfunction present in this animal model, our functional results can be representative of the alterations resulting as a consequence of obesity and diabetes mellitus 2.

Crystallographic, biochemical studies and solution structure by the small-angle X-ray scattering evidencing the dimeric formation of thioredoxin 1 from white leg shrimp Litopenaeus vannamei

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Thioredoxin (Trx) is a small 12 kDa redox protein that catalyzes the reduction of disulfide bonds in proteins from different biological systems. A recent crystals structures determination of white leg shrimp thioredoxin 1 from Litopenaeus vannamei (LvTrx) revealed a dimeric form of the protein mediated by a covalent link through a disulfide bond between the Cys 73 from each monomer (Campos-Acevedo, A. A, et al., 2013). In the present study, we use the SAXS-COACH device that consist in a HPLC (high-performance liquid chromatography) connected to the Small-angle X-ray scattering (SAXS), developed and optimized at the SWING beamline of synchrotron SOLEIL to determined the presence of the dimeric form of LvTrx at pH 7.5 in solution. Furthermore, X-ray-induced damage to disulfide bond of LvTrx was studied at atomic resolution at different transmission of 8% and 27% at 100 K (DLS, beamline I-24). The X-ray induces the cleavage of the disulfide bond of the catalytic site and the interface disulfide bond between Cys 73 at high X-ray dose, clearly established than the interface disulfide bond is not the product of a crystallographic artefact. In fact these studies confirming that the dimeric form is possible such as in the crystal as in solution.

Complex I activity decreases by low content of mitochondrial STAT3 obesity-induced

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Introduction. Recently reported the presence of STAT3 in mitochondria, protein that belongs to the STAT family. Mitochodrial STAT3 acts regulating complex I and II (C-I, C-II) activity of the electron transport chain. STAT3 activation depends on cytokines binding to their receptors on the cell surface and activates the JAK protein kinases that phosphorylate tyrosine residues conserved in STATs proteins[1]. STAT3 tyrosine-phosphorylated translocates to the nucleus and binds to the promoters of early response genes. Several regions of STATs are also phosphorylated in serine residues. STAT3 serine-phosphorylated moves to the mitochondria[2]. Furthermore obesity stress conditions are presented where there is a steady release of cytokines, including leptin; this saturates the leptin-receptor system causing insensitivity of it. In the correct binding of the hormone to its receptor, STAT3 targets mitochondria, to control the cellular metabolism and respiration. Therefore, the objective of this study is to demonstrate that low C-I activity obesity-induced is due to the absence of mitochondrial STAT3.

Materials and methods. A group of 10 Zucker rats (five lean and five fa / fa) with 11 to 13 weeks old was used as obesity model. Physiological data (systolic and diastolic blood pressure), heart and body weight, femur length, were obtained. Heart mitochondria were obtained by differential centrifugation, then Complex I activity was determined by oximetry and STAT3 content was analyzed by Western blot.

Results. Femur length (in cm) showed a difference indicated by the lean rats averaged 3.96 ± 0.26, on the other hand the fa / fa rats to 3.44 ± 0.26 ($p = 0.014$). The relationship between femur length and weight of the heart (LF / PC) in the groups lean and fa / fa, they showed an average of 0.294 ± 0.033 and 0.356 ± 0.041 ($p < 0.03$), respectively. Rat fa / fa blood pressure showed a trend of hypertension, that was recorded on average 150/120 ± 11.43/5.37 mmHg, and the lean group averaged 140/90 ± 7.63/3.7 mmHg ($p <0.001$). The activity of the C-I decreased by 69% (Lean 41 ± 2 $nmolO_2 \times min^{-1} \times mg^{-1}$; fa / fa 13 ± 1.7 $nmolO_2 \times min^{-1} \times mg^{-1}$, $p <0.001$). Finally, a reduction of 38% was detected in the content of total STAT3 heart tissue with respect to the condition of obesity and interestingly STAT3 was detected in mitochondria of lean rats, but not in obesity conditions.

Conclusion. Obesity promotes changes of the mitochondrial STAT3 content and thus a decrease in the activity of complex I, event which favors the reduction of mitochondrial respiration and thus a change in efficiency of energy production.

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Regulation of Deoxyxylulose phosphate synthase from *Arabidopsis thaliana*

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In plants, there are two metabolic pathways for the synthesis of isoprenoids: the mevalonate pathway and the MEP pathway. The MEP pathway takes place in the chloroplast. The first step of the pathway is catalyzed by the enzyme 1-deoxy-D-xylulose 5-phosphate synthase (DXS) which is a limiting step of the pathway.

Previousstudieshaveshownthattheexpression of thedifferent genes in thispathwayiscoordinated. In mutants of the pathway the proteinlevels of DXS increasebuttherest of theproteins are reduced. A similar effect is observed in the presence of fosmidomycin, an herbicide that blocks the second enzyme of the MEP pathway. These results have let us to suggest that this constitutes a feedback mechanism in response to the demand of the IPP and DMAPP final products. In the presence of fosmidomycin the half-life of DXS increases to more than 12 hours in comparison to a half-life of 80 minutes in the absence of this inhibitor. We concluded that DXS is posttranslational regulated.

To analyze any potential posttranslational modifications that the DXS protein could suffer, we performed 2D- gels protein analysis of wild type plants treated with and without fosmidomycin. Western blot anti-DXS analysis shows, differences in the migration of this protein in the samples treated with the inhibitor, several points were recognized by the DXS antibodies suggesting that posttranslational modifications that change its isoelectric point and migration might probably related to the DXS stabilization.

We are interested in whether there are differences between treatments, with and without fosmidomycin, in various aspects. For this purpose we have looked for DXS's interactors in both conditions by coimmunoprecipitations. We also know that DXS is more stable in presence of fosmidomycin, but we wanted to know if DXS is also more active in those conditions, for that reason we measured DXS enzymatic activity.

Discovery of the biosynthesis of arseno-organic molecules in *Actinobacteria*

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We have discovered the first example of a biosynthetic pathway for arsenorganic metabolites in bacteria. We have used an evolution-inspired genome mining approach to identify a set of genes in the model strains *Streptomyces coelicolor* and *S. lividans*66 that we predicted should be involved in the biosynthesis of an arsono or arsino metabolite, we demonstrate the incorporation of arsenic and AS-C bond formation using a synthetic biology approach in an heterologous host. Knock-out mutants and comparative metabolic profiling using HPLC-ICPMS led to the identification of the pathway products.

After confirmation of the incorporation of arsenic we mined actinobacterial genomes for arseno-organic metabolite biosynthetic gene clusters. We found that 10 % of the total actinobacterial genomes available in public databases, mostly mycelium forming genus have the genetic potential to produce arseno-organic molecules. This is to our knowledge the first example of a biosynthetic pathway for arseno-organic molecules in bacteria.

Furthermore, we believe that our genome mining approach will be helpful in the discovery of the biosynthetic gene clusters of novel classes of metabolites that cannot be detected by using current methods.

Antitumor activity evaluation of the protein extracts from the biological states of the house fly*Muscadomestica*.

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Abstract

Insects represent around 80% of all animal species in the world and they have survived to different environmental attacks along millions years. *Muscadomestica*can transport disease-causing organisms, but they could keep their normal physical conditions even living in the environment full of pathogenic microorganism, actually, these insects have been studied for its potential therapeutic proprieties because their biological stage have been shown cytotoxicity effects in cancer cells like breast cancer, liver cancer and colon cancer; however, this effect hasn´t been studied in cervical cancer cells.

The protein-enriched extracts of housefly larvae were evaluated using different assays like cell viability, cell proliferation and cell cycle measuring which demonstrated antitumor activity by effect in two protein-enriched extracts 50 and 100 μg/mL. In the cell viability assay using tripan blue staining, and treatment of 100 μg/mL at 12 hours, the percentage cell viability was 32.3%, but in the experiment at 24 and 48 hours, the percentage was 0%. By the other side, cell proliferation assay using crystal violet staining, at 12 hours, the percentage cell proliferation was 75.3% and for the next experiments were 87.8 y 77.8%. In the last assay, cell cycle measuring was found the greatest distribution in phase G_0/G_1, but in the next treatments with 100 μg/mL at 48 hours, cell cycle could not be measured because dead cells number were 81.3%.

These results show important alterations in Hela cells during its growth by its interaction with protein-enriched extractsand decrease in living cells and cell proliferation inhibition and cell cycle arrest in G_0/G_1 phase.

In addition, the protein-enriched extractswere characterized by unidimensional electrophoresis and western blot, we found proteins with range from 14 to 200 KDain house fly eggs and some of them were identified like glycoproteins using*Arachishypogaea* (PNA) y *Amaranthusleucocarpus* (ALL) lectins.

Key words: *M. domestica,* Protein, cytotoxicity.

Characterization of the transcription induced by the intoxication of Cry toxins in mosquitoes.

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Abstract:

The mosquito species *Aedesaegypti*is important for public health because of its role as a transmission vector of the Dengue virus, among others. The worldwide rise in the number of cases reported for this disease highlights the importance of finding ways to control mosquito populations. An alternative to the use of chemical insecticides is to apply preparations of Cry toxins from the bacterium *Bacillus thuringiensis*at rearing sites. These toxins have advantages such as high specificity of target insect, lack of toxicity towards humans and other organisms, and its biodegradable nature due to their proteic origin. It is now known that exposure of this mosquito to Cry11Aa initiates activation of the MAP kinase p38, and that knockdown of this protein increases the insect's sensibility to the toxin. This suggests that this kinase regulates defense mechanisms towards the effects of Cry toxins, which may include transcriptional changes. This has been observed in the nematode *C. elegans* in the presence of the toxin Cry5B, but it is not known if the genes involved in the defense suffer similar regulation in a different species. We undertook a time course intoxication assay with a LC50 of Cry11Aa for 12 hours. Using the Illumina platform, messenger RNA of the dissected midgut tissue from the exposed larvae was sequenced. Bioinformaticanalysis of expression data obtained indicate an incremental transcriptional response as the time of exposure to the toxin increases. Cellular functions enriched during the time course change, where the shorter time points analyzed show a reorganization of energy balance within the cell, and longer exposure times induce functions related to vesicle traffic, cellular morphogenesis, and lipid biosynthesis. During the time course studied there seems to an overall repression of functions related to oxidative stress, as a well as repression of cellular proliferation at short exposure times. These data will be verified through i*n vivo* silencing in *A. aegypti* larvae to confirm the role of these group of functions in the defense against Cry toxins.

Impact and mechanisms of shock wave application on human cells

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Shock waves for clinical practice consist of a single high-pressure peak with a steep onset and gradual decline into a pressure trough. They are generated in water and focused onto a specific tissue or cell suspension. Due to their extracorporeal application, focused shock waves have been proposed as a physical method to permeabilize cell membranes for gene transfer-mediated therapies.

The aim of this study was to analyzecell membrane permeation and transfection as well as the cell ultrastructure after *in vitro*shock wave treatment on the human breast adenocarcinoma cell line MCF-7 and the human embryonic kidney cell line HEK293.Shock waves were generated using an experimental piezoelectric device. Membrane permeabilizationusing shock waves was established by monitoring the entry of fluorescent dyes, confocal microscopy and fluorescence-assisted cell sorting analyses. The green fluorescent fluorochrome FITC Dextran-10kDa and the green fluorescent protein-codifying plasmid *pCX::GFPGPI*2 were used as reportersof cell permeabilization and transfection respectively.The cell ultrastructure wasanalyzed using scanning electron microscopy. Our results show the presence of nano- and micro-sized pores in cell membranes due to the shock wave treatments. The phenomenon was shown to be dose-dependent and related to the ability to induce macromolecular permeabilization and cell transfection.

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Improvement of enzymatic saccharification yield in *Arabidopsis thaliana* by expression of rice *SUB1A* transcription factor.

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Free or polymerized plant carbohydrates are the raw material for industrial production of bioethanol fuel. Soluble sugars can be directly fermented by microorganisms, but starch and cellulose must be enzymatically or physically deconstructed to sugar monomers, a process called saccharification. Currently, plant biotechnology is looking for strategies to improve plant carbohydrate content and/or susceptibility to saccharification.

A plant response where carbohydrate consumption and signaling is crucial for survival is during flooding stress. In rice, this response is mediated by *SUB1A*, a transcription factor from the *Ethylene Response Factors* (*ERFs*) Group VII gene family. *SUB1A* mRNA is rapidly induced when plants sense low-oxygen conditions and its expression directs plant transcriptome towards optimizing anaerobic metabolism, repressing starch consumption and retarding energy-consuming activities like elongation and flowering. Together this is called the Low-Oxygen Quiescent Syndrome (LOQS).

In this work, we employed the model plant *Arabidopsis thaliana* as a functional prototype to explore if overexpression of *SUB1A* and *SUB1C* (a closely related gene) can improve biomass saccharification. The rationale behind this research is that *A. thaliana* plants constitutively expressing *SUB1A* (OxSUB1A) may also display the LOQS low-starch consumption feature. To test this hypothesis, we employed the cellulose-hydrolyzing enzymatic cocktail Accellerase 1500 and thermostable amylase/amyloglucosidase to contrast glucose yield of OxSUB1A transgenics against wild-type plants. We also measured free sugars with DNS reagent and confirmed starch accumulation with I_2 histological staining.

First, we tested 25-day-old plants at the end of the day and found that OxSUB1A had 35% (±6 SE) more free-sugars, 20% (±8 SE) more glucose from cellulose and 80% (±8 SE) more glucose from starch than wild-type. *SUB1C* gene did not improved saccharification yield. Next, we followed mixed cellulose/starch saccharification yield on a diurnal experiment (0, 8, 16, 20 y 24 h) in 25-day-old plants and observed that OxSUB1A had more glucose after saccharification than wild-type (80-120%) at all times. Finally, we followed saccharification yield on a developmental experiment and detected that saccharification yield was higher in OxSUB1A only in juvenile stages (170%±23 SE, 2-week-old; 98%±22 SE, 3-week-old; 63%±14 SE, 4-week-old) and that in mature stages, no significant difference from wild-type could be detected (-1.8%±10 SE 4-week-old). We also measured in a texturometer that OxSUB1A offered less resistance to deformation (2.34±0.26N) than wild-type (3.47±0.35).

In order to find a probable molecular mechanism for this phenotype, we measured transcirpts by qPCR of key genes involved in starch degradation (SEX4 and GWD1), juvenile transition (SPL3, SPL5) and flowering time (FUL, SOC1) but found no expression changes, indicating that starch accumulation is not orchestrated by previously known molecular mechanisms.

We conclude that *SUB1A* is an interesting target for manipulation of carbohydrate content in plant breeding for improved saccharification yield.

Plant pathogen interaction of agave wilt: The response of the *Agave tequilana* Weber var. Azul to the infection of *Fusarium oxysporum*

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The agave´s wilting associated with *Fusarium oxysporum* is one of the major diseases of the blue agave (*Agave tequilana* Weber var. azul), the crop used to produce “Tequila” in Mexico. To date, little is known about the *A. tequilana* – *F. oxysporum*interaction, nevertheless to design a new control strategy of the fungus,it is necessary to know which defense mechanisms are effective against the pathogen and which are not.Although it has been reported that *F. oxysporum* has a pattern of general attack, it’s difficult to extrapolate the actual knowledge to blue agave because of its anatomical and physiological characteristics. During the early stages of *F. oxysporum*infection, plant triggers different mechanisms of defense that attempt to interrupt the establishment of the compatible interaction. The aim of this study was to evaluate plant defense mechanisms that are early induced and those associated with resistance to infection by *F. oxysporum* in *A. tequilana*.In order to test the response of the plant in compatible and incompatible interactions, three different strains of *F. oxysporum* were used: 1) Pathogenic strain (**PAT**), 2) Non-pathogenic strain (**NoP**) and 3) Pathogenic host-specific strain *Fusarium oxysporum* f. sp *lycopersici* (**FOL**).Early Defense mechanisms evaluated were hypersensitive response (HR) and strengthening of the cell wall in agave roots. Pieces of primary or secondary roots were sectioned and stained for evaluated ROS production, programed cell death (PCD) and callose and lignin accumulation in cell wall to the 24, 48 and 72 hours after inoculation with the fungus.Resistance-associated plant defense mechanisms evaluated were productions of pathogenesis-related proteins (PR proteins), phytoanticipins and phytoalexins. These proteins and compoundswere extracted from agave roots at 1, 3, 6, 9, 15 and 30 days after inoculation of the fungus.Regarding early defense mechanisms, induced HR observed with **PAT**was greater thanwith the other two strains. The strengthening of the cell wall was observed with the inoculation of the three strains, and was higher with **FOL**. With respect to the mechanisms of resistance, differential response between the three strains of *F. oxysporum* was observed. The initial response to the inoculation with **PAT** and **FOL** was similar in terms of the production of PR proteins, phytoalexins and phytoanticipins. However, it was observed that over time the response was differentiated with **FOL**, andthat could be interpreted as an incompatible interaction. From these results it was possible to identify effective and ineffective defense responses of*A. tequilana*to infection of *F. oxysporum.*

MicroRNA profiling of Arabidopsis AtGRDP1 overexpression line reveals De regulation of mir159 and mir160 in response to ABA

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BIOPROSPECTINGOF YEAST STRAINS CAPABLE OF PRODUCING MICROBIAL OILS AS FEEDSTOCK FOR BIODIESEL

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Introduction. Currently, the world was witnessing a major problem regarding non-renewable energy sources, as there is a decrease in oil reserves and the increase in the costs of search and retrieval. Another of the most important issues are the environmental consequences of the gases that are released by petroleum fuels, this together, leads us to consider as an alternative source of energy to biofuels. During the past decade has focused attention on the biodiesel, a renewable and environmentally friendly biofuel, because its combustion produces only CO_2 and water. Biodiesel is rather an attractive alternative because of its characteristics: biodegradable, non-toxic, renewable and clean, for similar properties to those of conventional diesel (1).The objective of this work was to isolate and characterize a yeast strain capable of producing microbial oils as a raw material for biodiesel.

Methods. The isolation of strains was performed on YPD agar by streak plate methods. Microscopic identification of the strains was performed staining with crystal violet. Cell growth was determined by optical density at 580nm. The measure of the production of microbial oils was achieved using a semi-quantitative method developed by Thakur *et al* in 1989 (2) and with a quantitative technique according to Bligh and Dyer modified by Li-Xia Pan *et al* in 2008 (3). The identification of yeast strain was performed by 26S rDNA sequencing and comparison of identity in the BLAST database (4).

Results and discussion. Twenty five yeast strains were isolated from various samples of soils and fruits, to select the best oleaginous strains was implemented a spectrophotometric semiquantitative assay of total lipid production, only four strains classified as **C0**, **M2**, **MR1**, and **M1**, which presented the higher absorbance in seniquantitative assay were selected, these strains were assessed quantitatively through biomass yield and lipid content, obtaining the following results for each strain, respectively: 3.928 g/L, 69.30%, 6.676 g/L, 57.14%, 2.928 g/L, 97.85%, 4.856 g/L and 44.08% of biomass production and lipid percent. The molecular identification of the aforementioned strains was also carried out using 26S ribosomal DNA sequencing. The results were comparing with the GenBank database, and thus the identification was achieved: strain **C0** corresponded to *Kluyveromycesmarxianus*, strain **M2** was identified as *Issatchenkiaorientalis*, strain **MR1** was identified as *Candia glabrata* and strain **M1** was identified as *Pichiakudriavsevii*. It is worth noting, that none of these strains has been previously reported as oleaginous, so characterization of these yeasts, in this aspect, is novel.

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Don't be afraid of mass spectrometry (MS) – Strategies to generate and analyze MS data in a 'normal' biochemistry laboratory

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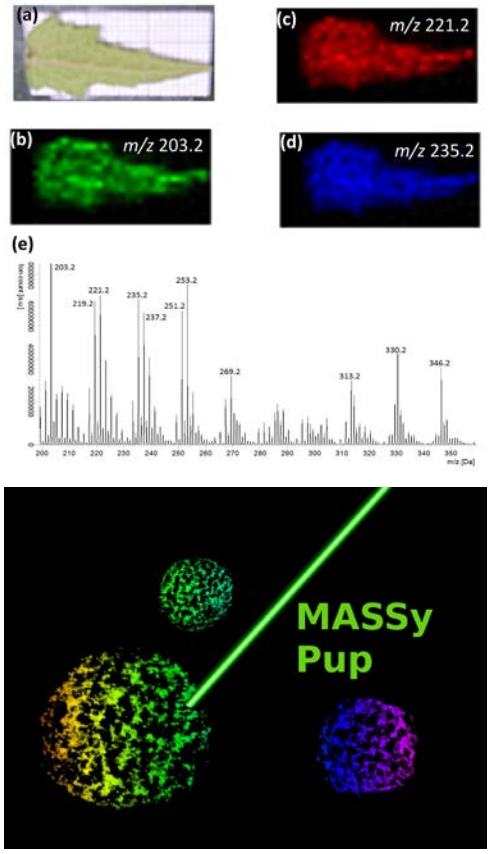
Mass spectrometry (MS) has become a central tool in modern biochemistry, because its selectivity and sensitivity permit the detailed analysis of a broad range of bio-molecules. High-throughput strategies such as proteomics and metabolomics complement genomics, in order to gain a more complete understanding of biological systems.



Mass spectrometry imaging (MSI) and ambient ionization strategies such as desorption electrospray ionization (DESI) and low-temperature plasma ionization (LTP) provide new insights about the localization and the dynamics of molecules in living organisms.

Although there is no doubt about the utility of MS, high investment and running costs often frighten away potential users and the complicated data analysis deters interested researchers.

Thus, in our lab we develop strategies to facilitate the entry for the use of MS in life sciences.

We constructed a low-cost, thought powerful, ambient MSI system, which can be used to study the distribution of compounds on surfaces or biological tissues [1].

Further, we compiled a free and open source MS software distribution, which supports the analysis of MS, proteomics and metabolomics data by standard users [2].

We also will report on our experiences with miniature mass spectrometers, an important new trend for getting MS into any biochemistry laboratory.

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Colloidal Silver and cell viability association with markers of oxidative stress: heme oxygenase 1 Catalase 8 isoprostane and iron.

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Introduction: Exposure of cells to colloidal silver (CS) induces lipid peroxidation and oxidation of lipids multiple studies have shown that silver nanoparticles generate oxidative stress (OS) Even the CS acts as a xenobiotic and antigen, stimulating chemical defense and immune in both reactive oxygen species (ROS) are produced which induce the cell to activate antioxidant mechanisms such as catalase and heme oxygenase-1 (HO-1), involved in the defense against EO, it is well known that HO-1 attenuates the toxic effects of metals and ROS and HO-1 in most chronic degenerative diseases is induced, hence its importance to public health **Objective:** Analyze EO induction by exposure to CS, also associate the EO parameters with cell viability **Methods:** Cells from a primary culture of human lymphocytes were exposed to different doses 0 (negative control), 0.036µg /mL, 0.36µg/ mL and 3.6 µg/mL of CS and times (0.5 hrs., 2 and 24 hrs.) hydrogen peroxide 3% was used as positive control. The number of cells and cell damage was quantified by the mitochondrial activity (MTT), cell viability (VC) using trypan blue and quantification of total protein by Bradford technique. Lipid oxidation by quantification of 8-isoprostane (8-ISO) and induction of HO-1 using kits from Enzo Life Sciences, and catalase activity was determined by the Folch reagent and iron (Fe) quantified by fenantronile technique. Data were analyzed with SPSS version 18 statistical package to determine the association between the parameters analyzed **Results:** The CS induce EO, caused loss of VC and MTT activity, which between them were associated r = 0.764 p≤0.0001, and both also showed a negative association with the exposure time (r = -0.640 and r= -0.771 p ≤0.0001) and with the concentration of CS (r = -0.395 and r = -0.345 p≤0.0001). EO markers like catalase also present a negative association with both the VC and the MTT (r = -0.698 and r=-0.554 p ≤ 0.0001) and a positive association with the time r = 0.737 p ≤0.0001. EO markers like catalase also present a negative association with both the VC and the MTT (r = -0.698 and r=-0.554 p ≤ 0.0001). The 8-Iso a product of lipid oxidation was positively associated with MTT, the VC (r = 0.455 and r = 0.464 p ≤0.0001), catalase (r = 0.543 p≤0.037), and with the HO-1, this latter from 0.5, up to 24 hrs showed strong association with r = 0.925 and r = 0.964 both with p≤0.0001; with respect to the concentration at 2 hrs, the HO-1 and 8-Iso were negatively associated, r = -0.556 p ≤0.031 r = -0.731 and p ≤0.002. Moreover, the Fe at 0.5 hrs was associated with catalase r = 0.743 p≤0.002, the HO-1 r = 0.811 p≤0.0001, and 8-Iso r = 0.718 p≤0.003 and finally the Fe at the concentration of 0.036µg/ mL present a high association with all parameters except with the HO-1. EO thereby induces less time and low concentration of CS, suggestive of a primary depolarizing effect leading to oxidation of membrane lipids where Fe is involved. **Conclusion** The CS participates in the oxidation of lipids in particular the presence of the 8-Iso involved in cell viability and MTT activity and in the induction of HO-1and catalase. These parameters can be useful tools for studying the EO development in the chronic degenerative diseases, by exposure to xenobiotics such as CS.

The metallothionein gene from the white shrimp *Litopenaeus vannamei*: Characterization and expression in response to hypoxia

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Aquatic animals often encounter variations in oxygen tension that leads to the generation of reactive oxygen species (ROS) which can have harmful effects on the organism. Under these circumstances some organisms have evolved to tolerate hypoxia. In mammals, metallothioneins (MTs) have a protective function against hypoxia-generated ROS. Here we report the complete gene for metallothionein from the shrimp *Litopenaeus vannamei* (LvMt). This MT gene is differentially expressed in hemocytes, intestine, gills, pleopods, heart, hepatopancreas and muscle, with the highest levels in hepatopancreas and heart. LvMt mRNA induction is triggered by hypoxia in hepatopancreas (1.5 fold-change) and gills (4 fold-change) after 3 h at 1.5 mg DO L^{-1}. The LvMt gene structure resembles the homologs from other aquatic invertebrates and terrestrial animals, possessing three exons interrupted by two introns and contains response elements for the metal response transcription factor 1 (MTF-1), hypoxia-inducible factor 1 (HIF-1) and p53, among others in the promoter region. In response to hypoxia, HIF-1/MTF-1 might participate in the induction of MT to contribute towards the tolerance to ROS toxicity. MT importance in aquatic organisms may include not only metal-detoxifying but also ROS-detoxifying processes.

Keywords: metallothioneins, ROS, expression, hypoxia, shrimp

A peptidethat regulatesthe motilityofsea urchin sperm,activates themitochondrial metabolismmediated by the CarnitinePalmitoylTransferasel (CPT-l)

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Sea urchin sperm has only one mitochondrion, which,in addition to being the main source of energy, may modulate changes in the intracellular calcium concentrationto regulate its motility and possibly the acrosome reaction. Speract is a decapeptide thatdiffuses from the jelly, the outer layer of the egg, that upon binding to its receptor in the sperm flagella produces anincrease inpHi,andstimulates the sperm motility and respiration, among other physiological changes(Beltrán*et al*., 2007; Darszon *et al*., 2011).

The stimulation of*Strongylocentrotuspurpuratus*spermwithsperact induces a depolarization of the sperm mitochondrion and increases the levels of NADH,raising the sperm pH_iindependently of Ca^{2+} (García-Rincón, unpublished).

It has been suggested that sea urchin sperm obtain energy from the oxidation of endogenous lipids (Mita& Nakamura, 1998). Free fatty acids (FFA),which supply the β-oxidation, are incorporated into the mitochondrial matrix possibly through the shuttle of long chain fatty acids (Inglis & Stewart, 2006).In order to determine whether the increases in NADH induced by speract comes from the β-oxidation, we decided to expose the sperm to different concentrations of the CPT-I inhibitors, perhexiline or etomoxir,and evaluate NADH levels in response to speract. We found that the two CPT-I inhibitors decrease the levels of NADH induced by speract in a dose-dependent manner.We conclude that the increase in pH_iinduced by speract stimulates the transport of FFA into mitochondria regulated by the CPT-I that feeds the β-oxidation,which in turn increases the mitochondrial NADH ($NADH_{mit}$) levels and suppliestheenergy for sperm motility.

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Cyclophosphamide induces oxidative stress in brain, liver and kidney of normal rats treated

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Antineoplasic drugs are toxic to non-cancerous tissues; these effects reduce efectivity, dose and time of the treatment and the quality of life of patients. The molecular mechan- isms implicated in toxicity have been only recently studied. The toxicity of antineoplastic treatment can be induced by oxidative stress; however few studies has been performed in this sense.

Cyclophosphamide is an alkylating agent able to induce cross-linking of DNA and fre- quently used in cancer treatment. Only some studies has been reported about molecular mechanisms about the cyclophosphamide toxicity.

In this work, male rats Wistar (200-250 g) were treated with 3 weekly doses of cyclophos- phamide (60 mg/kg, intraperitoneal); other rats group were administered simultaneously a daily dose of the antioxidants: alpha-tocopherol (100 mg/kg, ip), ascorbic acid (100 mg/kg, ip) and N-acetylcysteine (100 mg/kg,ip). At the indicate time, liver, kidney and brain were removed and homogenized to assess lipid peroxidation by measuring thiobarbituric acid (TBARS) species; total antioxidant capacity using the myoglobin-induced oxidation of ABTS. Induction of apoptosis was evaluated by caspase 3 activity. Likewise, the activity of antioxidant enzymes superoxide dismutase (SOD), gluthatione peroxidase (GPX) and cat- alase (CAT) were measured by spectrophotometric methods.

High lipid peroxidation and apoptosis were found in liver, brain and kidney of rats treated with cyclophosphamide. Whereas, treatment with antioxidants was able to prevent lipid peroxidation and apoptosis induced by cyclophosphamide treatment.

Total antioxidant capacity induced by cyclophosphamide treatment was high in liver, low in brain and unaffected in kidney. Antioxidant treatment was able to avoid changes in total antioxidant capacity in the organs.

Cyclophosphamide treatment altered antioxidant enzymes activity. SOD activity was lower in liver and higher in kidney and brain of rats treated with cyclophosphamide. The activity of CAT was higher in liver and kidney of treated rats with the antineoplastic; while in brain we were unable to detect CAT with the method used. GPX activity was lower in liver and higher in kidney and brain of treated rats.

The antioxidant treatment was able to prevent changes in SOD activity of kidney and brain; Changes in CAT and GPX activities of liver and kidney also were prevent with the antioxidant treatment, in rats treated with cyclophosphamide. The activities of SOD and GPX in brain of rats treated with cyclophosphamide remain high even with treatment with antioxidants.

The results indicate that the treatment with three weekly doses of cyclophosphamide was able to induce oxidative damage and apoptosis, and modify the total antioxidant capacity in liver, kidney and brain. Daily administration of antioxidants during cyclophosphamide treatment protects against oxidative damage and induction of apoptosis caused by the treatment. Oxidative stress was partially corrected by treatment with antioxidants.

C-Phycocyanin prevents renal mitochondrial dysfunction induced by cisplatin

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Cisplatin (cis-diamminedichloroplatinum, CP) is a widely used antineoplastic drug for thetreatment of various cancer types. However, its use is limited by its nephrotoxicity withabout 25–35% of patients after a single dose of CP treatment.The mechanism of CP-induced nephrotoxicity is not completelyunderstood; however, several mechanisms, including hypoxia, free radicals, inflammation, apoptosis and mitochondrial dysfunction are thought to be involved. We previously demonstrated that the antioxidant C-phycocyanin (C-PC) prevent renal damage induced by CP. In this way, the aim of the study was to determinate the potential of C-PC (30 mg/kg i.p.)to preventthe kidney mitochondrial dysfunction induced by CP (22 mg/kg i.p.). The mitochondrial dysfunction caused by CPwas characterized by oxygen consumptionemploying a Clark electrode. It was employed different substrates to induce state 1, malate:glutamate (Complex I) and succinate (Complex II), besides the state 3 was induced with ADP and Carbonyl cyanide p-[rifluoromethoxyl]-phenyl-hydrozone (FCCP) to induce Vmax. It was calculated therespiratory control ratios (RCR), adenosine diphosphate (ADP)/ oxygen (O) ratio,loss of membrane potential and calcium handling,aconitase activity and catalase activity (CAT). Our group is the first one to demonstrate that C-PC pretreatment prevents the mitochondrial dysfunction caused by CP in mice.

Does Senescence Associated Secretory Phenotype (SASP) profile change when senescence is induced by different ways?

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Cellular senescence isusuallycharacterized by a proliferation arrest dueto a maximal round of cellular replications(Replicative Senescence, RS) or to the exposition to diverse stimuli that can prematurely induce this cellular state, such as oxidative stress (Stress-Induced Premature Senescence, SIPS) or proteasome inhibition (Proteasome Inhibition Induced Premature Senescence, PIIPS). Senscence has beenassociated with aging and multiple age-related diseases as such as cancer or neurodegenerative disorders including Alzheimer and Parkinson diseases.

All senescent cells exhibit the same hallmarks,namely proliferation arrest, SA-betagalactosidase activity and cellular cycle downregulatorsoverexpression, along with cytokines, growth factors, chemokines and matrix metalloproteinasessecretion, which altogether are known as Senescence Associated Secretory Phenotype (SASP). SASP has been related with chronicinflammation leading to cellular transformation and neurodegenerative diseases, and has been well characterized in RS and SIPS but not in PIIPS.SASP is considered a communication mechanism between cells, so diverse SASP profiles might be able to induce different responses in the neighbor cells. Therefore, the aim of this work was to determine SASP components in PIIPS and compare them to RS and SIPS in order to determine if SASP componentsare different depending on the stimuli used to induce senescence.

Senescence was induced in primary lung mice fibroblasts in 3 different ways: 1) RS, by allowing the cells to proliferate until they became senescent, 2) SIPS, treating the cells with H_2O_2 and 3) Inhibiting the proteasome activity with epoxomicin. SASP was analyzed using a commercial cytokine array (RayBiotech). After analyzing 62 different cytokines important differences were observed between PIIPS and the other senescence types. While SIPS and RS showed an increase in the secretion of most cytokines, in PIIPS only 13 cytokines were incremented. Interestingly, SASP pro-inflammatory profile was conserved in the 3 types of senescence.Suggestingthat SASP profile from PIIPS cells might induce a different immune response than SIPS and RS, and that SASP would be a stimuli-dependent attribute of senescent cells.

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Auranofin-induced oxidative stress causes redistribution of the glutathione pool in *Taenia crassiceps* cysticerci.

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In the parasite lineage of Platyhelminthes the redox homeostasis is dependent on thioredoxin-glutathione reductase (TGR), a mixed-function selenoenzyme that acts transfering electrons from NADPH to the oxidized states of both GSSG and thioredoxin. TGR has been proposed as a pharmacological target because in these organisms is the only enzyme involved in the reduction of both GSSG and oxidized Trx. Previously, we studied the effect of TGR inhibition by auranofin (an antirheumatic gold compound) on the viability of *Taenia crassiceps* cysticerci. It was demonstrated that micromolar concentrations of auranofin in the culture medium were high enough to fully inhibit TGR and kill the parasites (Parasitol Res. 2010. 107:227-31). In this work we have analyzed the dynamic of changes in the glutathione pool following the addition of auranofin, as well as the effect of N-acetyl cysteine (NAC) and buthionine sulfoximine (BSO).

The results obtained showed that, as result of the presence of the gold compound, the total concentration of glutathione (GSH + GSSG) decrease, concomitant with a diminution in the GSH/GSSG ratio. Such changes were simultaneous with the formation of GSH-protein complexes as well as with the export of GSSG from cysticerci. The incubation of cysticerci in the presence of both auranofin and NAC, a promotor of GSH biosynthesis, prevents all the above changes, mantaining the viability of the parasites. By contrast, the simultaneous addition of auranofin and BSO, an inhibitor of GSH biosynthesis, results in an encourage in the effects of the gold compound.

These results suggests the lethal effect on *T. crassiceps* cysticerci due to auranofin can be explained, at least partially, as a consequence of major changes in the glutathione status resulting in an irreversible damage, probably by an increase in the oxidative stress.

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The lipogenesis attenuation by hydrogen peroxide avoid the liver steatosis obesity-related but increase the mortality by starvation

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The mechanisms that determine the metabolic alterations that lead to conditions characteristic of obesity and starvation are not completely known. By producing a null mutation in the gene encoding catalase, we have identified hydrogen peroxide (H_2O_2) in the liver as an important regulator of metabolic disorders that result from from obesity and starvation. Mice lacking catalase fed with normal food weighed and maintained their normal levels of glucose and triglycerides. However, whenthese mice were fedwith a high fat diet increased their weight and became obese as wild typemice (WT), but did not develop hyperglycemia and hepatic steatosis. The expression pattern of genes associated with metabolism in liver are consistent with an increase in glycolysis and lipolysis,and a decrease in gluconeogenesis and lipogenesis. On the other hand, catalase deficient mice fasted for 24 hours showed similar metabolic changes, although the decrements in body weight and blood glucose levels were as those determined for WT mice. Interestingly, a regime of fasting or food restriction on neonate mice markedly reduced the life expectancy of those lacking catalase, indicating that the metabolic alterations caused by the increase inH_2O_2 compromise the life of the organism under these conditions. Interestingly, mice lacking catalase have a shorter lifespan without showing the characteristics of premature ageing. We have found that the levels of active AMPK were higher in the liver of mice lacking catalase than in the one of WT. Our results suggest that H_2O_2 produced in liver peroxisomes causes an up-regulation in AMPK activityresulting inreduced lipogenesis and increased utilization of energy reserves (i.e., fatty acids), bothwith significant implications in the metabolic alterations associated with obesity, starvation and longevity.

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CHANGES IN WATER AVAILABILITY INDUCE FOLDING IN INTRINSICALLY DISORDERED STRESS PROTEINS FROM PLANTS.

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Late Embryogenesis Abundant (LEA) proteins are a broadly distributed group involved in plant tolerance to water deficit. Most of them belong to the hydrophilins because of their high hydrophilicity and content in small amino acids. Hydrophilins, including LEA proteins, are predicted to be part of a wider group of proteins known as intrinsically disordered proteins (IDPs). By partial dehydration and freeze-thaw *in vitro* assays, it has been shown that some LEA proteins are able to protect other proteins from the effects of water limitation and it was suggested that this might occur through protein-protein interactions. Given the unstructured character of LEA proteins, we hypothesized that their putative flexible nature plays a critical role in the interaction with their partners, allowing them to interact with diverse molecular targets. We also have proposed that this structural flexibility might be modulated by the cell water status, thus promoting selection of specific conformations needed to interact with specific targets depending on the condition. To get insights into their structure and its relation to their function, we have characterized the structural properties of two Arabidopsis group 4
LEA proteins (AtLEA4-2 and AtLEA4-5) using Circular Dichroism and Nuclear Magnetic Resonance. We showed that both are intrinsically unstructured in solution over a wide range of temperatures; however, their intrinsic potential to form secondary structure was exhibited by treatment with a structure inducer, which promoted a significant α-helix formation (from 5% in its native state to 44% α-helix). A decrease in water availability also induced folding in both LEA proteins. Likewise, conditions inducing molecular crowding (a condition more similar to cellular environments) also led to conformational changes. The significant higher gain in structure for LEA4 proteins than for other LEA proteins with similar physicochemical characteristics upon water content changes indicated that this is a property related to their particular amino acid sequence. The structural characteristics found for group 4 LEA proteins in this study show their structural flexibility and suggest their involvement in the recognition of molecular partners needed to be protected from the deleterious effects of water deficit. The relation of the structural properties of AtLEA4 proteins to their function as protectors of native proteins from the deleterious effect caused by water limitation will be discussed.

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Protein-ligand interactions to understand the role of the mutations in the Shwachman-Diamond Syndrome

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Protein-ligand interactions are essential in all cellular mechanisms including ribosome biogenesis,the process that involves the production of functional ribosomes. In *S. cerevisiae*, a late step of ribosome maturation in cytoplasm requires the coordinated action of the GTPase Efl1 and Sdo1 to release the anti-association factor Tif6. The presence of Tif6 in the surface of 60S subunits prevents its association with the small subunit to form the mature ribosome. The human orthologues of Efl1 and Sdo1 are EFTUD1 and SBDS, respectively. The latter is mutated in the Shwachman-Diamond Syndrome (SDS), a disease characterized by skeletal abnormalities and predisposition to developleukemia. In this work, we studied the physical interaction between the Efl1 and Sdo1 using different approaches. Photo-crosslinking experiments that incorporate non-natural amino acids in Sdo1 residues equivalent to those mutated in the Shwachman-Diamond Syndrome demonstrated that they directly interact with EFL1. Yeast two-hybrid experiments allowed us to map domains 2-3 of Sdo1 with domains 1-2 of Efl1 as the regions of interaction between both proteins. Finally, using SAXS (small angle x-ray scattering) it was possible to obtain the radius of gyration (R_g) of the complex. Together these experiments allowed us to propose a structural model of the complex formed between Efl1 and Sdo1; based on the homology Efl1 shared with the elongation factor 2 (EF-2) and the crystal structure of the archaea Sdo1. The importance of the interaction between these proteins was highlighted by the fact that in the presence of Sdo1 the $K_M$the GTPase for GTP decreased half compared to the value of the enzyme alone. Although, the K_M is a good measurement of the affinity of an enzyme for its substrate it is not a real affinity constant. Further studies of the affinity of Efl1 for guanine nucleotides using stopped-flow FRET (Fourier Resonance Energy Transfer) demonstrated that the dissociation constant (K_d) for GDP in the presence of Sdo1 increased twice. Suggesting that Sdo1 acts a positive regulator of Efl1 diminishing the binding of GDP and thus favoring its release. Finally, using fluorescence anisotropy we quantitatively measured the interaction of these two proteins. SDS mutations weaken the interaction with EFL1. These results suggests that mutations in SDS abrogate Efl1 regulation due los the loss of interaction between the two proteins.

Thrombin generation as parameter of response to therapeutic agents in patients with bleeding disorders.

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Introduction. The thrombin generation assay (TGA) is a global functional test that reflects the hemostatic capacity of the blood coagulation mechanism as a result of the interplay between procoagulant and anticoagulant factors. It has been used in patients with bleeding disorders to assess thrombotic or hemorrhagic risk and its modification by therapeutic agents. The objective was to investigate the usefulness of TGA as parameter of response to purified factor VIII (FVIII) and activated prothrombin complex concentrate (APCC) treatment in patients with severe hemophilia A (HA) positive to inhibitors.

Methods. We studied 189 patients with severe HA. (FVIII:C <1%) suspected of – or known as – carrying an inhibitor to FVIII clotting activity (FVIII:C). The study was approved by the corresponding local ethics committees. Written consent was obtained from all participants. Inhibitors to FVIII were investigated by the Nijmegen–Bethesda (N–B) method. Thrombin generation was measured with the calibrated automated thrombogram in inhibitor-positive plasmas previously spiked and incubated with FVIII or APCC. The following parameters were obtained: Lag-time, endogenous thrombin potential (ETP), peak, time to peak (ttpeak) and rate of thrombin generation (rate).

Results. Lag-time: 59% of normalization with 2U/mL of FVIII in oposite to 100% with 1-2 U/mL ACCP. ETP: 33% and 87% of type I and type II/III inhibitors responded to FVIII compared to 83.7% that responded to 1-2 U/mL of APCC. Peak: Normalization were achieved only in 10% of the cases. The average increase was 23% and 51% with FVIII and APCC, respectively. Correlation between ETP and Peak with FVIII (r = 0.85) and APCC (r = 0.55) was observed. Ttpeak: It was shortened by FVIII in 50% of plasmas with type I inhibitor and in 80% of type II/III inhibitor but in 100% of cases with APCC. Rate: The average rate was 19.0 nM / min (9% of normal) with FVIII and only in two cases the increase was greater than 70%. Average rate was 53.4 nM/min (25% of normal) with APCC, and only in one case the increment was greater than 100% of normal.

Conclusions. The most useful parameter of response to therapeutic agents was the ETP. A differential behavior in all parameters according to the type of inhibitor, was observed when plasmas were incubated with FVIII. Lag-time were longer than baseline values in approximately a half of plasmas with type I inhibitor, maybe by the effect of the inhibitor, which did not occur when incubated with APCC. In all cases, the response to APCC was better than to FVIII.

Physicochemical study of the transcriptional repressor NagC from Escherichia coli

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NagC is a transcriptional repressor of the ROK family. It regulates the transcription of the divergent *nag*E-BACD operon.It is involved in the repression of amino sugar metabolism and it is essential for cell growth under amino sugars as the sole carbon and nitrogen source and for cell wall recycling. The DNA binding site is a Helix-Turn-Helix domain located at the N-terminal end. The inducer binding-site is near the C-terminal end and it has been shown to be *N*-acetylglucosamine 6-phosphate (GlcNAc6P). This ligand allows the expression of the *nag*operon. Theoperator sequences arewell known [Plumbridge, 2012] but its interaction with NagCprotein at the atomic level and the involved equilibrium constantsremain to be studied.

Before studying NagC binding constants we tested different conditions of ionic strength and pH to stabilize NagC in solution. We studiedfluorometrically the NagC affinity for different operator sequences and for its inducer, GlcNAc6P, Fluorescent emission originated in the unique Trpresidue (Trp176), by exciting at 280 and recording the emission at 310 nm. This signal has a good quantum yield. NagC interaction withDNA or the inducer consistently quenches the fluorescence emission.DNA binding to NagC was assayed withtwo differentdsDNA fragments of its known operator sequences. A complex oligomeric equilibrium was found over different experimental conditions.

NagC titration curve with dsDNA which contains the *nag*Es.o. sequence shows a single hyperbolic binding component, with a K_{dis} of 1.32 ± 0.06µM.The titration with the *nag*B operator sequence showsan apparent negative cooperativity (*h* of 0.75 ± 0.02, $K_{0.5}$ of 3.1 ± 0.5 µM). Negative cooperativity is due the presence two binding species, each with a different affinity.Finally we found that the bindingof the inducer to the ligand-free NagC is cooperative; fitting theinducer binding to the Hill equation yields a Hill number of 1.65 ± 0.11 and a$K_{0.5}$of 30 ± 1.2 µM; this result corresponds to a reasonable cooperativity for a dimer. GlcNAc6P titration using the DNA saturated form shows an increased affinity and positive cooperativity; this can be explained as a binding to a conformer of greater oligomeric order.In support of the oligomeric equilibrium observations, we used SDS-PAGE with cross-linked NagC samples previously equilibrated over different ligand conditions. Tetrameric forms where found when the protein interacts with any of its ligands, in contrast to the ligand-free samples that only show monomeric and dimeric conformers. This is consistent with the apparent negative cooperativity observed over DNA binding and the positive cooperativity observed on inducer titration.

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Effect of low-glucose cultivation on the *Entamoeba histolytica* energy metabolism

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INTRODUCTION. *Entamoeba histolytica* is an enteric microaerophilic parasite which causes amebal cholitis and extraintestinal abscesses in endemic human populations. The parasite life cycle includes two phases: the infectious cyst and the invasive trophozoite. During host infection and intestinal colonization, trophozoites are exposed to adverse conditions including nutrient deprivation. Although the effect of glucose deprivation on energy metabolism has been studied in several cellular models, it has been barely studied in *E. histolytica*. This work is focused to determine the metabolic changes in the amebal energy metabolism upon growth under low glucose concentration.

METHODS. *Entamoeba* was grown in TYI medium with 0, 2, 5, 10 and 55 mM glucose added, being the latter the control condition. The parasites have been adapted to grow under those conditions for at least 15 months. Along this time, the enzyme activities, metabolite concentrations and fluxes of glucose catabolism were determined.

RESULTS. The cellular protein did not vary in amebas grown under all conditions (0.92 mg protein/ 10^6 cells). At low glucose growth (0, 2 and 5 mM) the trophozoites have a smaller intracellular volume than those grown with 10 and 55 mM glucose (1.5 versus 13 μL /10^7 amebas, respectively).

Amebas grown without added glucose consumed more glucose of the medium (11.6%) than those grown under control conditions (6.8%). The kinetic parameters (*Vmax* and *Km*) of the glycolytic enzymes were determined in cytosolic fractions of the different amebas. The *Vmax* values of 6- phosphoglucomutase, aldolase, 3-phosphoglycerate mutase and pyruvate phosphate dikinase diminished while hexokinase increased 2-folds. The glucose transport did not significantly change its *Vmax* or *Km* values except in amebas grown at 0 mM showing an increased affinity (0.5 mM versus 4.2 mM in control cells). The glycolytic intermediates glucose-6-phosphate, fructose-6- phosphate, dihydroxyacetone phosphate, glyceraldehyde-3-phosphate, pyruvate and ATP did not change. Glycogen content showed a 90% decrease in amebas at 0, 2 and 5 mM glucose indicating a highly active glycogen degradation.

Finally, the glycolytic flux measured as ethanol production was lower (4-5 nmol/min*mg cell protein) in low-glucose amebas (0 and 2 mM) in comparison to the other amebas which show 32 - 45 nmol/min*mg protein.

CONCLUSION. It is concluded that under low-glucose growth, glycogen degradation and the expression of a high affinity glucose transport overcame the decrease of external glucose to maintain amebal survival.

Analysis of differential compartmentalization between paralogous gene products: its role in functional diversification.

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Gene duplication has been considered the most important evolutionary process for the origin of new genes. After duplication the possible evolutionary processes that can guide the fate of the duplicated copies are: neofunctionalization (the creation of a new function), pseudogenización (loss of function), subfunctionalization (distribution of ancestral gene functions between the two copies). Protein subcellular relocalization may contribute to functional diversification of the products of gene duplication.

Bat1 and Bat2 have differential subcellular compartmentalization as Bat1 is located in the mitochondria while Bat2 is in the cytosol. The “ancestral type” protein *Lk*Bat1 has the same subcellular localization of Bat1; the mitochondria.

To determine the role Bat1 and Bat2 differential subcellular localization this work has aimed to construct relocalized Bat1 and Bat2 mutants to analyze their phenotype under aerobic and anaerobic conditions and the consequences (advantages/disadvantages) of the different subcellular compartmentalization of these proteins. To relocate Bat1 to the cytoplasm the mitochondrial signal sequence was removed and for Bat2 relocalization to the mitochondria Bat1 presequence had to be inserted in Bat2 coding sequence using the Delitto Perfetto approach.

Our results indicate that in biosynthetic conditions under aerobic and anaerobic conditions BAT1 has a preferentially biosynthetic role, which is not affected by its subcellular localization. Under aerobic conditions Bat2 has a minor role in biosynthesis which is only evident in a bat1Δ bat2Δ double mutant and which is not dependent on Bat2 subcellular localization. Under anaerobic conditions Bat2 displays a better biosynthetic capacity when it is located in the mitochondria. In the catabolism under aerobic and anaerobic conditions Bat1 plays a minor role in VIL catabolism and it’s not influenced by its localization. Bat2 plays the major role in catabolism. Under aerobic conditions there is a negative effect when Bat1 and Bat2 are located in the mitochondria, this effect is diminished when BAT1 is deleted or relocalized to cytoplasm.

Polymorphisms in mitochondrial genome affect the regulation of Cytochrome b synthesis in Saccharomyces cerevisiae

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The third mitochondrial respiratory complex or bc_1 complex couples the oxidation of ubiquinol, the reduction of cytochrome c and the transfer of four protons to the intermembrane space. The bc_1 complex in Saccharomyces cerevisiae contains ten subunits, with Cytochrome b (Cob) as the only subunit codified in the mitochondrial genome (Zara et al, 2009).

Translation of the COB mRNA is possibly regulated by Cbp3 and Cbp6 (Gruschke et al, 2011). They act on the 5' untranslated region (5'UTR) of the COB mRNA and also interact with the newly synthesized Cob protein. Cbp3 and Cbp6 interact with the early subcomplex formed by Cob and the chaperon Cbp4. When the nuclear subunits Qcr7 and Qcr8 assemble with Cob, Cbp3 and Cb6 are release to activate again the COB mRNA translation (Gruschke et al, 2011; Gruschke et al, 2012).

In this work, we observed that regulation in the synthesis of Cob depended on the yeast strain used. We evaluated two lab strains: BY4742 and D273-10b. We observed on a BY4742 strain that in the absence of the subunit qcr7 (qcr7Δ), Cob synthesis dramatically decreased. In contrast, in the D273-10b strain the mutant qcr7Δ showed normal levels, although the cells could not respire. We evaluated whether the information for the differential phenotype was on the mitochondrial DNA or on the nuclear genome. After exchange of the mitochondrial genomes we determined that a D273-10b strain with a BY4742 mitochondrial DNA could not synthesize Cob in qcr7Δ cells, indicating that the difference in the regulation of Cob synthesis is present on the mitochondrial genome.

We observed a similar phenotype when evaluated the effect of deletion of Cbp3 and Cbp6 over synthesis of Cob: on the strain BY4742 cbp3Δ and cbp6Δ mutants showed reduced levels of Cob synthesis, whereas on the D273-10b strain cbp3Δ and cbp6Δ mutants showed normal levels of Cob synthesis, even when the cells did not respire.

Because the differences in Cob synthesis between the two studied strains were due to some information on the mitochondrial genome, we sequenced the 5' UTR of COB in the two strains, and observed 6 point mutations on nucleotide sequence. In addition, the BY2742 mtDNA has 5 introns in COB while the D273-10b has 2 introns. Next we are going to test if the information for the differential regulation of Cob synthesis is present on the COB 5'-UTR or the introns.

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DIURNAL RESTRICTED FEEDING SCHEDULES PROMOTES CHANGES IN NF-kB PRESENCE IN LIVER OF WISTAR RATS.

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Introduction:. Restricted feeding schedules (RFS) modify the physiology of the organisms at several levels, such as metabolism, cell cycle and immune response. Previously, our laboratory described that RFS, that is related to caloric restriction by 2 hours of feeding access daily during 3 weeks, promotes increased apoptosis as well as cell proliferation in liver tissue of rats. However, it does not know the mechanisms through caloric restriction modify the survival rate of liver cells. In this regard, nuclear factor kappa B (NF-□B), which is a family of transcription factors implicated in cell survival and inflammation could be involved in the increased cellular turnover observed under RFS. The subunit p65 of NF-□B is phosphorylated and dimerized to be translocated to the nucleus, in order to promote gene expression of its target genes, such as anti-apoptotic and proliferation genes, while the subunit p50 inhibits gene expression of its target genes.

Objective: To evaluate the adaptations promoted by diurnal RFS in body weight, liver weight and presence of p65 in liver homogenates and nucleus fraction.

Materials and methods: Male Wistar rats of 180-220 g at the beginning of the experiment were kept in groups of 4 on a cycle 12/12 light/dark (light-on at 9:00 h) at constant temperature (21° C) with free water and free food access (5001 rodent diet from LabDiet) until the beginning of the experiment. Rats were randomly assigned to two different feeding conditions: 1) Ad-libitum (AL) - Control animals with free access to food and water during the 24-h period. 2) Diurnal RFS - Experimental animals which food availability was limited to 2h daily from 12:00 to 14:00 h. Body weight (BW) and food ingestion was measured every day. After three weeks, different subgroups of animals were sacrificed at 3-h intervals, for a 24-h period to obtain liver tissue samples, which was weighed fresh and then homogenized to be analyzed. Nuclear extracts were obtained by ultracentrifugation. Total and phosphorylated p65 were analyzed by Western Blot.

Results: Rats under RFS had an average body weight (BW) gain of 13.5%, while AL group had a weight gain of 67.6%. RFS group had an average daily caloric intake of 50% less than AL group. The AL group showed an average liver/BW 9.82% higher than the RFS group. The presence of both, total and phosphorylated p65 in liver showed changes in a daily profile in RFS group vs AL group, and also had lower average of the total presence in RFS group vs AL group ($p<0.05$).

Conclusions: RFS is a caloric restriction model that modifies metabolism and liver tissue dynamics. Total and phosphorylated p65 in the liver are sensible to RFS. Subunit p65 of NF-□B could play a role in the increased hepatic cell turnover observed under RFS.

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IDENTIFICATION OF GENES INVOLVED IN VIRULENCE OFpUM505 PLASMID FROM *Pseudomonas aeruginosa*

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The pUM505 plasmidof 123 kilobases wasisolatedfrom a clinical strain of *Pseudomonas aeruginosa*(1). pUM505 possesses a pathogenicity island (PAI) of 78 genes, 64 of them have been found in the chromosomal PAPI-1 and PAPI-2 PAIs of *P. aeruginosa* PA14, a virulent clinical isolate.(1)Moreover, it has been reported that PA14 usesthe same virulence factors to cause disease in plants (*Arabidopsis thaliana*), animals (mice) and nematodes(*Caenorhabditiselegans*)(2). However it is unknown whether pUM505 is involved in virulence, whereby the aim of this study is to determine ifthe plasmidincreases the virulence of the *P.aeruginosa*PAO1 standard strain and to identify the genes responsible for this property. Using as models of virulence lettuce leaves and *Dictyosteliumdiscoideum*cultures, pUM505 showedincreased virulence of the PAO1 strain. To identify genes involved in virulence a gene bank was constructedby digesting the plasmid and ligating the fragments to the vector pUCP20. A library of 120 clones was obtained in tha Top10 strain of *E. coli*and PAO1 of *P. aeruginosa..*Four clonesof this library were found to increase virulence in both*E. coli*and*P. aeruginosa.*The cloned inserts of thesetransformants wassequenced, and several ORF´s of pUM505 were identified. The ORFs 2, 17 and 42 (*hop*) have homologues that have been reported involved invirulence. The conclusion is that the plasmid pUM505 increasesthe virulence of its hosts due to someORF´soutsidethe pathogenicity island. These ORF´s are currently under study.

1. Ramírez-Díaz et al, 2011. *Plasmid* 66:7-18.
2. Mahajan-Miklos et al, 1999.*Cell* 96:47–56.

Virulotypingof *Salmonella enterica*strainsisolated from meat and dairy products from Michoacán

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*Salmonella enterica*is the causal agent of the most widespread foodborne bacterial illnesses in humans with high rates of morbidity and mortality, and is a major economic and public health issue worldwide. Several virulence genes involved in the invasion and infection mechanismsof*S. enterica* have been described (*inv*A*, rmb*A*, ssa*Q*, sop*B*, sop*E,and *sip4-F*) thatare encoded in several pathogenicity islands located in the chromosomal DNA of the pathogen. *Virulotyping*is a new scheme of genotyping based on the detection of virulence-associated genes, whichhas provento be an efficient tool for identification and separation of strains/clones of *S. enterica*.Therefore, the objective of this project was to evaluate the association between virulotyping and other typing systems (MLST and Serotyping),with the geographic region in the state of Michoacánfrom which the strains of *S. enterica* were isolated, with theaim of possiblycontributing to optimize the epidemiological surveillance systems. We analyzed90 strains of *S. enterica*isolatedfrom meat and dairy products that were collected between 2008 and 2011 in the state of Michoacán. These strains were previously genotyped by MLSTand their provenances and serotypes were known.The invA*, rmb*A*, ssa*Q*, sop*B*, sop*Eand *sip4*-F genes were amplified by PCR assaysin all studied strains.According to the presence or absence of amplification products of genes associated with virulence, 11 different virulotypes were observed and namedalphabetically as A through K. Virulotype A contained all of the virulence genes analyzed and is widely distributed throughout the state, thus having the highest pathogenic potentialfollowed by virulotypes B and C with the largest number of virulence-associated genes, except for genes*inv*Aand *Sop*E, respectively. The highest diversity of virulotypes was observed in the port of Lázaro Cárdenas, and in the regionsof Morelia and Apatzingán. Virulotype and sequence type (ST) of tested strains were unrelated. Because in all cases at least three virulence-associated genes were found, our results show that the studied strains of*S. enterica* have a high pathogenic potential,which is relevantfor public health given the presence of these strains in foodstuffs of daily consumption in the state of Michoacán. Virulotypingcan be added toserotyping as a genotyping tool for epidemiological surveillance services.

Effects of 17β-estradiol on the internalization of *Staphylococcus aureus* into bovine mammary epithelial cells

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Staphylococcus aureus is the infectious agent mainly responsible for mastitis. Acute or chronic inflammatory processes of mammary gland distinguish this pathology. The incidence of mastitis in dairy cattle is highest at the drying off period and parturition,which are characterized by changes in the levels of sex steroids such as 17β-estradiol (E2). This hormone,besides its role in reproductionalsoregulates the innate immune response, the mammary gland growth and its involution by apoptosis ofthe mammary epithelial cells. These effects are mediated by specificreceptors that recognize and bind to E2.There are two classical estrogen receptors (ERs): ERα and ERβ. In general, E2 has been associated with anti-inflammatory effects in diverse organs and cell types; and this could be the reason by which during parturition cows are more susceptible of infections, such as mastitis caused by *S. aureus.*However, the role of E2 on the innate immune response of bovine mammary epithelial cells (bMEC) during*S. aureus*infection has not been studied. In addition,*S. aureus* has the abilityto invade different types of non-professional phagocytes, escaping from the host lysosomal degradation machinery and persisting within the intracellular location for long time periods. In this work we evaluated the effect of E2 on the internalization of a *S. aureus* strain (ATCC 27543from bovine mastitis)into a primary culture of bMEC. Cells weretreated 24 h with different concentrations of E2 (ranging 1-500 pg/ml), and then were infected during 2 h with*S. aureus*.Previously, we performed viability assaysin order to determine possible cytotoxic and antibacterial effects of E2 against bMEC and *S. aureus,* respectively. Using MTT and trypan-blue exclusion assays, we did not detect cytotoxic effects of E2 on bMECafter 24 h, neither antibacterial effect (after 8 h) determinedbyturbidimetric analysis, CFU counting or using thelive/dead baclight bacterial viability kit.Our data showed that inbMEC treated 24 h with E2 (50pg/ml), *S. aureus*internalization was reduced (~50%). By flow cytometryanalysis we detected that E2 (50pg/ml) increased theintracelllular ERα abundance (~20%) in bMECs, andimmunocytochemistry supportedthese results.In order to determine if soluble products secreted by bMEC treated with E2 could be responsible of reducing *S. aureus* internalization, we also analyzed the extracellular bacterial viabilityafter 2 h of interaction with E2-treated bMEC culture media. Extracellular bacterial viability was reduced (~20%) in bMEC treated with E2 (50 pg/ml). Altogether these results suggest that E2 is reducing bacterial internalization in bMEC through the modulation of innate immune response of these cells, and the ERα is involved in these effects.However, other experimental approaches that are being carried out, such as the analysis of inflammatory elements, are necessary to support these conclusions.

Metagenomic analysis of bacterial diversity in kefir and tibi grains

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Kefir grains, which are composed of various microorganism held together in an exopolysaccharide matrix calledkefiran, produce a fermented milk beverage that is self-carbonated and contains a low percentage of alcohol; tibi grains on the other handgrow in a jaggerysolution in water. Tibi grains are similar to kefir grains being an association of microorganisms, held together by an exopolysaccharide matrix called dextran, and products formed during the fermentation process.In recent years, microecosystem analysis has been revolutionized by metagenomic technologies.

The aim of this study was to determine the bacterial diversity of kefir and tibi grains collected in Mexico by 16S rDNAmetagenomic analysis, of grains acclimated to simple culture media, lactose for the kefir grains and sucrose for the tibi grains. DNA was extracted from each grain, kefir grains grown in milk, kefir grains grown in lactose ,tibi grains grown in piloncillo and tibi grains grown in sucrose. V3-16S rDNA libraries were made by PCR and characterized by ion semiconductor massive sequencing in Ion Torrent PGM equipment. Metagenomic analysis was done using the QIIME pipeline.

Four bacterial Phyla were detected in kefir grains grown in milk:Bacteroidetes (57.58%), Firmicutes (39.57%), Proteobacteria (1.49%) and Fusobacteria (0.77%); as well asfour in kefir grains grown in lactose:Bacteroidetes (52.18%), Firmicutes (44.59%), Proteobacteria (1.60%) andFusobacteria (0.94%).At the genus level, the sequencing results show a high diversity in the bacterial composition within the kefir grains grown in milk and lactose, where there were predominantly composed of *Prevotella sp*49.82% and 46.02%, respectively.The α diversity analysis, which could evaluate bacterial diversity in every sample, using Shannon and Simpson indexes, showed that the diversity present in kefir grains grown in milk is more abundant than the diversity of kefir grain grown in lactose.Three bacterial Phylawere detected in tibi grains grown in jaggery: Firmicutes (84.11%), Proteobacteria (8.81%) andBacteroidetes (6.18%); and three in tibi grains grown in sucrose Firmicutes (50.77%), Proteobacteria (36.81%) andBacteroidetes (11.73%). At the genus level, thetibi grains grown in jaggerywere predominantly composed of *Lactobacillus sp*(68.24%) and the tibi grains grown in sucrose were predominantly composed of *Clostridium sp*(32.56%). Theα diversity analysis, showed the diversity present in tibi grain grown in jaggery is more abundant than the diversity of tibi grain grown in sucrose.

The β diversity analysis showed that the bacterial community of kefir grains growing in milk and lactose are more similar between them then the communities of tibi grains growing in jiggery and sucrose.Funding byCinvestav y CONACyT 163235 INFR-2011-01 for JGM.

IDENTIFICATION OF NOVEL CIPROFLOXACIN RESISTANCE GENES OF pUM505 PLASMID FROM *Pseudomonas aeruginosa*

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The pUM505 plasmid was isolated from a clinical strain of *Pseudomonas aeruginosa;* it has a length of 123 kilobases and contains 138 encoding regions[1]. DNA sequence analysis revealed that pUM505 lacks antibiotic resistance genes reported to date; however, when the plasmid was transferred to *P. aeruginosa* PAO1 standard strain, conferred resistance to ciprofloxacin (Cp), an antibiotic of the quinolone family[1]. In an attempt to identify genes responsible for resistance to Cp the *orfs* 35, 36 and 37 from pUM505 were clone and transferred to *Escherichia coli* J53-2 and to PAO1 strains, but they only conferred resistance to *E. coli*, suggesting that additional genes are require for Cp resistance in PAO1[2]. The aim of this study is identify additional genes to the *orfs* 35-37 of pUM505 involved in Cp resistance. Therefore, pUM505 was transferred to a *P. aeruginosa* PAO1SR (streptomycin resistant, Sm^R) strain and a transposon-insertion mutant bank was generated by mobilizing suicidal plasmid pFAC (containing the Himar1::Gm^R transposon) from *E. coli* S17-1 (pFAC) to PAO1SR (pUM505). From a bank of 12, 000 clones, 12 mutants which showed a sensitive or hypersensitive phenotype to Cp were identified. DNA sequence analysis of these mutants showed that *orf*57 (*xerC*), *orf*58, *orf*59 (*xerC*), *orf*85, *orf*86 and *orf*87 genes were disrupted by this transposon. These data suggest than, pUM505 plasmid contains genes related to Cp resistance by two possible mechanisms, one conferred by site-specific recombinases (*xer*C) and another by a quinolone efflux pump.

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Cell wall caracterization and imnunesensig of no-albicans species.

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The species of *Candida*genus are the causative agent of candidiasis. In Latin America, the six species more frequently isolated from bloodstream samples are *C. albicans, C. tropicalis, C. parapsilosis, C. guilliermondii, C. glabrata*and *C. krusei.*The fungal immune sensing is a key step in the establishment of a protective anti-fungal immune response, and among the first events that trigger this interaction is the recognition of the fungal cell wall. The *C. albicans* cell wall has been thoroughly studied, but little is known about the wall organization and composition in other *Candida*, such as *C. guilliermondii, C. tropicalis, and C. krusei.* The cell wall of*C. albicans*, is conformed of two well defined layers:an inner layer composed of chitin, β1,3- and β1,6-glucans, and an outer layer rich in proteins modified with *N*- and/or *O*-linked mannans. This components are absent in mammals cells; therefore, are recognized by a variety of components of the innate immune system.Here, we found that the cell wall of *C. albicans, C. tropicalis* and *C. guilliermondii* has similar levels of mannan, glucan and chitin, but *C. krusei* contains higher levels of chitin and lower glucan content. The phosphomannan content was similar in*C. albicans* and *C tropicalis*, but lower in*C. krusei* and *C. guilliermondii*. In addition, we found that the wall porosity is higher in non-*albicans* species. According with the differences in the wall composition, the studied *Candida* species stimulated differential cytokine production when co-incubated with human mononuclear cells. Together, these results indicate that *Candida* species have subtle, but significant, differences in the cell wall that affect the interaction with innate immune cells.

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Inhibitory activity of *Flammulina velutipes* extracts against the *S. schenckii*: cell - host interaction

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Fungal infections have augmented recently due to uncontrolled use of antibiotics and increase of immune compromised patients. In spite that fact that there are many different antifungal substances, the majority of them are toxic for human body. For instance, the incidence of mycosis caused by *Sporotrix schenkii* has increased in Mexico; nevertheless, the effective treatment has not been established yet. The substances from natural sources, in the other hand, present almost no side effects. Particularly, *Flammulina velutipes* extracts have shown the high content of mannose, which is known as a potent inhibitor of cell - host adhesion [1], but the possibility of the extracts to inhibit the adhesion, is still unknown. The present study addresses the characterization of extracts from two strains of *F. velutipes* and examine its capacity for inhibition of the adhesion of fungus to host cell.

Experimentally, two strains of *F. velutipes* were used: AQF-1 (the botanic garden of Saint-Petersburg, Russia) and ATCC 34574, Epithelial cell (ATCC L929). The mycelium of *F. velutipes* was obtained in YPG medium. The extract was obtained by ultrasound [1]. Quantification of proteins in the extract was made by Lowry (1954). Con A and WGA lectins were used to identify glycosilated proteins [2]. Moreover, adhesion assay of yeast *S. schenckii* (MP103) with epithelial cells at 60min were made in absence or presence of the extract (50, 100 and 150µg/mL). After the interaction, the count of no-adhered yeast was realized.

The results shown, that both strains of *F. velutipes* present similar macro- and micromorphology. The yield of the mycelium biomass per liter of medium was 2.5±0.3 for strain AQF-1 and 1.80±0.27 for ATCC34574 and the yield of the extract in % of dried mycelium 4.8±0.5 and 3.0±0.6 respectively. The results for protein quantification in the extract in % were 2.9±0.2 and 3.1±0.3 for AQF-1 and ATCC 34574 respectively.

Moreover, both extracts presents similar protein pattern i.e. four mayor common proteins of 85.5, 43, 26.3 and 22 kDa, and three common glycosylated proteins: 100, 41.3 and 23 kDa.

The adhesion assay indicate that the extract of *F.velutipes* presents inhibitory activity in dose independent manner for strain AQF-1 and in dose dependent for ATCC34574.

Conclusions: The extract of *F.velutipes* presents inhibitory activity of *S. schenckii* adhesion, the strain AQF-1 shows more activity, finally an important finding was identification of glycoproteins from *F.velutipes* which has not been described before. It is necessary to perform additional tests with other pathogenic fungi and characterize other components which can be involved in this activity.

References.

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Entamoeba histolytica Infection Modulates the Expression of microRNA in Human Colon Cancer Cells

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Human amoebiasis is one of the most prevalent parasitic diseases worldwide, with nearly 50 million people affected, mainly in developing countries. The protozoan parasite responsible for amoebiasis is *Entamoeba histolytica* that infects the gastrointestinal epithelium and causes colitis, dysentery and extra-intestinal abscesses. Parasite resistance or susceptibility reduction to the drug metronidazol treatment has stimulated the search for new control strategies, which require a better understanding of infection development and host-parasite interactions. MicroRNAs (miRNAs) are small regulatory RNAs that bind the 3′-untranslated region of target mRNAs, acting as critical regulators of gene expression at the posttranscriptional level. To date, miRNAs have been found to be critically implicated in the outcome of diverse diseases, but little is known about host miRNAs regulation in response to parasitic infection.

Here, we evaluated change in the expression of host miRNAs following interaction of SW480 human colon cancer cell line with *E. histolytica* trophozoites using miRNA microarray technology (TaqMan MicroRNA Arrays). Results showed that five miRNA species out of 384 miRNAs were significantly upregulated after 45 minutes interaction, namely hsa-miR-526b, hsa-miR-643, hsa-miR-150, hsa-miR-615-5p and hsa-miR-525. Gene ontology enrichment analysis of miRNA-targeted genes revealed that several pathways and molecular functions were modulated in SW480 cells interacting with trophozoites, including mechanisms associated with apoptotic signalling pathway, cellular differentiation, regulation of cell death, lipid metabolic process, among others. Thus, our data suggest that alteration in miRNA levels likely plays an important role in regulating intestinal epithelial cells functions upon *E. histolytica* infection. These results also suggest that identified miRNAs could represent promising target for the control of *E. histolytica* infection.

Potential Biomarkers forDuchenne Muscular Dystrophy and Carrier Detection

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Abstract

Duchenne Muscular Dystrophy (DMD) is the most severe neuromuscular disease and common dystrophynopathy, affects 1:3500 living males. An estimate of 400 new cases take place in Mexico every year. DMD is a monogenetic disorder, caused by mutations in the *DMD* gene, located in Xp21.2 locus, which encodes a subsarcolemal protein, dystrophin, an integral part of the dystrophin-associated protein complex (DAPC). Dystrophin protects muscle from movement-induced damage, therefore dystrophin deficiency results in chronic degeneration of the muscle. Since there is no cure for DMD, prevention strategies are aimed by means of carrier detection and genetic counseling. In addition, multidisciplinary management has improved life standards for DMD patients. In order to know the utility of biomarkers in the diagnosis of male DMD patients and carrier detection, we evaluated serum levels of myostatin, follistatin, matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1). A descriptive case-control study was performed. The study included 21 DMD patients in ambulatory stage and non-DMD control subjects. In addition, 17 female carriers and female non-carriers control subjects were included however, follistatin levels were not evaluated. Compared to control subjects, DMD patients had significantly lower serum myostatin levels (1.02 ng/ml vs. 4.24 ng/ml, $p<0.001$), however they showed higher follistatin levels (1.40 ng/ml vs. 0.79 ng/ml, $p<0.001$), higher MMP-9 levels (1071.20 ng/ml vs. 532.40 ng/ml, $p<0.001$) and higher TIMP-1 levels (402.34 ng/ml vs. 376.70 ng/ml, $p<0.398$). Female carriers had lower serum myostatin levels as well (2.70 ng/ml vs. 2.94 ng/ml, $p<0.538$), meanwhile higher TIMP-1 and MMP-9 levels showed no significant difference. We propose that a decrease in myostatin levels in DMD patients and female carriers could be caused by muscle destruction and the lack of feedback signals that activate satellite cells, therefore follistatin levels are increased. On the other hand, an increase in MMP-9 and TIMP-1 levels in DMD patients reflect tissue remodeling and are consistent with reports in other populations. The role of these molecules as potential progression biomarkers in DMD and carriers detection warrants further study.

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Diffuse reflectance spectroscopy as a possible tool to complement liver biopsy for grading hepatic fibrosis in paraffin-preserved human liver specimens

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A diffuse reflectance spectroscopy-based method to score fibrosis in paraffin-preserved human liver specimens has been developed and is reported here. Paraffin blocks containing human liver tissue were collected from the Pathology Department of the General Hospital of Mexico and included in the study with patient's written consent. The score of liver fibrosis was determined in each sample by two experienced pathologists in a single-blind fashion. Spectral measurements were acquired at 450-750 nm by establishing surface contact between the optical probe and the preserved tissue. According to the histological evaluation, four liver samples showed no histological evidence of fibrosis and were categorized as F0, four hepatic specimens exhibited an initial degree of fibrosis (F1-F2), five liver specimens showed a severe degree of fibrosis (F3), and six samples exhibited cirrhosis (F4). Interestingly, the human liver tissue showed a characteristic diffuse reflectance spectrum associated with the progressive stages of fibrosis. In F0 liver samples, the diffuse reflectance intensity gradually increased within the wavelength range of 450-750 nm. On the contrary, F1-F2, F3, and F4 specimens showed a corresponding 1.5, 2, and 5.5-fold decrease in the intensity of diffuse reflectance with respect to F0 livers. At 650 nm, all the stages of liver fibrosis significantly differed from each other. To our knowledge, this is the first study reporting a distinctive diffuse reflectance spectrum for each stage of fibrosis in paraffin-preserved human liver specimens. These results suggest that diffuse reflectance spectroscopy may represent a complementary tool to liver biopsy for grading fibrosis.

Physicochemicalcharacterizationand antimicrobialactivity of propolis collected in the apiary "Las agujas" from the "La primavera" forest

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Propolis are a mixture of resins and plant exudates collected by bees, which has been used widely and popularly as a medicinal remedy since ancient times because theirown antimicrobial, antifungal, antiviral and antiparasitic activity, which vary according to their natural geographic source.

The objectives of this study are quantifying polyphenols (bioactive molecules) of propolis extract obtained from the apiary "las agujas", "La primaveraforest"; and analyze the antimicrobial activity against *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative) bacterias, and *Candida albicans* yeast, using the Dalgard& Peek's bioassay.

Propolis was collected during the winter by traps placed in hives. Defatted propolis was obtained by soxhlet extraction with solvents. The defatted propolis was solubilized in ethanol. The ethanolic extract was concentrated in a rotary evaporator under reduced pressure and lyophilized. The material obtained was reserved for characterization study and testing.

The results indicate that propolis has an equivalent polyphenol content of 38 wt% (phenols: 268.2 mg A.G.E./g and flavonoids: 111.2 mg Q.E./g of propolis extract). The UV absorbance profile of propolis is atypical compared to others reported in the literature. The bioassays were performed in triplicate for each of the mentionedmicroorganisms and showed that the extract completely inhibited the growth of all of them, in a doseresponse fashion. We conclude that the propolis analyzed has antimicrobial activity potentially applicable in infections caused by pathogenic bacteria and fungi.

Cytotoxicity ofdefensinsγ-thionin (*Capsicum chinense*) and PaDef (*Perseaamericana*var. *drymifolia*) againstcancercell lines

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Cancer isa majorhealth problem worldwide.InMéxicois thethird leading cause ofdeath,being the breast and cervical cancer the most prevalent. Conventional therapieshavea low therapeutic indexand severe side effects; thus, it is necessaryto develop alternative therapies. Antimicrobial peptides (APs) are part of the innate immune defense mechanism of many organismsand have been considered as promising candidates to treat cancer because they have the ability to inactivate a wide range of cancer cells. Theanticancer activities ofAPsincludethe induction of apoptosis, modification of cell cycleas well asthe lysis ofthe cytoplasmic membraneand autophagy induction.In this work, weassessthe cytotoxicity ofplant APs; the defensinsγ-thionin(*Capsicumchinense*) andPaDef(*Perseaamericana*var.*drymifolia*) against differentcancer cell lines,andwe analyzedtheirpossible mechanism of action.Defensinswerechemically synthesizedandcytotoxicityassayswere performedonHeLacells (cervical cancer), MCF-7 (breast cancer),bMEC(bovine mammary epithelialcells) and PBMC (peripheral blood mononuclear cells). The APγ-thionin(200µg/ml) significantly inhibitedHeLa cellviability(77%), andin lesser extentto MCF-7cells(24%). Interestingly,bMECand PBMC viabilitywas unaffected, suggesting thatcytotoxic effects of γ-thioninare selectivetocancer cell lines.Furthermore,thedefensinPaDef(300µg/ml) decreased the viabilityof HeLa cells(33%); however,the viabilityof bMECwas affected(63%) but thePBMC viabilitywas unmodified. To determinethepossible mechanism of actionofdefensinγ-thioninonHeLacells, the apoptosis rate was measured by flow cytometryusing propidium iodide/Annexin-V double staining. The apoptosis rates were 77% and 87% after the treatment of the cells during 24 h with 100 and200 µg/mlof γ-thionin,respectively.On the other hand,the cell cycleof HeLa cells treated withγ-thioninwas analyzedby flow cytometry using propidiumiodideDNA staining. The results showedthat the cell cyclewas arrested at G1/S phase with 25 and 50 µg/ml of γ-thionin. With theseresults, we concludethatthedefensinsγ-thioninandPaDefhavecytotoxic effectsagainst the cancercell lines evaluated. Also, the defensinγ-thioninshowedaselective effectagainstcancer cellsthrough theinductionofapoptosisandcell cycle arrest.

New treatment for breast and colorectal cancers inhibiting mTOR pathway and glycolysis by promoting apoptosis and autophagy.

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Cancer is one of the leading causes of death worldwide, its treatment is expensive and the secondary consequences for patients are numerous. Carcinogenesis is a multistep process, where several events participate in the dysregulation of the good functioning of the cell. Our research proposes an alternative treatment for breast (BC) and colorectal cancers (CRC), based on the correction of the tumoral cell metabolism leading to apoptosis and eventually to autophagy. The pharmacological scheme consisted in the synergy of three drugs: doxorubicin, metformin and F3 (D/M/F3). The first one, is a well known chemotherapeutic; metformin is a broad-spectrum drug which inhibits mTOR, an important protein complex involved incell growth and proliferation through AMPK and AKT pathways, and indicates lack of energy and nutrients availability. The third drug is an analogue of pyruvate, which stops glycolysis by inhibiting the enzyme lactate dehydrogenase (LDH).*In vivo* results showed that the formulation consisting of D/M/F3 reduced significantly the size of the tumor in both cancer types in comparison to the positive control (doxorubicin). Even more, the toxicity of the drugs'cocktail in animalsis considerably lower than the regular commercial medicines used for these kinds of cancers. Furthermore, by flow cytometry analyses (Annexin V test), it was observed that D/M/F3 treatment on the breast cancer cell line MDA-MB-231, increases apoptosis death and reduces necrosis death after 24 hours of exposure, diminishing the known toxicity of doxorubicin. Our *in vitro* experiments suggest that the mechanism of action of the three drugs together interfere with the regulation of apoptosis and autophagy in the tumoral cell, correcting these two cell death mechanisms. Additionally, a plus of our treatment is the price of the medicines, which are very accessible for public in general. In summary, we are presenting an innovative and creative alternative to treat BC and CRC targeting mTOR and glycolysis in cancer cells in*in vitro* and in two different murine models.

Age-related reduction of muscle mass and strength in fast but not slow skeletal muscle. The role of costamer-nuclei proteins interactions.

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Skeletal muscle (SM) is the major tissue present in vertebrates. Its principal function is generating mechanical energy from chemical energy. SM differentiates in a process called myogenesis and SM full development ends shortly after birth, growing as a consequence of hypertrophy. During senescence, the reduction of muscle mass and strength has been widely considered one of the major causes of disability in older animals. Sarcopenia, has been considered to be the main cause loss of strength of muscle and increased fatigability. There are two general types of SM that has to be considered to study the mechanical properties of muscle (Slow-oxidative, low fatigue) and (Fast-glycolytic, high fatigue) which are differentially affected by aging, therefore the molecular mechanism involved leading to sarcopenia has different origins. Two different rat groups; one with 4 months (adult young) and 440g of weight and the other with 15 months (old rats) and 760g of weight where used for SM mechanical studies, using representative muscles; for fast (Extensor digitorium Longus) and slow (soleus) contraction. Dorsal-SM was used to isolate sarcolemma, sarcoplasmic reticulum and nuclei for biochemical studies, to quantify sarcopenia and costamere-nuclei connectivity (collagen, protein marker determination of nuclei and costamers) were used. Our results show that fast SM of old rats has a 60% reduced force by unit of mass compared with the equivalent of young rats, where muscle fatigue follows the same pattern. On the other hand, the tension decay during a tetanic stimulation (fade) shows a different shape indicating a potential impairment of SERCA1 activity that is under study. Slow SM does not show a significant difference between groups.

The costameric proteins associated to the sarcolemma-nuclei mechano-transmission are compromised during aging, might be with a mechanism similar to the one observe for some muscular dystrophies as we have previously studied (A. Solares-Pérez, R. Álvarez, R. H. Crosbie, J. Vega-Moreno, **Joel Medina-Monares**, F. J. Estrada, A. Ortega, R. Coral-Vazquez, Cell Calcium 2010.)

Distal colon microbiota and Short chain Fatty Acids in association with Mexican Childhood Obesity

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Background

Obesity is a leading public health problem in developed and developing countries. Nowadays 30% of the human population is obese.Colon microbiota provides an additional energy through fermentation of undigested carbohydrates, in the form of Short Chain Fatty Acids (Acetic, Propionic and Butyric Acids) (SCFA). Dysbiosisof the microbial communityof the distal colon in Mexican children affect the nutrient absorption, andmight contribute importantly to the developmentof obesity.

Method

The profile of the microbial diversity of normal-weight (n=82), overweight (n=29), and obese Mexican children (n=80) (age 9–11 years)wasdeterminedusingthe hyper variable region V3 fingerprint from the bacterial 16SrDNA.Bacterial genomic DNA was extracted from fecal samples and suitable libraries were made byPCR. 16S rDNA libraries were characterized byion semiconductor massive sequencing in Ion Torrent PGM equipment.Metagenomic analysis was done using the QIIME pipeline. Levels of Short Chain Fatty Acids (Acetic, Propionic and Butyric) were measured by High Performance Liquid Chromatography (HPLC).

Results

From the metagenomic analysis of the massively sequenced amplicon libraries, four types of phylum were identified such as Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria.Proteobacteriawas more abundant in Normal-weight in comparison to Overweight, and it was more abundant than in Obese children. HPLC analysis revealeda significantly lower (p=0.023) concentration of Butyric acid in obese children than in Normal-weight children; about a 38% decrease in Butyric acid level compared to Normal-weight. In overweight children, the levels of propionic acid increased significantly (p= 0.025) than obese children.

Conclusion

In this study, we conclude that a distal colon microbiota dysbiosisaffects the metabolism of undigested food in Mexican overweight and obese children. We found a higher abundance of *Faecalibacterium*, in the same groups, whichhas higher capacity to harvest energy from undigested carbohydrates. In addition, we found less SCFA in obese and Overweight children. On the other hand, we observed proteobacteria was more abundant in Normal-weight, particularly *Succinivibrio*which counterbalance the excess of energy production made by other bacteria.

Study of the rearrangement of the canonical RNAD canonical enzymes during mRNA decay.

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In *Escherichia coli*, the transcription of RNA messengers needed for protein synthesis is carried out by the multi protein complex RNA polymerase. Later on, the events of decay and degradation are made by a different multi enzymatic complex called the RNA Degradosome (RNAD), which carries out this process in a regulated manner, giving rise to the phase of post-transcriptional regulation of gene expression. The canonical RNAD of *E. coli*is assembled by the resident enzymes Ribonuclease E (RNase E), whose structured amino-end contains endoribonuclease activity, and whose carboxy-end functions as scaffolding to organizethe addition of the DEAD-box RNA helicase enzyme, whose function requires ATP (RhlB);the addition of enolase,a glycolytic enzyme, and the addition of the inorganic phosphorus-dependent exonuclease polynucleotide phosphorylase (PNPasa). We have developed a system based on the Förster Resonance Energy Transfer phenomenon (FRET), to obtain evidence of proximity among the RNAD enzymatic components. The FRET is the transfer of energy from an excited fluorophore, which acts as a donor, to another lower energy acceptor fluorophore. In this phenomenon, the transfer efficiency is inversely proportional to the sixth power of the distance between the acceptor molecules and donors, and it gets a typical resolution between 10 to 100 Å (1-10 nm). We have reported with our FRET system, the evidence of proximity between the components of the RNAD *in vivo* in *E. coli*. In this work we report evidenceof rearrangements in the RNAD, when the conditional decay of the messenger RNA for the *ptsG* glucose transporter is triggered. The decay-degradation of *ptsG* mRNA is an excellent example of post-transcriptional regulation, which is presented in *E. coli*under conditions ofhigh glucose-6-P concentrationwhich is toxic for the bacteria. For the regulated decay-degradation mediatesa sRNA which is stabilized by the Hfqchaperone protein.During interaction of the sRNA-Hfq complex with the RNase E, the endonuclease function degrades the *ptsG* mRNA messenger targeted by the sRNA. Although there is a clear evidence of participation of RNase E, it is still necessary to prove the involvement of the RNAD in the process. The rearrangements detected *in vivo* by our FRET system, support the participation of the RNAD canonical components in *E. coli*. Work financed by Cinvestav-IPN; CONACyT-24269-CB-2005-01-48795, and CONACyT-CB-2010-01-152857.

Dynamics and chaotropic agents effects on the amyloidogenic lambda 3r light chain studied by NMR spectroscopy

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Amyloidosis is a heterogeneous group of diseases characterized by amyloid fibril deposition in target tissue, specifically, primary amyloidosis (AL) is the most common systemic amyloidosis. AL is characterized by pathologic fibrillar production of light-chain immunoglobulin (Ig), causing organ dysfunction. One of the most amyloidogenic germ lines of Ig is the lambda 3r. Two mutants have been derived from this germ line, the single mutant C34Y (3rC34Y) and the triple mutant P7D, C34Y and W89A (3rCWP7D). These mutants have different stability and propensity for amyloid fibril formation. However, is unclear the correlation between protein dynamic and amyloidogenic propensity. In this work, we analyze the intrinsic dynamic for the two constructs in order to get a better understanding of the amyloid fibers formation process. We have used dynamic Nuclear Magnetic Resonance (NMR) experiments at different time scales. Contrary to common sense, NMR experiments at millisecond time scale dynamics, show that the more stable protein (3rC34Y) has more intermediate exchange than the more unstable protein (3rCWP7D). On the other hand, at slow time scales (hydrogen exchange and real time experiments) we observed a correlation between protein dynamic and thermodynamic stability. This work stress out the important of the dynamic time scale in protein conformation studies.

An intramitochondrial PKA modulates placental steroidogenesis in a cAMP independent manner.

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Progesterone synthesis by the human placenta is essential for the maintenance of pregnancy, but the signal transduction cascades as well as the endocrine, paracrine or intracrine stimulus that regulate placental steroidogenesis are not completely understood. However, the most studied signaling pathway is the one mediated by cAMP/PKA, since PKA has been implicated in the phosphorylation of transcription factors and proteins related to steroidogenesis [1].

In order to assess the overall contribution of PKA in progesterone synthesis and protein phosphorylation in syncytiotrophoblast mitochondria of human placenta (HPM), the PKA isoform, its distribution in different submitochondrial fractions and its response to specific inhibitors or activators in the context of steroidogenesis was searched.

The results showed that PKA α catalytic subunit was distributed in all the submitochondrial fractions, whereas βII regulatory subunit was the main isoform observed in both the outer and inner membranes. The PKA located in the inner membrane showed the highest activity. Moreover, PKA seems to be tightly associated to the mitochondrial membrane fraction, but most important, its dissociation cannot be favored by the known holoenzyme activators (db-cAMP), as it occurs in the classical model of PKA activation. With respect to progesterone synthesis and protein phosphorylation, both events are clearly modified by inhibitors of the PKA catalytic subunit (H89) but are neither sensitive to inhibitors of the regulatory subunit (RpcAMP) nor to activators of the holoenzyme (cAMP, db-cAMP). The attempt to activate elements of the signaling cascade located upstream to PKA, like a soluble adenylate cyclase, did not modify HPM steroidogenesis either.

The unresponsiveness to the classical holoenzyme activators prompts us to consider that PKA of HPM could already be active and it is only liable to be inhibited but probably not to be further activated. Moreover, the lack of catalytic subunits release in the presence of db-cAMP, suggests that the activation of PKA do not necessarily means dissociation [2].

Our results suggest that the presence of an intramitochondrial PKA could be an essential component as part of a complex transduction cascade related to steroidogenesis. However, PKA activation and its repercussion on steroidogenesis could be reached by a mechanism different from that observed in the cytosolic PKA.

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Role of p53 on tumor energy metabolism

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Introduction.p53 is a transcriptional factor involved in several tumor processes. Recently it has been determined that under normoxic conditions (21 % O2) p53 negatively modulates tumor glycolysis decreasing the content of glycolytic proteins mRNA and on the contrary, positively modulates oxidative phosphorylation (OxPhos) enhancing mRNA of glutaminase and SCO2. Unfortunately, the proteomic, kinetomic and fluxomic analysis of both energy pathways were not determined to remark the role of p53 as metabolic modulator. In this study we evaluate the role of p53 on the energy metabolism of HeLa cells wild-type (HeLa-L, low p53 level) and on p53-over expressing HeLa clone (HeLa-H, high p53 level) by measuring protein contents, kinetic activities and fluxes. Knowing that tumor cells develop in hypoxic areas, studies were performed under normoxic (21 %O2) and chronic hypoxic (0.1% O_2) conditions.

Results. In normoxic conditions (21% O_2), p53 increased (60%) the OxPhosrateof HeLa-H as well as mitocondrial membrane potential ($\Delta\Psi m$,65%) and the mitocondrial protein contents (3- 4 times) of ND1, COX IV, ATPsynthase, PDH and GA compared to HeLa-L, whereas glycolysis was similar in both HeLa types. Under chronic hypoxia, HeLa-H OxPhos functionality (rate flux, □□m and protein contents) was severely diminished (80- 90%) versus normoxia; on the contrary, hypoxia did not modified HeLa-L Oxphos suggesting that p53 may be involved in the activation of mechanisms (i.e., autophagy activation) for hypoxic induced-OxPhos impairment. Regarding glycolysis, hypoxia increased HeLa-L flux through HIF-1αstabilization whereas in HeLa-H, p53 prevented the high glycolysis mediated byHIF-1α. Underlying the mechanism associated with the p53-mediated mitochondrial impairment, mitophagy activation was evaluated. We found that PUMA, Bnip3, Atg3 and Lamp1 increased (2-4times) in HeLa-H vs. HeLa-L.These changes correlated with a substantial decrement (75%) in the mitochondrial content and a reciprocal increment (50%) in the lysosome content.

Conclusions.p53 favors the oxidative phosphorylation under normoxia. In contrast, under hypoxia p53 induces mitochondrial degradation mechanismsresulting in a low oxidative phosphorylation.

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Characterization of a hyaluronidase from thevenom of the spider*Brachypelma verdezi*

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The spider venom is defined as a “multicomponent system”, because is constituted of low and high molecular weight compoundsas nucleic acids,organic acids, acyl-polyamines, peptides and proteins[1]. One of the venom proteinsis the hyaluronidase, an enzyme that is also widely distributed in natureas corporal components in fluids of mammalians, animal venoms, insects, leeches and bacteria[2]. The hyaluronidases, depending on the natural source, have specific hydrolytic cleavages for substratessuch as hyaluronic acids and/orchondroitin sulfates[3,4,5].
In medicine, the hyaluronidaseshas therapeutic uses for spreading or facilitating the diffusion of medical drugs in to the body; so that, itis interesting to search fornovel sources of hyaluronidases with different substrate specificities in nature having higher physicochemical stability and enhanced enzymatic activities.One source of hyaluronidases that we have explored comesfrom the venoms of *Brachypelma* species or also called Mexican Tarantulas. Previously, we have obtained a hyaluronidase from *B. vagans*[6]; however, it has low stability at acidic pH, which diminished its hydrolytic activity. Recently, we observed a more stable hyaluronidase from the specie *B. verdezi*. In this work, we compared the biochemical properties and molecular constitution of the hyaluronidases from *B. vagans* and *B. verdezi*. Both enzymes were separated through out different chromatographic methods and their biological activities compared. Our results show that the enzymatic activity of the hyalurodinase from *B. verdezi* was twice higher than that of*B. vagans*. Furthermore, using molecular biology techniques such as the polymerase chain reaction and rapid amplification of cDNA ends, we found a transcript that codes for a hyalurodinase from *B. verdezi*. The mature protein sequence of this enzyme containsonly threeresidues different to that of *B. vagans*. The better specific activity and pH stability from the hyaluronidase from the venom of *B. verdezi*points to be used as acandidate for medical applications.
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COMPLEX IV HETERODIMERIC COX2 IN *Polytomella* sp: IMPORT AND PROTEIN-PROTEIN INTERACTIONS

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Gene transfer from the mitochondrion to the nucleus is an ongoing evolutionary process, that explains why extant mitochondrial genomes are highly reduced, encoding only a few set of proteins required for oxidative phosphorylation. These proteins exhibit high hydrophobicity, so it is believed that the functional migration of the corresponding genes to the nucleus has been limited by this parameter. However, the chlorophycean algal lineage, that includes the colorless alga *Polytomella* sp., has a mitochondrial DNA lacking the *cox2* gene that encodes subunit II (COX2) of cytochrome c oxidase. In *Polytomella* sp. this gene was split into two genes, *cox2a* and *cox2b*, which encode proteins COX2A and COX2B respectively. A feature of these subunits is an additional sequences located at one of its ends, at the N-terminal region for COX2B and at the C-terminal region for COX2A. These extensions do not have similarity to any other COX2 sequence reported so far. In addition, COX2A has a pre-sequence of 130 amino acids in the N-terminal region. It has been proposed that the extensions are involved in the formation of the heterodimer COX2A/COX2B and in its structural stabilization. In order to characterize the interaction of proteins COX2A and COX2B and to determine the importance of the extensions, interaction assays were performed, such as affinity chromatography and Far-Western blotting with the recombinant proteins. The results indicate that the COX2B extension is necessary but not essential for the association of COX2A/COX2B. Both proteins are synthesized in the cytosol, imported and assembled, so we explored this process in vitro, using isolated *Polytomella* sp. mitochondria. Our results suggest that COX2B is imported directly into the intermembrane space, while COX2A follows an energy-dependent import pathway. In addition, the MTS of the COX2A precursor is edited. This is the first time that the in vitro import of split COX2 subunits into mitochondria has been achieved. Work supported by CONACyT (128110) and DGAPA-UNAM (IN 203311).

Analyses of the oligomeric organization of paralogous proteins in *S. cerevisiae*: Leu4 and Leu9 a specific case

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Introduction

We have previously proposed that diversification of *GDH1* and *GDH3* encoded NADP-dependent glutamate dehydrogenases and the *LYS20* and *LYS21* encoded homocitrate synthases could result into formation of hetero-oligomericisozymes showing peculiar biochemical properties, which could play an important role under certain environmental conditions. Following the same scheme we studied the mitochondrial α-isopropylmalate synthases Leu4 and Leu9 in *S. cerevisiae*,these isozymes are codified by *LEU4* and *LEU9*genes respectively.

Results

Our results indicate that mitochondrial α-isopropylmalate synthases form a Leu4/Leu9 hetero-oligomericisozyme "*in vivo*". Biochemical and physiological characterization of the Leu4/Leu4, Leu9/Leu9 and Leu4/Leu9 isoforms was carried out.

Leu4 and Leu9 subcellular localization and differential expression, contribute to the formation of homomeric or heteromericisozymes with differential leucine sensitivity, this is related with a physiological and metabolic impact that could constitute an adaptation to facultative metabolism in yeast.

Surprisingly the formation of the heteromericisozyme Leu4/Leu9 is preferred those of Leu4/Leu4 and Leu9/Leu9.

Analyses of the α-isopropylmalate synthase isoforms *Kl*Leu4 and *Kl*Leu4BISpresent in the ancestral type yeast *Kluyveromyces lactis* and Leu4 and Leu9suggesta functional diversification in *S. cerevisiae*determined by subcellular compartmentalization, expression profiles and kinetic properties of each one of the isozymes codified by these genes.

Conclusions

LEU4 and *LEU9* paralogous genes retention and further diversification has resulted into formation of three isozymes giving versatility to the physiological role of α-isopropylmalate synthases.

Our results support the proposition that constitution of hetero-oligomers plays a fundamental role in paralogues retention. For the case of Leu4 and Leu9 formation of heterodimers plays a central role in the homeostasis of leucine biosynthesis.

Paralogous enzymes could enrich the repertoire of isozymes able to meet and resolve modifications in the environment.

DEAD-box RNA-helicases from *Bacillus subtilis* display differences in RNA-unwinding activity.

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RNA DEAD-box helicases are enzymes with RNA-unwinding activity. Due to its activity, RNA DEAD-box helicases are implicated in all the cellular processes involving RNA remodeling, such as, translation, RNA splicing, maturation, and degradation. These RNA helicases are into the DEAD group due to their Asp- Glu-Ala-Asp sequence motif. *B. subtilis* has four RNA DEAD-box helicases: Dead, CshA, CshB and YfmL. Thus far, only recombinant CshA has been biochemically characterized, it possess bidirectional RNA unwinding activity. In vivo, deletion of cshA, cshB or yfmL result in cold sensitive phenotypes but, the individual contribution of each enzyme to the cell function, remains unveil. To get insight details of the function of those enzymes, we obtain and purify His- tagged CshB and YfmL from *E. coli*. We performed standard RNA unwinding assays using 5´-overhang and 3´-overhang RNA duplexes as substrate, CshA was included in the experiments as control. Our first results demonstrate that CshB displays activity only when 3´overhang duplex is used, opposite to CshA that has bidirectional activity. YfmL present a partial activity showed as intermediate structures between RNA duplex and single strand. To determine if the protein C-terminal domain of these helicases, is involved in the catalytic function and preference for the substrate, we attempt to make chimeric proteins by fusing the N-terminal domain of YfmL with the C-terminal domain of CshA and YfmL. So far, we have cloned the fusion PCR products into a maintenance plasmid. In the next weeks we will analyze for protein expression and purification of the chimeric helicases.

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Heterologous expression of *Debaryomyces hanseniicatalase* genes in *Saccharomyces cerevisiae.*

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Debaryomyces hansenii has two catalase genes (*Dh*CTA and *Dh*CTT), and both exhibit higheractivity than those of *Saccharomyces cerevisiae*. We have previously shownthat *D. hanseniic*atalase T activity is present only in cells on late stationary phase growing on rich media with glucose, whereas cells growing on non-fermentable carbon sources as ethanol exhibit higher catalaseA activity. However, the corresponding transcripts are always present.In order to do a more detailed study of the expression of these orthologous yeasts genes, two plasmids with either catalase T or A from *D. hansenii* coding genes were constructed, including1000 bp upstream and 400 bp downstream to keep the 5´-UTR, the promoter and the 3´-UTR. The genes were amplified by PCR and cloned into pRS316. Anacatalasemic double mutant of *S. cerevisiae* was transformed separately with both constructions. Our results show that both genes successfully complement catalase activity, measured by spectrophotometry, zymograms and RT-qPCR. The expression of *Dh*CTAgene decreases the doubling time of the complemented mutant when it is grown on YP-ethanol medium, whereas *Dh*CTTincreases the maximum biomass reached when grown on rich medium (YPD). The present report shows the differences seen between wild type *S. cerevisiae* strain and the complemented strains, growing in rich media with a fermentable (glucose) or a non-fermentable (ethanol) source of carbon, and in presence or absence of NaCl.We found that *Dh*CTT gene in *S. cerevisiae* genomic background is regulated in the same way as its orthologous gene,*Sc*CTT1, but has10-foldincrease in activity compared to the wildtype*S. cerevisiae* strain.On the other hand,the *Dh*CTA gene in *S. cerevisiae* genomic background is regulated as in *D. hansenii* background, but the activity levels are closest to the baker´s yeast. The expression of both *Dh*CTAand *Dh*CTT genes improves the resistance to a hydrogen peroxide shock in exponential growth phase, being *Dh*CTTthe gene that confers better resistance.

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MEDIATOR as a link between development and abiotic stress response in plants

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Improving abiotic stress tolerance is a central goal of crop breeding.Abiotic stress decreases crop yields by negatively affecting plant growth and development. Independent effortshave been made to elucidate the molecular components of stress response pathways, as well as the mechanisms controlling developmental programs. One current challenge is to understand how physiological stress responsesand growth regulation are coordinated.

In response to stress perception in reproductive tissues, plants alter their growth to maximize yield and balance resource consumption between vegetative and reproductive development.Studies have shown that an important subclass of small non-coding RNAs, microRNAs (miRNAs), play important roles during plant growth, developmental transitions, and determination of cell identity. They were also shown to be involved in the modulation of responses to biotic and abiotic stress of numerous crops, such as maize, wheat and rice, and in *Arabidopsis*. For example, the microRNA miR156acts upstream of the *SPL* transcription factors, which themselves regulate miR172, to control vegetative and reproductive development (Wu *et al.*, 2009). One isoform of miR156is also induced in response to heat-shock, in order to modulate growth responses to this abiotic stress (Stief*et al.*, 2014).

A recent study in our lab has shown that the MED12/CCT and MED13/GCT subunits of the CDK8 module of Mediator act upstream of the miR156-*SPL*-miR172 cascade (Gillmor *etal.*, in review). While theCore Mediator complex acts as a transcriptional co-activator,providing a bridge between RNA pol II and transcription factors, the CDK8 module of Mediator typically acts as a repressor. By performing RNAseq of mRNA and smallRNAtranscriptomes of mutants in components of the CDK8 module, we have discovered that several miRNAs that respond to abiotic stress are missregulated in mutants of this complex. As a signal integrator that regulates transcriptional outputs, the CDK8 module of Mediator is a candidate for a link between developmental programs and the stress response pathways. We are currently evaluating the importance of the CDK8 module in phosphate homeostasis, by characterizing phosphate responses in CDK8 module mutants, as well as dissecting the role of CDK8 module genes in transcriptional regulation of phosphate-responsive miRNAs and mRNAs.

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Small RNAs regulate the response to injury of the filamentous fungus *Trichoderma atroviride*

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Wound response in multicellular eukaryotes is essential for survivaland is highly conserved in plants and animals [1]. In our laboratory we recently discovered that the filamentous fungus *Trichodermaatroviride*responds to injury by triggeringhyphalregeneration and the formation of asexual reproductive structures [2]. Our transcriptomic analysis revealed that the mechanism of response to this stimulus is very similar to that of animals and plants, suggesting that it is highly conserved amongst the three eukaryotic kingdoms. Additionally, several recent studies have reported that post-transcriptional regulation by microRNAs is involved in the response to injury in animals and plants [3,4].

Based on this background we decided to evaluate injury response in mutants of theRNAisynthesis machinery of *T. atroviride*. The∆*dcr2* and ∆*rdr3*strains presented a dramatic defect in regeneration ability and asexual reproduction in response to injury. To understand the molecular processes affected by the absence of the RNAipathway, we performedtranscriptomic analysis of the WT and ∆*dcr2* strains subjected to injury,showing that signaling processes, DNA repair and cell cycle progression are essential to overcome this stress and are affected in the ∆*dcr2* mutant. Even more interesting was the presence of a population of small RNAs of 21-22nt in response to injury in the WT, which is absent in ∆*dcr2* mutant, implicating them as a product of the Dcr2 enzyme.

Ourresults indicate that gene regulation by small RNAs is essential to respond to injuryin *T. atroviride*and that this mechanism is highly conserved in all three kingdoms. This phenomenon gives us the opportunity to use a simple biological model to understandthe complex process of tissue repair, which could culminate in enhancing regenerative therapies in humans.

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BAT1 &*BAT2* paralogoussubfunctionalization is determined by expression divergence and chromatin reorganization in the yeast *Saccharomyces cerevisiae*

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Expression divergence between duplicate genes has long been a subject of great interest to geneticists and evolutionary biologists, because it is considered an important step in the emergence of a new gene from a redundant duplicate. It has been hypothesized, that expression divergence between duplicate genes could be the first step for the preservation of redundant duplicates. Moreover, expression divergence between duplicate genes increases with evolutionary time resulting in different expression profiles that determines their functional diversification. Expression divergence was observed when analyzed by three regulatory mechanism: i) *cis*-regulation of transcription mediated by sequences present in the upstream region; ii) *trans*-regulation mediated by regulatory proteins binding to *cis* elements, such as transcription factors (TF); and changes in chromatin organization, such as nucleosome positioning. Many global studies have addressed the effectof expression divergence on functional diversification of paralogousgenes which originated from gene duplication with the aim to understand how *cis* and *trans* regulatory elements have evolved.Here we investigate the role of expression patterns and chromatin divergence of*BAT1*& *BAT2*paralogous genes, which encode branched chain amino acid aminotransferasesin order to undertand functional diversification of the paralogous enzymes. Divergence of the *cis* elements found in *BAT1* and *BAT2* promoters, should allow different *trans* interactions and expression profiles. *BAT1* expression is not determined by the quality of the nitrogen source and is similar in both, repressing(glutamine) and non-repressing nitrogen sources (GABA). However it is repressed by the combined action of valine-isoleucine and leucine (VIL), showing a biosynthetic expression profile. Accordingly, *BAT1* transcriptional activation is determined through action of Leu3 and Gcn4 andindirectly by Gln3. Expression repression is observed in the presence of the end product V I L and it could be triggered by the negative effect of Put3 and through nucleosome sliding at the -1 position.*BAT2* expression is determined by the quality of the nitrogen source, repressed in glutamine and induced in secondary non-repressive nitrogen sources such as GABA and V I L. *BAT2* transcriptional activation is achieved through the combined action of Leu3, Gln3 and Ure2 transcriptional modulators. Expression repression in glutamine is achieved through a novel Gln3-independent mechanism, triggered through the negative action of Leu3. This negative action of Leu3 is not dependent on theα-IPM intracellular concentration, suggesting a peculiar Gln3-independent repression mechanismmediated by the quality of the nitrogen source.Displacementofthe +1 nucleosomeby the Swi/Snfchromatin-remodelingcomplex,could also play a role in the Leu3-dependent responsetothenutrientsignalofthenatureofthenitrogensource.In this case, the above describeddual role of Leu3 as activator or repressor is the key for the *BAT1* &*BAT2* expression divergence.Our results indicate that expression divergence plays a major role inparalogues functional diversification, revealing that subfunctionalization could be triggered by the selection of transcriptional activators that could act as repressors or activators under the same physiological conditions, resulting on opposed regulation of paralogous genes, and thus differential physiological role of their encoded products. On the other hand, we identified the critical changes in the chromatin structure of the pertinent promoters, such as nucleosomes sliding or displacement that determine their expression divergence.

In search for *cis*-regulatory elements activated by Dengue virus infection in *Aedes aegypti* midgut cells

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Dengue is themost importantvector-borne viral disease in the world.*Aedes aegypti*is the major vector for the transmission of this virus. As the virus spreads to variousmosquito tissues,the expression profile of infected cells is modified. Multiple virus infected mosquito midgut transcriptome profiles have been determined aiming to identify patterns of expression and specific factors potentially involved in virus-vector interactions that could constitute molecular determinants for Dengue virus transmission. However, information is currently lacking regarding the full set of protein-DNA interactions that may participate in establishing the observed expression profiles generated by virus infection in mosquito midgut cells.Also,the mechanism of transcriptional regulation of many genes whose expression is modified in the presence of Dengue virus remains unknown. The identification of *cis*-regulatory sequences will provide fundamental knowledge for a better understanding of the signaling pathways that are modified during Dengue virus infection and the mechanisms by which the transcriptional program of mosquito midgut infected cells is altered.In order to identify *cis*-regulatory elements, and their putative cognate binding factors, we carried out open chromatin profiling of AAEL006536, one of the genes more strongly activated by Dengue virus infection in*A. aegypti* midgut cells. Chromatin from infected and non-infected mosquito midguts was extracted andfractionated. The DNAobtained was then analysed by qPCR to detect nucleosome depleated regions in the upstream region of the gene. We identified two open chromatin sites upstream of this gene only in chromatin from infected midguts. One region overlaps with the reported transcription start site, as was expected, and the second lies further upstream of the TSS. The position and chromatin structure of this last region is consistent with a promoter function.We will further discuss the composition of these newly identified *cis*–regulatory sequences.

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Polyamines and Abscisic Acid: a new link between two pathways involved in stress responses in *Arabidopsis thaliana*.

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Polyamines (PAs) are small organic polycations with key roles in both development and stress responses in plants. The mainly four ubiquitous PAs in plants are putrescine (Put), spermidine (Spd), spermine (Spm) and thermospermine (tSpm). Besides their role in plant development, PA are also involvement in abiotic stress such as salinity and drought, and the evidences indicate that transcriptional regulation of genes coding enzymes involved in PAs biosynthesis is mediated by the phytohormoneabscisic acid (ABA). Nevertheless a detailed mechanism or the exactly crosstalk between both pathways is still unknown. In this study using genetic approaches, we provide evidence of a feedback mechanism between PAs and ABA pathways. We challenged to ABA treatment (0.5, 1, 2 and 4 μM) different T-DNA insertional mutants for *AtADC1* and *AtADC2*. The Arginine decarboxylase (ADC) is a rate-limiting enzyme that catalyze the first step of polyamine (PA) biosynthesis, and there are two genes in *A. thaliana*: *ADC1* and *ADC2.* The clearest result was the hypersensitivity of *Atadc2* to ABA treatment reflected in a decreased in two parameters: germination rate and development of green cotyledons. Interesting, *Atadc1* showed a minor hypersensitivity to ABA treatment in comparison with its paralog *AtADC2*. Because is already known that the double mutant *adc1/adc2* is lethal, in order to provide more evidence about the role of PA in ABA pathway an artificial microRNA that potential silenced both genes was generated. We transformed with the microRNA both mutants background (*adc1* and *adc2*) and the results showed that microRNA silenced lines are more susceptible to ABA and salinity (NaCl 125 mM). Besides, the transcriptional profile of different players in ABA signaling such as: ABI3, ABI5, and WRKY2 displayed in the wild type, adc1 and adc2 backgrounds during ABA treatment suggest an interesting feedback mechanism between PAs and ABA pathways. Also we are evaluating the phenotype during ABA and abiotic stress in T-DNA insertional mutants of others genes of the PAs biosynthesis such as: *AtSAMDC4*, *AtSPDS2* and *AtSPM* in order to provide novel evidence of the crosstalk between both routes.

Tumor-soluble factors derived from the breast cancer cell line ZR75.30 mimic a TNF pro-inflammatory transcriptome and phenotype in primary human endothelial cells.

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Tumor microenvironment and inflammation play an important role in carcinogenesis and particularly in metastasis. Chronic inflammation favors cancer development through an altered expression of chemokines, cytokines, gowth factors and their receptors. This unique microenvironment includes extracellular matrix and a variety of normal cells such as endothelial cells (EC). Activated NFκB transcription factor emerges as a mayor link between inflammation and tumorigenesis and may be a key signaling pathway that contributes to preneoplastic and malignant cell phenotype capable of escaping apoptosis, promoting tissue remodeling and angiogenesis, suppressing immune surveillance and promoting cell proliferation and survival. Tumor Soluble Factors (TSF) from cancer cell lines can promote angiogenesis and a pro-adhesive phenotype in HUVECs, activate the NFκB pathway and induce the expression of cell adhesion molecules (CAMs) mimicking the effects of TNF. In the present study we analyzed the transcriptome of a pro-adhesive phenotype of HUVECs treated with ZR75.30-TS and through in silico analysis we used the changes in expression of molecular regulators to predict the related canonical pathways. We found that the exposure of primary human endothelial cells to TSF secreted by the human breast cancer cell line ZR75.30 induces a pro-adhesive phenotype and promotes changes in molecular regulators related to inflammatory response, wound healing, extracellular space, cytokines, metabolism and cell communication. The changes in molecular regulators and the predicted phenotypic profile mimics the ones induced by TNF. The main molecular regulator that emerges from the in silico analysis was NFkB, providing further support to the notion that this transcription factor plays a central role in the recruitment of normal cells to tumoral progression.

Caenorhabditiselegans as a model to study the involvement of microRNAs in lipo-toxicity.

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The lipotoxic effects of obesity are considered as contributing factors in cancer, diabetes and cardiovascular disease, but little is known about the mechanisms leading to the dis- ease state at the molecular level. MicroRNAs are ssRNAs of 19-24 bases in length that are part of the extended family of noncoding RNAs that inhibit mRNA translation and/or stability. MicroRNAs are key participants in diverse organismal and cellular events, such as development, differentiation, aging and metabolism. In order to elucidate the participa- tion of microRNAs in lipotoxicity, we are using the worm nematode *Caenorhabditiselegans* as a model, as its carbohydrate and lipid metabolisms are highly similar to that observed in mammals, and to the fact that its genome contains over 300 microRNAs, half of them iden-tical to those found in humansand, finally, that it can be fed with high-carbohydrate diets.

We established a model of lipotoxicity by feeding the worm with increasing amounts of glucose (from 0 to 100 mM glucose added to the growth medium), that resulted in the ac-cumulation of this sugar and that led to the formation of lipid droplets in the worm´s tis-sues. The size of the worms grown with added glucose was increased, while their progeny was decreased. As it has been reported that the expression of miR-34-5p was upregulated in the liver of mice fed a high-fat diet (PNAS 2012, 109: 9828-32) and that *C. elegans* has an homolog of this microRNA, we were able to document that the expression of cel-miR-34-5p was increased when the worms were grown in media with sugar added.

As a way of identifying the Transcription Factors that are mediating the upregulation of cel-miR-34-5p in medium with glucose added, we analyzed the promoter region of cel- miR-34 by bioinformatic means and predicted the existence of a putative binding site for SBP-1, an homolog of the mammalian SREBP-1c (Sterol Regulatory Element-Binding Pro- tein-1c), a protein that is involved in the regulation of lipogenesis.

In order to evaluate if SBP-1 regulates the expression of cel-mir-34, we silenced the ex-pression of SBP-1 by RNAi and found that the expression of cel-mir-34 was downregu- lated in worms grown in glucose-added medium, suggesting that SBP-1 directly or indirect- ly is regulating the expression of cel-mir-34. We will confirm this result by using an *sbp-1* mutant and a cel-mir-34-promoter::GFP fusion construct.

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Cortactin deficiency causes increased ROCK1-mediated actin-contractility and decreased adrenomedullin secretion leading to enhanced endothelial permeability

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Endothelial cells regulate several processes such as leukocyte transmigration and vascular permeability, to do so, they need to reorganize their actin cytoskeleton in cooperation with different actin binding proteins. Cortactin (cttn), an F-actin binding protein, has been implicated in actin dynamics and endothelial permeability, however,the molecular mechanism by which cttn contributes to these phenomena are notclear. To analyze cortactinrole in permeability, we generated a cortactinknockdown (cttn-KD)human endothelial cell line using shRNA technology. Western blot experiments reported increased expression of Rho Kinase 1 (ROCK1) and enhanced Myosin Light Chain 2phosphorylation at the serine 19 residue which would lead to increased actomyosin contractility, in concordance to this, F-actin staining revealed increased number of actin stress fibresincttn-KD cells. Our results are further supported by *in vivo* data from cttn-knockout mice, which showed higher ROCK1 signal in tissue sections from brain, colon and lungs. These mice also had reduced adrenomedullin (ADM) levels in sera which contribute to increased permeability by reducing intracellular cAMP levels. Permeability assays demonstrated that ADM treatment of both cell types can completely rescue the effect in permeability, while treatment with ROCK inhibitor Y-27632 partially did it. Our results suggest a potential role for cortacinin the regulation of endothelial permeability by controlling ADM secretion and RhoA/ROCK1 mediated contractility.

Asymmetric Cell Division of Corneal Epithelial Cells.

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Cell polarity, mitotic spindle orientation and asymmetric cell division play a crucial role in epithelial cell self-renewal and differentiation. Yet, little is known about the molecular programs that control these processes in corneal epithelial cells. We have studied asymmetric cell division using the RCE1(5T5) cell line, which mimics corneal epithelial differentiation. Immunostaining experiments, in which we studied the mitotic spindle orientation in growing cultures and confluent epithelia, showed that, in vitro, epithelial cells are polarized and undergo apico-basal cell divisions, which constitute 25% of the total mitoses. This ratio was maintained during the proliferation, as well as in stratified epithelia. Furthermore, immunostaining with antibodies raised against the cell fate determinant Numb or against spindle orientation-regulatory protein Insc, showed that these proteins were asymmetrically localized in vertical mitosis. Since 10% of mitotic cells showed this asymmetric distribution, we suggest that such cell population may correspond to the epithelial stem cells in cell culture. Numb also showed an asymmetric segregation in some horizontal mitosis. Using RNA from central corneal and the limbus of adult rabbits, we found by semi-quantitative RT-PCR that the expression of Insc, NuMA and LGN, which are involved in mitotic spindle orientation, is 1.2 to 1.5 times higher in limbal epithelium than in central cornea. Together, these results suggest that asymmetric cell division in adult, occurs predominantly at limbal epithelium, and open the possibility that niche determines the pattern of division through extrinsic signals. To further demonstrate the regulation of asymmetric cell division, we analyzed some factors involved in this process, such as ECMs, as well as the participation of Notch and Wnt signaling, and their corresponding ligands, during differentiation of mammalian corneal epithelium.

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Does IFC-305 compound could be modulating the Kupffer cell phenotype?

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Macrophages are directly related to immediate response mechanisms for systemic imbalances, as occurs on hepatic-pathologies like cirrhosis. The role of macrophages depends on the ability to have a full spectrum of activation with different phenotypes classified generally as M1-proinflammatory and M2- antiinflammatory. In the case of Kupffer cells, resident macrophages of the liver, present the same M1/M2 activation states and this is driving recently the search for new therapeutic options focusing its efforts to find molecules that modulate these phenotypes (*Ramachandran P, J Hepatol: 56:1417, 2012*). In contrast, it is known that cirrhosis and liver diseases in Mexico are a national health problem, that is why Dr. Chagoya, along with members of her lab have worked on developing a drug called IFC-305 that showed the ability to reverse and hepatic-protects experimental cirrhosis. We have demonstrated that the IFC-305 *in vivo* could modulate de immune response and change de number of Kupffer cells in a model of CCl_4 cirrhosis in rats that involves a chronic inflammation resolution. Actually we are working on elucidate the action mechanism of how does IFC-305 could be modifying the phenotype of Kupffer cells that are exposed to LPS (M1 classical activation) and to IFC-305 over an *in vitro* model of primary cells culture.

Kupffer cells were isolated from normal rats by collagenase digestion and Percoll density gradient differential centrifugation. Kupffer cells were incubated in fresh Dulbecco's Modification of Eagle's Medium containing LPS 10µg/mL and/or IFC-305 for 48 h, We measured NO secretion after LPS stimulation by Griess method and arginase enzymatic activity by spectrophotometry. Arg I and iNOS protein expression were detected by Western-blot.

Kupffer cells were characterized as (HIS36+ CD11b/c+) by IMF. The levels of NO increase with LPS and decrease significantly by the effect of IFC-305 co- administration on the Griess assay and IMH results. Arginase enzymatic activity showed that Control (0.303± 0.086 mg urea/ 4x106cells) differs significantly ($p < 0.05$) from IFC 5mM (1.276 ±0.117) which increase significantly and the same occurs when we co-administration of IFC 100µM + LPS (2.970 ± 0.171); IFC 5 mM + LPS (0.780 ±0.097). Protein expression detected by Western-blot showed that iNOS expression decrease average (20 arbitrary units of density) in the presence of IFC-305 vs LPS; meanwhile arginase I increase average (5-10 arbitrary units of density) over the control.

Our *in vitro* study showed that lipopolysaccharide (LPS) enhanced M1 phenotype of Kupffer cells, meanwhile the IFC-305 alone and co-administred with LPS could activate macrophages to M2 phenotype augmenting the arginase expression and its activity modulating the Kupffer cell phenotype.

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HS1 regulates the neutrophil extravasation cascade during inflammation by converging PKA signaling into integrin activation

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Neutrophil extravasation is a critical step in innate immunity in response to tissue injury or invading pathogens. Inflammatory signals activate β2-integrins and facilitate neutrophil adhesion onto the endothelial apical surface and intraluminal crawling to the site of diapedesis. Hematopoietic cell-specific lyn substrate (HS1), the cortactin homologue in hematopoietic cells, regulates actin dynamics at the immune synapse and in neutrophils during migration. However, it is not yet known if HS1 plays a role in the regulation of the neutrophil extravasation cascade. Investigating HS1-deficient mice by intravital microscopy of the inflamed cremaster muscle, we found an increased rolling velocity and a strong inhibition of neutrophil adhesion and transmigration. Additionally, HS1-deficient neutrophils showed disturbed polarization in response to both tumor necrosis factor-a and keratinocyte-derived chemokine (KC). These effects were not due to disturbed expression of adhesion molecules but could rather be explained by disturbed Rap1 activation causing reduced β2-integrin-dependent neutrophil adhesion in response to KC treatment. Interestingly, this process was dependent on PKA activation since PKA inhibition blocked KC-induced Rap1 activation. The importance of PKA for HS1-mediated support of extravasation was corroborated by the finding that PKA activation increased whereas inhibition reduced transmigration of WT neutrophils but not of HS1-KO neutrophils. However, HS1 is not a direct substrate of PKA but it co-immunoprecipitates with phosphorylated VASP. This interaction is also inhibited after PKA inhibition and may thus provide an important scaffold for Rap1 and β2-integrin activation. Our results establish HS1 and PKA as critical signalling molecules that coordinate the molecular machinery required for Rap1/β2-integrin activation and efficient neutrophil transmigration.

Allostery and cooperativity, together forever?

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The GlcN6P deaminase (EC 3.5.99.6) catalyzes the isomerization-deamination of glucosamine 6-phosphate (GlcN6P) releasing fructose 6-phosphate (Fru6P) and ammonium ion (NH^+_4). In some species is a regulatory metabolic step because of both its hompotropic cooperativity and its allosteric activation by *N*-acetylglucosamine 6-phosphate (GlcNAc6P). The most studied deaminases share the same Rossmann-like fold, nevertheless was found in GlcN6P deaminase from *Shewanella oneidensis* (SonNagBII) an entirely different fold, the SIS fold. This is an interesting case of homoplasy, displaying functional convergence, both in allosteric regulation as in homotropic cooperativity.

We made a structural model of SonNagBII on basis of the crystallographic structure of its homologous from *S. denitrificans*. The enzyme is a homodimer, with one active site per subunit and a single allosteric per dimer, located in the intersubunit space. The location of these sites was identified by molecular docking and subsequently verified by site-directed mutagenesis. The expected binding stoichiometry was confirmed by direct binding experiments. Our results demonstrate that homotropic cooperativity and allosteric activation by GlcNAc6P depend on different molecular mechanisms since both functions can be experimentally dissociated by nanoencapsulation of the enzyme in silica gel, by site-directed mutagenesis and by allosteric inhibition by citrate. This experimental dissociation is not possible in the deaminases with Rossmann-like fold because of the structural relation of both transitions.

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"Studies on functional divergence between *Saccharomyces cerevisiae ALT1* and *ALT2* using *Kluyveromyces lactis KlALT1* and *Lachancea Kluyveri LkALT1* as "ancestral type yeast"

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Gene duplication has a relevant role in evolution, since diversification of paralogous genes allow the emergence of new or specialized functions from the preexisting ones.

Saccharomyces cerevisiae experimented whole genome duplication (WGD) about 100 millon years ago. Comparisons between *Saccharomyces* and *Kluyveromyces* linages suggest that the last linage diverged after whole genome duplication of *Saccharomyces*. It is thus possible to consider that *Kluyveroyces* physiology its more similar to the ancestor which did not underwent duplication and gave rise *Kluyveromyces lactis* and *Lachancea kluyveri* linages, these is why both are considered as "ancestral type yeasts".

Aminotransferases constitute an interesting model to study diversification of paralogous genes since aminotransferases constitute biosynthetic and catabolic pathways whose opposed action relies on a single catalytic site. *ALT1* and *ALT2* are two paralogous genes present in *S. cerevisiae* genome. These paralogs encode 65% identical proteins. Previous results showed that only Alt1 displays alanine aminotransferase activity. Alt1 its localized in the mitochondria and Alt2 in the citosol . *ALT1* is alanine-induced showing an expression profile of a gene encoding an enzyme involved in amino acid catabolism, conversely, *ALT2* expression is alanine-repressed, indicating a role in alanine biosynthesis, although the encoded-protein has no alanine aminotransferase enzymatic activity. Since a double *alt1*$^{-}$ *alt2*$^{--}$ mutant is not an alanine auxotroph it can be concluded that there exists a yet unidentified alternative pathway for the alanine biosynthesis.

K. lactis and *L. kluyveri* only have one *ALT1-ALT2* ortholog, identified by sequence homology and sinteny: *KlALT1* and *LkALT1* respectively. Through the characterization of the null mutant of the orthologous genes, we discovered that *KlALT1* and *SkALT1* are the principal pathways of alanine catabolism, in each one of these yeasts, although in both cases an alternative alanine biosynthetic and catabolic pathway is present, although it has not been identified yet. Purification and kinetic analysis of KlAlt1 and LkALt1 shows that both display alanine aminotransferase activity, suggesting that the biosynthetic and catabolic capacity displayed by alanine aminotransferases has been exclusively delegated to Alt1.

The characterization of *KlALT1* and *LkALT1* and their encoded products will allow the proposition of a model for the divergence of *ALT1* and *ALT2*.

Physicochemical properties correlate with evolutionary categories ofbacterial expansins.

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Expansins are a family of proteins with plant cell wall remodeling activity. The role of expansins in plant physiology is well known, however, only recently their function inprokaryotes has begun to emerge. Whole-genome sequencing availability has revealed thepresence of expansin-like homologue sequences in organisms other than plants.Interestingly, only a handful of species have been found to contain expansins, among thesefungi and plant-pathogen bacteria.In order to evaluate the phylogenetic relationships of expansin-like proteins, a BLASTpsearch for *Bacillus subtilis* EXLX1 against the non-redundant nr database (NCBI) wasperformed. From this search, 282 sequences were retrieved mainly from bacteria (48.2%) and fungi (32%), and in lesser proportion from other eukaryasuch as amoeba (4.8%), oomycetes (8.1%), tunicates (0.7%), plants (0.7%), heterolobosea(0.4%), and metazoa (0.4%).We were interested inevaluating canonical expansin-like homologues from bacteria, therefore, only forty-four proteins thatexhibit the canonical two-domain structure (Barwin-like endoglucanases and PHL pollenallergen) according to the Superfamily database, were considered. We determined the phylogenetic relationships,domain architecture and physicochemical characteristics of expansins from prokaryotic species. We have found that bacterial expansins were acquired from horizontal gene transfer probably from plants. Twenty-two proteins were composed of the two-domain structure only, and the rest of the expansins were modular proteins with extra domains with activities related to cellulose degradation. Most of the expansins were either basic or acidic proteins that differ at the opposite face of the polysaccharide binding surface. Thisfeature correlates with the type of bacteria that produces them, this is: acidic expansins were found in Gram negative bacteria, whereas basic expansins are produced by Gram positive bacteria. This acidic/basic trait could have implications on the interaction of the microorganism with its plant host during plant infection.

In vivo detection of compounds from organisms by mass spectrometry using low-temperature plasma (LTP) ionization

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Mass spectrometry (MS) has become one of the most important tools for the analysis of biomolecules, such as natural products, peptides and proteins. For MS based analyses, the molecules need to be converted into ions. This process takes place in an ionization source.

Novel ambient ionization sources permit the detection of compounds directly from samples with little or no sample pre-treatment [1].

Among these techniques, Low Temperature Plasma (LTP) ionization arises as a promising technique for *in vivo* analysis of compounds, because it allows the direct detection of biomolecules whilst causing only mild damage on the studied organism. However, currently there is no LTP source commercially available.

Figure 1. LTP device for the detection of compounds directly from a tobacco plant.

Therefore, we built a prototype in the Laboratory of Biochemical and Instrumental Analysis. Contrary to devices reported in the literature, our design has a variable voltage and plasma temperature. Further, the diameter of the plasma beam is adjustable [2]. We use our instrumental set-up for the direct analysis of biological tissues and imaging applications [3].

Recently, we detected nicotine directly from *Nicotiana tabacum* plants, and monitored changes in the concentration of this metabolite, caused by mechanical damage (see Fig. 1). Our findings demonstrate that LTP-MS offers a novel analytical approach towards the *in vivo* detection of biomolecules.

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STUDY OF THE EXPRESSION OF GENE *Ccs* (*Capsanthin-capsorubin synthase)* AT DIFFERENTE MATURATION STAGES IN TWO DIFFERENT MORPHOTYPES OF HABANERO PEPPER (*Capsicum chinense Jacq.)* BY RT-PCR REAL-TIME TECHNIQUE.

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ABSTRACT

Chilli (*Capsicum spp.)* is one of the most important vegetable crops in Mexico, forming part of their culture to consume different types as ingredients of various dishes, due to their diverse tastes, smells, colors and pungency that characterize the Mexican food. Habanero chili (*C. chinense Jacq.)* is an economically important crop for vegetable growers of the state of Yucatán, ranking second after the tomato crop, interest in this crop does not focus solely on their economic importance and human consumption; has also been shown that the chili is an excellent source of natural colorants, minerals and vitamins A, C and E. The natural dyestuffs are based on the content of carotenoids and other. Carotenoids molecules of 40C which synthesized in the plant plastids in the carotenoid synthesis pathway..

The aim of this study is based on analysis of the *Ccs* gene (*Capsanthin-capsorubin synthase*), which gives training xanthophylls such as capsanthin and capsorubin, in the pericarp of fruits of chile habanero (*C. chinense Jacq*.) In two different morphotypes (Orange and Red) and at different maturation stages. The results show differences in expression levels between morphotypes and differences between maturation stages of each one; both showing higher levels of expression in the late stages of maturation; this low expression in less mature stage could be due to the accumulation of other carotenoids found much earlier in the biosynthetic pathway of carotenoids.

Detection of mutations related to Multidrug-Resistencia in clinical *Mycobacterium tuberculosis* from Mexico by next generation sequencing technology.

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Tuberculosis (TB) causes more deaths worldwide than any other single infectious disease. In 1993 The World Health Organization (WHO) declared tuberculosis a global public health emergency. TB is reappearing as a public health crisis, and several factors contributed to this increase, including the development of multi-drug-resistant (MDR) strains that has complicated the development of efficacious regimens. MDR tuberculosis is defined as disease caused by *Mycobacterium tuberculosis* with resistance to at least isoniazid and rifampicin. In Mexico, MDR has been detected, the WHO estimated an increase of MDR TB from 6.3% in 2011 to 9.0% in 2013. It has been suggested that resistance arises from the acquisition of mutations or occasional small chromosomal deletions in the genome of *M. tuberculosis* and selection pressure from antibiotic use provides a competitive advantage for mutated strains. Efforts to reduce tuberculosis transmission in Mexico requires identifying and treating those who already have the disease. To reduce TB diagnosis time and improve drug therapy, new molecular tools are urgently needed. Next-generation sequencing (NGS) might be one of these tools by providing a more precise diagnosis and optimizing drug treatment based on the genetic makeup of the pathogen. In this study, we used NGS to identify potential biomarkers for drug resistance on dozens of genes that are putatively associated with drug resistance. Many studies have identified markers of drug resistance using pure strains of *M. tuberculosis.* Here, we seek to identify known and potentially new markers of drug resistance directly from a clinical sample i.e. sputum from patients with tuberculosis. We have carefully selected 150 genes some of which have been previously associated with drug resistance, and designed probes to create genomic libraries of each of these genes for NGS DNA sequencing using the GAII (Illumina) platform.DNA was extracted from sputum and preliminary results of 9 genomic regions in 16 samples, indicate a total of 114 mutations in strains resistant to the first and second lines of treatment. Of these, 94.8% of all mutations were in complete agreement with drug susceptibility tests and 7.1% were new mutations that have not been previously reported. For example, we observed that previously reported mutation, 1401 A>G, on *rrs* was associated with resistance to amikacin, but resistance to this antibiotic was also associated with new mutations on *rrs* such as 931 A>C. Our current studies will deliver a comprehensive list of genetic markers on *M. tuberculosis* that would speed diagnosis and drug selection in the near future potentially in an all-inclusive diagnostic test.

Analysis of the effects of HPV16 E2 expression on the cell cycle in HaCaT cells.

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The papillomavirus E2 protein is essential for the viral life cycle. Several functions of this protein are well known, such as its participation in the viral replication, in the segregation of the viral genome during mitosis and as a transcriptional regulator of the viral E6/E7 gene expression. However, HPV16 E2 protein may also regulate a large number of genes in the host cell, either by direct recognition of sequences in gene promoters or interacting with several cellular proteins. By these interactions HPV16 E2 protein regulate cellular processes including apoptosis, cell cycle and RNA processing. Our research group demonstrated by pull down assays the interaction between HPV16 E2 protein and TAF1, a protein member of the TFIID complex. Additional results showed that HPV16 E2 expression in C-33A cells modifies the transcription of hundreds of genes. A group of genes whose expression was affected corresponded to proteins whose expression is dependent of TAF1, suggesting that the interaction E2-TAF1 could be responsible for this effect. Also we demonstrated that E2 protein has the ability to modify the TAF1 binding and the expression of several Cyclins, probably affecting cell cycle progression. In this work we are evaluating the effect of the expression of HPV16 E2 protein on cell cycle progression in HaCaT cells. Using flow cytometry we determined changes in the cell cycle profile in HaCaT-wt cells or HaCaT-E2 lentiviral transduced cells. Preliminary results suggest that the expression of E2 does not modify the cell cycle profile in HaCaT cells growing asynchronically, indicating the need to control the levels of other cell cycle regulators to observe an effect, possibly by synchronizing the cell culture in G0/G1 or S phase.

The nuclear higher-order structure (NHOS) defined by the topological relations DNA-nuclear matrix is species-specific.

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The nuclear DNA of metazoans is organized in topologically constrained, supercoiled loops anchored to a ribonucleoprotein compartment or substructure known as the nuclear matrix (NM) that results from extracting the nucleus with no-ionic detergents, high salt and DNase. The composition of the NM is rather complex since more than four hundred proteins have been associated with this compartment while some constituents are common to most cells and others are tissue-specific. DNA loops are attached or addressed to the NM by regions known as MARs that have no specific consensus sequence but are usually rich in AT and contain repetitive sequences. MARs have been operationally classified in constitutive-structural, resistant to high-salt extraction, and temporal-facultative non-resistant to high-salt. The former are also known as LARs that define structural loops anchored to the NM. The LARs-mediated DNA-NM interactions constitute a set of topological relations determining a higher-order structure within the cell nucleus (NHOS). It is a fact that chromatin proteins such as histones do not participate in the DNA-NM interactions. Our lab has provided evidence that the NHOS results from thermodynamic constraints acting upon the primary structure of DNA, the longest and most rigid known natural polymer. Such constraints impose the need for dissipating structural stress along the double helix by looping and supercoiling otherwise it would break or become denatured. There is important evidence that replication, transcription and processing of primary transcripts occur in specific macromolecular complexes organized upon or relative to the NM. Our lab has provided evidence that the structural DNA loops correspond to the replicons in vivo and that the NHOS is tissue-specific (see reference). However, we have also reported that transcription of equivalent genes occurs efficiently despite their tissue-specific local NHOS. Therefore, our previous findings pose the question of the biological meaning of the NHOS since it might only be the result of physicochemical factors acting upon a generic DNA and a tissue-specific NM so that nuclear physiology simply adapts itself to operate upon a diverse NHOS or on the contrary, biological necessity imposes constraints upon the NHOS and so it becomes the subject of natural selection and thus conserved at least between closely related species. We have developed and applied an experimental approach based on topological principles for the coarse-grained, large-scale comparative study of the NHOS in two closely related species: rat and mouse. Our results indicate that like the karyotype the NHOS is species- specific, thus not conserved along evolution.

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Systemic activation of innate immunity induced by LPS inhibits long- and short-term plasticity and increases phosphorylation of TrkB receptor in the MF-CA3 synapse of the hippocampus.

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Innate immunity constitutes the first line of defense against pathogens. This immunity is able to recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs). Activation of PRRs promotes production and secretion of cytokines and other soluble factors inducing an inflammatory environment. In mammals, innate immunity can interact with multiple tissues. In the central nervous system (CNS), microglia is the most important cell type from innate immunity and it may play distinct roles in neurophysiology. Lipopolysaccharide (LPS) is a cell-wall component from gram-negative bacteria that is recognized by Toll-like receptor (TLR)-4, a potent stimulator of innate immunity that unleashes inflammation. After systemic activation of innate immunity, microglial cells become active and they can respond to and produce a wide range of soluble factors affecting physiology of the neural circuits. The hippocampus is a highly organized structure of the limbic system involved in learning and memory. This structure is composed by dentate gyrus (DG) and cornuAmmonis 1, 2, 3 (CA1, CA2 and CA3), all of them involved in synaptic transmission. The pyramidal cell layer of CA3 area is innervated by the so-called mossy fibers (MF), a bundle of axons from granular cells of the dentate gyrus that represents an important substrate for learning and memory in physiologic conditions. However, the molecular mechanisms induced by activation of innate immunity underlying modulation of neurotransmission in this circuit are still unknown. Using a murine model of intraperitoneal administration with LPS (i.p. 1mg/kg) in mice of 4-8 week old, our field recordings made in CA3 area from acute hippocampal slices show that systemic activation of innate immunity inhibits long-term potentiation (LTP) after 12 and 24 hours of challenge. The post-tetanic potentiation (PTP) and paired pulse facilitation (PPF), two forms of short-term plasticity, also were affected in LPS-treated mice. These results indicate that innate immunity can modulate synaptic transmission. In addition, our results of western blot obtained from mechanically isolated CA3 sections show that LPS is able to induce the activation of TrkB receptor, as well as the phosphorylation of Akt and CamKII in the same area, suggesting that neuroprotective and pro-survival mechanisms are activated in hippocampus after systemic inflammation.

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Sirtuin modulation protects against spinal motor neuron loss induced by chronic excitotoxicity*in vivo*

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder whose pathological hallmark is the loss of motor neurons (MN) and paralysis. Although the cause of the selective death of MN is unknown,excitotoxicity mediated through the activation of glutamate receptorspresent in spinal MN, mainly of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)type,is an important mechanism(*Expert OpinTher Targets*11:1415-1428, 2007). We have designed an*in vivo* model of chronic spinal excitotoxicity in rats,by infusing AMPA directly in the spinal cord,that permits to assess the potential protective action of drugs(*J Neuropathol Exp Neurol* 66:913-922, 2007). Sirtuins (SIRTs) are histone deacetylases that depend on NAD+ to perform their catalytic functions and have been shown to be neuroprotectors, particularly SIRT1. Thus, SIRT1 activators such as the polyphenolic compoundsresveratrol (RSV) and quercetin (QCT), have been shown to be beneficial in an *in vitro*transgenic model of ALS (*EMBOJ* 26:3169-3179, 2007). So, we studied the effects of RSV and QCT infusion in our *in vivo* model of chronic spinal neurodegeneration induced by AMPA, using the surgical, behavioral, histological, and immunohistochemical procedures previously described.AMPA [1 mM] infusion caused a progressing paralysis during 10 days, initiating in the ipsilateralhindlimband manifested as an increasingly reduced time to fall fromthe Rotarod.At this time, the number of MNs was reduced by ~90% inthe ipsilateral side and by ~50% in the contralateral side. The glial marker GFAP was increased in both sides of the spinal cord, which indicates astrogliosis, andthe acetylated lysine 16 of histone 4 (H4K16ac, a specific marker of SRT1 activity) was markedly reduced in both sides.RSV and QCT infusion per se, at a dose of 1 nmole/day, did not alter the motor behavior or the number of MNs.Both RSV and QCT, when coinfused with AMPA, significantly increased the time to fall from Rotarod and also significantly decreased MN loss, but QCT showed a better protective effect. Gliosis was not reduced,andthe reduction of H4K16ac immunochemical stainingproduced by AMPA was prevented in the ipsilateral side. We also tested the effects of SIRT1 inhibition with EX527 at 1 nm/d dose. EX527 alone did not cause any effect, and when coinfused with AMPA protected similarly to RSV in both the Rotarod performance and MN number. We conclude that SIRT1 activation using RSV and QCT prevents MN loss against chronic excitotoxicity*in vivo* and that this opens new therapeutic strategies for ALS.The contradictory effect of EX527 may be due to the fact that this inhibitor is selective for SIRT1 and thereforethe inhibition could result in areduced NAD+ consumption, which under excitotoxic conditions may stimulate other protective NAD+-dependent mechanisms in the stressed MNs.

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TRPV1channelregulationbysteroidal molecules andits analgesic effect

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TRPV1 channels are important mediators of nociceptive and inflammationprocesses. They can be activated by several types of physical and chemical stimuli including high temperatures, low extracellular or high intracellular pH and by a direct interaction with compounds such as capsaicin[1]. Most endogenous molecules known to interact directly or indirectly with TRPV1 are positive regulators of the activity of the channel. However, relatively few endogenous negative regulators have been identified[1]. Previously, we identified cholesterol as an endogenous inhibitor of capsaicin-induced currents by virtue of its interaction with a cholesterol recognition amino acid consensus (CRAC) sequence localized in its S5 segment[2]. For other TRP channels (i.e. TRPM3), there is evidence regarding the role of steroids such aspregnenolonesulphate as modulators of their activity[3]. Since cholesterol is the precursor of several steroidal molecules, and since it shares structural features with these molecules, we explored if TRPV1 could be directly regulated by these steroidal molecules in a similar fashion to cholesterol. By applying pregnenolone, progesterone, testosterone and β-estradiol to excised membrane patches of HEK cells expressing TRPV1, we determined that activation of TRPV1 by capsaicin is not altered by these steroids. However, we assessed if steroidal molecules regulate TRPV1 expression through long term effects. We found that the chronic treatment with progesterone and pregnenolone reduces TRPV1 expression. Moreover, in behavioral experiments, we found differences in the pain threshold induced by capsaicinintradermal paw application between male and femaleC57BL/6J mice. In these experiments the male animals were more susceptible to capsaicin-induced pain than the female mice, suggesting that the hormonal difference between sexes could serve as a protective factor against this type of pain. Furthermore, the co-injection of capsaicin with progesterone reduces pain (measured as the paw-licking time) in male mice. Theseresults give knowledgeabout the analgesic effect ofsteroidal molecules through the regulation of TRPV1 expression.

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Striato-nigralcircuits control sugar-induced inflexible intake

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Inthe last decades obesity has reached alarming rates across the world. While excessive caloric intake constitutes the leading cause of this epidemic, the identities of the neurobiological mechanisms underlying overeating are still unclear. Sugar-sweetened beverages constitute the main source of added calories in most Western diets, and are strongly associated with weight gain, obesity, and long-term metabolic disease. However, the neural circuits underlying inflexible sugar intake – i.e. the perservative intake of sugar despite its deleterious health consequences – remain to be identified. It has been shown that dopamine release into the dorsal aspect of striatum is critical for the formation and expression of inflexible behaviors, and that sugar intake produces significantly greater levels of dopamine efflux when compared to artificial sweeteners. In the present study we aimed at characterizing the effectors acting downstream of dopamine release to generate inflexible behaviors. Accordingly, we developed in rodents a sugar-induced inflexible behavior model, and combined different techniques (genetic, neurochemical, optogenetic, and pharmacogenetic)to unveil the pathways downstream to dopamine release controlling behavioral inflexibility associated to sugar intake. Our results show that: 1) Repeated exposure to sugar, but not to non-nutritive sweeteners, results in the acquisition of inflexible intake patterns, specifically perseverant intake despite satiation or taste adulteration; 2) Optogenetic and cell-specific ablation studies show that sugar-induced inflexible intake is mediated by medium spiny neurons in the dorsal striatum expressing D1 receptors (D1-MSN); and 3) Activation of nigral circuits increases sensitivity to reward devaluation by opposing the stimulatory influence of striatal D1-MSN. In summary, our data reveals a new neural pathway via which repeated glucose exposure leads to less sensitivity to the deleterious aspects of high-sugar intake.

Anti -tumor and Immunomodulatory Effect of 6-Pentadecyl Salicylic Acid in an *in Vivo* Model

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The 6-pentadecyl salicylic acid (6-SA) is obtained from *Amphipterygiumadstringens*. It has been used as anti-inflammatory, antioxidant and for the treatment of gastric ulcers, stomach cancer and *Helicobacter pylori* infection (classified as a type 1A carcinogen by IARC).It was found that 6-SA significantly reduces prostate tumor growth by inhibiting the angiogenesis and human hepatic cancer by inducing endoplasmic reticulum stress and apoptosis in immune-deficient mice. It also increases the lymphocytes and macrophages of the bronchoalveolar fluid in mice instilled with diesel exhaust particles. However, there are no studies regarding the anti-tumoral and immune-modulatory effects of 6-SA in an immune-competent *in vivo* model. Here, we investigate the antitumorogenic and immune modulatory effect of 6-SA in an immune-competent Balb/C mice model. Balb/c female mice were inoculated subcutaneously with $1x10^5$ 4T1 (mouse mammary carcinoma) cells in the mammary fat pad. After 5 days, the animals were treated with 2 mg/kg of 6-SA intravenously every alternative day for 1, 2 or 3 weeks. The animals were scarified and the blood was collected by cardiac puncture, the tumor-draining lymph nodes and the tumors were isolated. The lymph node and blood mononuclear cells were immunophenotyped for CD4+, CD8+, CD19+, CD335+ and F4/80+ cells by flow cytometry. The results show that 6-SA significantly reduced the tumor diameter and weight after 14 days of treatment without any significant changes in the body weight. Immunophenotyping analysis showed an increase in the proportion of CD4+, CD335+ and F4/80+ cells in circulating blood and CD4+ cells in lymph nodes after one week of treatment. An increase in proportion of F4/80 + cells in blood and CD8+, F4/80+ in lymph nodes were noticed after 2 weeks of treatment whereas after three weeks of treatment the proportion of CD4+, CD335+ and F4/80+ cells increases in lymph nodes. These results show that 6-SA exerts anti-tumoral effect with an immunomodulatory activity in an immune-competent *invivo* model.

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LAS NANOPARTÍCULAS DE DIÓXIDO DE TITANIO SON INTERNALIZADAS MEDIANTE FAGOCITOSIS EN CÉLULAS GLIALES.

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Las nanopartículas de dióxido de titanio (NPs-TiO_2) se utilizan en la fabricación de numerosos productos de uso y consumo humano. Debido a su tamaño, entran por el sistema respiratorio, la piel y la boca, y se acumulan en varios órganos, entre ellos el cerebro. Nosotros previamente demostramos que las NPs-TiO_2 se internalizan, inducen estrés oxidativo y daño mitocondrial en células gliales de rata (U373) y humanas (C6); sin embargo, todavía no se conocen los mecanismos involucrados en su internalización. Por lo tanto, el objetivo de este trabajo fue determinar los mecanismos de internalización de las NPs-TiO_2, así como su localización en las células U373 y C6.

Para evaluar su acumulación intracelular se usó microscopía electrónica de transmisión (TEM) y microscopía de lapso de tiempo. Con el fin de determinar si el proceso de internalización está mediado por fagocitosis, se determinó la expresión de las proteínas caveolina-1 y CSP-1 por citometría de flujo, y se utilizó un inhibidor de la fagocitosis (citocalasina D).

Los resultados mostraron que las NPs-TiO_2 se internalizan en ambos tipos celulares. Las NPs-TiO_2 se unieron a las membranas celulares, y se internalizaron a tiempos muy cortos después de la exposición de las células, 10 y 30 min para las células C6 y U373, respectivamente. Durante el proceso de endocitosis, se observó la formación de pseudópodos y vesículas intracelulares, indicando que el proceso está mediado por fagocitosis. No se observó una localización específica de las NPs-TiO_2 en algunos organelos celulares, sino se localizaron en el citoplasma dentro de fagosomas. La internalización de las NPs-TiO_2 se inhibió fuertemente por la citocalasina-D. La endocitosis se asoció con un incremento en la expresión de la caveolina-1 y de la CSP-1.

En conclusión, las NPs-TiO_2 se internalizan por un proceso de fagocitosis en las células gliales, lo cual está relacionado con su fuerte efecto citotóxico en estas células.

Diethyldithiophosphate(DEDTP) Induces Changes in Tumor Infiltrating Lymphocytes, Macrophages and Natural Killer Cells in 4T1 Murine Breast Cancer Model

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Thediethyldithiophosphate(DEDTP) is a dialkylphosphate(DAP) used for domestic and industrialpurposes. It has a half-life of about 80 h, and producesdietylthiophosphate(DETP) anddietylphosphate(DEP). It is known thatorganophosphorus compounds(capable of generating DAPs asbiotransformationmetabolites), produce deleterious effectson the immune systemofthe exposed population.Previous studies, inhuman peripheral bloodlymphocytes*in vitro*, demonstrated thatDAPsuncoupleactivation responseandT lymphocytes proliferation. Other assay*in vivo*, inC57mice, found that exposure toDEDTPincreasestumorsizeof BMKcells, increases the proportion ofTlymphocytesand altersthe secretion ofinterleukin (IL)-6,IL-10 and IL-2. Therefore, ourobjective is to determinethe effect ofDEDTPon T lymphocytes, macrophages and natural killer cells (NKs) infiltrationin4T1breastcancertumorsin mice.To this end, weusedfemaleBalb/c micefrom6-8 weeks old, mice wereexposedfor 8 daysto0.01g/kgi.p.ofDEDTPand inoculatedon day 5s.c.with1x10^{5}4T1cells.Tumorswere obtainedat days5 and 20post-inoculation andthe percentage ofhelperlymphocytes (CD4), cytotoxic (CD8),macrophages (F4/80, CD206) and natural killer cells(CD335)infiltratedin the tumors and lymph nodes was evaluatedby flow cytometry. We observed that miceinoculatedwith4T1cellsduring exposure toDEDTPhad an increaseinthe number ofCD4$^+$lymphocytesandF4/80$^+$macrophagesanda decrease inthe proportionofCD335$^+$NKcellsafter 5 daysoftumor challengeintheproximallymph node. Interestingly, 15days after DEDTP treatment the population ofTCD3$^+$lymphocytes, macrophages CD206$^+$andCD335$^+$NKcells in theproximalganglionwere increased.In tumors,exposureto DEDTPincreasedinfiltrationofCD4$^+$andCD206$^+$cellsand decreasedthe ratioofNKCD335$^+$andmacrophageF4/80$^+$cellsafter 5 daysoftumor developmentcompared tothe animalswiththetumorbutwithoutexposuretoDEDTP. After 15days of the infiltrationof TCD3$^+$cells,CD206$^+$/F4/80$^+$andCD335$^+$NKtumorswas increased compared tounexposed animals.These data demonstratethat DEDTP could act as an immunomodulator of infiltrating immune cells in tumors from exposed animals, although this effect are not decisive against a 4T1 tumor challenge, this is probably due to the inherent aggressiveness of the 4T1 cell line and the Th2genetic background of Balb/c mice, but suggest that DEDTPmay have a relevance in bacterial or parasitic infectious challenge.CONACyTgrant 153468.

CYP2E1 induction leads to oxidative stress and cytotoxicity in cerebellar granule neurons

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Increasing evidence suggests that brain cytochrome P450 (CYP) can contribute to the in situ metabolism of xenobiotics. In the liver, xenobiotics can be metabolized by CYPs into more reactive products that can damage hepatocytes and induce cell death. In addition, normal CYP enzymatic activity produces reactive oxygen species (ROS) that contribute to cell damage through oxidative mechanisms. CYP2E1 is a CYP isoform that can generate ROS and induce cytotoxicity in multiple tissue types. The aim of this study was to determine whether CYP2E1 induction by ethanol, acetaminophen or isoniazidcan induce significant brain cell impairment. Exposure of primary cerebellar granule cultured neurons to the tree chemicals, increased CYP2E1 expression. The treatmentwith acetaminophen resulted in reactive oxygen species (ROS) production and cell death and these effects are inhibited with a pre-treatment of
4-methyl pyrazole or diallyl sulfide, CYP2E1 inhibitors, or to a mimetic of superoxide dismutase/catalase, Eukarion-134. To analyze whether the antioxidant response of cerebellar granule neurons is sufficient to protect against the ROS generated by isoniazid or ethanol induction of CYP2E1, we lowered cellular GSH levels with L-buthioninesulfoximine (BSO). This BSO treatment depletes glutathione but it does not compromise cell viability. A significant increase in ROS production was found in BSO-treated cells after ethanol or isoniazid exposure at the time when CYP2E1 is induced.These effects were attenuated by simultaneous exposure to CYP2E1 inhibitors or antioxidants. In some treatments the neuronal damage was larger suggesting that the damage is also a product of idiosyncratic drug reactions. These results contribute significant data abouthow typical liver enzymes like CYP2E1 could represent a risk of in situ neuronal damage.

Analysis of profibrosing molecules in cells stimulated with cigarette smoke extract.

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Introduction: Idiopathic Pulmonary Fibrosis is a chronic, progressive and lethal disease that occurs in adults between 55-75 years old of unknown etiology, it is known that smoking is the main risk factor. From microarray analysis on lung epithelial cells stimulated with cigarette smoke extract (CSE) overexpression of CCL2is detected. CCL2 is a chemokine responsible to recruitfibroblasts, fibrocytes andmonocyte chemoattractant, stimulate the production of IL-6 and collagen in fibroblast, the overproduction of collagen can be mediated by TGF-β, IL-6 in turn can inhibit apoptosis of fibroblasts. The CCL2 Knockout mouse model develops an attenuated hepatic fibrosis and Knockout mouse of its receptor (CCR2)shows an attenuated bleomycin-induced pulmonary fibrosis. **Hypothesis:**CSE induces proliferation of lung epithelial cells and overexpression of TGF-β1 and CCL2. The effects of CCL2 in turn induces alpha smooth muscle actin and IL-6overexpression in fibroblasts.**Objectives:** Validate whether the CSE induces overproduction of TGF-β1 and CCL2 in lung epithelial cells and analyze in turn if mediates alpha smooth muscle actin, and IL-6overexpression in lung fibroblasts. **Materials and Methods:** A549 lung epithelial cells were stimulated with CSE, real time PCR was done to validate the expression of CCL2, the rate of proliferation of lung epithelial cells was analyzed with WST-1. CCD25 lung fibroblasts was stimulated with media from lung epithelial cells stimulated with CSE and real time PCR analysis was made for expression of alpha smooth muscle actin and IL-6. **Results:** The CSE induces proliferation of lung epithelial cells, overexpression of CCL2 and overproduction of TGF-β1. Conditioned media from stimulated lung epithelial cells with CSE induces alpha smooth muscle actin and IL-6 overexpression in fibroblasts. **Outlook:** With a Knock Down model of CCL2 in pulmonary epitelial cells we will analyze whether CCL2 mediates the overproduction of TGF-β, alpha smooth muscle actin, collagen and IL-6 in fibroblasts stimulated with media from epithelial cells stimulated with CSE.

Smad transcriptional co-factors Ski and SnoN exhibit a highly dynamic turnover in normal *versus* transformed hepatocytes

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Transforming growth-factor beta (TGF-β) signaling maintains tissue homeostasis. Alterations in this pathway can lead to carcinogenesis, autoimmune disorders, organ fibrosis and vascular disorders. TGF-β is also the main signal known to control Ski and SnoN proto-oncogenes protein stability. Ski and SnoN are the nuclear TGF-β/Smad co-repressors, they play important roles regulating cell proliferation and differentiation, and their aberrant expression can lead to insensitivity to TGF-β signals and hence to malignant transformation. The role of Ski and SnoN in tumorigenesis still remains ambiguous : elevated expressions of Ski and SnoN have been reported in many human cancer cells and tissues, consistent with their pro-oncogenic activity, but there is also evidence of a potential anti-oncogenic activity for both proteins. Herein, we set to deeply characterize Ski and SnoN protein regulation within the hepatic context, using a comparative model of normal and transformed hepatocytes (HCC, hepatocarcinoma). We show that the co- repressors protein levels, stability, subcellular localization, and regulation by TGF-β, show profound differences between the normal and the HCC condition. We demonstrate that HCC hepatocytes show high Ski and SnoN protein levels due to an important increase in their stability. Surprisingly, in normal hepatic cells Ski and SnoN protein levels are low and exhibit a highly dynamic turnover. Even though TGF-β is the main stimulus known to regulate Ski and SnoN protein stability, we found that changes in the cytoskeleton and cell adhesion importantly regulate Ski and SnoN protein stability, exclusively in normal hepatocytes. Contrarily, Ski and SnoN from HCC-derived hepatocytes are refractory to changes in the cell environment. These data demonstrate that Ski and SnoN protein regulation shows profound differences between normal and HCC hepatocytes. Also importantly, we provide evidence that despite high levels of Ski and SnoN co-repressors in HCC, TGF-β - dependent genes are highly expressed, suggesting that high levels of the co-repressors in HCC do not necessarily imply a repression on TGF-β-dependent genes as believed. Despite their high expression in HCC cells, the role of Ski and SnoN as oncogenes remains elusive since they do not appear to interfere with the cytokine signaling, making these two co-repressors unlikely targets for therapeutic intervention in HCC.

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GPI/AMF inhibition blocks the development of the metastatic phenotype of mature multi-cellular tumor spheroids.

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Introduction. Epithelial-mesenchymal transition (EMT) and cellular invasiveness are two pivotal processes for the development of metastatic tumor phenotypes. The metastatic profile of non-metastatic MCF-7 cells growing as multi-cellular tumor microspheroids (MCTSs) was analyzed by determining the contents of the EMT, invasiveness and migratory proteins, as well as their migration and invasiveness potential and capacity to secrete active cytokines such as the glucose phosphate isomerase/AMF (GPI/AMF). As control, the same analysis was also performed in MCF-7 and MDA-MB-231 (highly metastatic, MDA) monolayer cells, and in stages IIIB and IV human metastatic breast biopsies.

Results. The proliferative cell layers (PRL) of mature MCF-7 MCTSs, MDA monolayer cells and metastatic biopsies exhibited increased cellular contents (2-15 times) of EMT (β-catenin, SNAIL), migration (vimentin, cytokeratin, fibronectin) and invasiveness (MMP-1, VEGF) proteins *versus* MCF-7 monolayer cells, quiescent cell layers of mature MCF-7 MCTS and non-metastatic breast biopsies. The increase in metastatic proteins correlated with substantial elevated cellular abilities for migration (18-times) and invasiveness (13-times) and with the higher level (6-times) of the cytokine GPI/AMF in the extracellular medium of PRL, as compared to MCF-7 monolayer cells. Interestingly, the addition of the GPI/AMF inhibitors erythrose-4-phosphate or 6-phosphogluconate at micromolar doses significantly decreased its ***extracellular***activity (>80%), with a concomitant diminution in the metastatic protein content and migratory tumor cell capacity, and with no inhibitory effect on tumor lactate production or toxicity on 3T3 mouse fibroblasts. The present findings provide new insights into the discovery of metabolic inhibitors to be used as complementary therapy against metastatic and aggressive tumors.

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Theflavonoidepicatechinactives the PI3K/Aktpathway in healthymouse'shearts

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ABSTRACT

Cardiac hypertrophy (CH) is specifiedas an increase in heart size, which is associated with cardiac failure and premature death.Initially, the CH is an adaptive response that plays cardioprotective and repair functions to stimuli such as hemodynamic overload.Among the causes of hemodynamic overload are hypertension, aortic stenosis, myocardial infarction, intense exercise and pregnancy. There are two types of CH; the pathological and the physiological CH. The first one is accompanied by interstitial fibrosis, apoptosis, ischemia, fetal gene overexpression and alterations in the myocardialstructure, which leads to a malfunction of the heart. The second one occurs in growth, pregnancy and intense sport practices; in these situations the heart size increases, without interstitial fibrosis, withincreased capillary density and the heart has a normal function.It has been reported that IGF1 receptor (IGF1R) activation is required in the physiological CH. The activation of IGF1R induces activation of the PI3K/Akt pathway; then,Akt phosphorylates mTOR which activates p70S6K, who finally regulates muscle protein synthesis.Furthermore, several studies have shown that the flavonoid epicatechin (EPi) is capable of activating the PI3K/Akt pathway in different cell cultures, it would be interesting to assess the effect of the flavonoid EPi in healthy mouse's heart. Objective.To evaluate the effect of the flavonoid EPi in the PI3K/Akt pathway in healthy mouse's heart.Methodology. The flavonoid EPi was administered at a dosage of 1mg/kg body weight for 15 days every 12 hrs. The heart weight/body weight ratio was carried out to determine heart growth.The presence of pathological and physiological CH markers were determined by Western blott.Masson trichrome staining was performed to define the possible existing fibrosis. Results. We found statistically significant enlargement of the heart ($p = 0.0001$) in mice treated with EPI. No pathological CH markers (β-MHC, ANP and BNP) were observed in mice treated with EPi.The expression levels of pPI3K, pAkt and pmTOR proteins were increased in the cardiac tissue of mice treated with EPi.Presence of fibrosis was not founded in histological sections from any of the experimental groups. Conclusions. These results show that the administration of EPi actives the PI3K/Akt pathway in healthy mouse's hearts and induces physiological CH but no pathological CH.

Exploring the role of Rho GTPases on leptin-induced migration, of breast cancer cells in culture.

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Leptin is an adipocyte-secreted hormone involved in the regulation of food intake and metabolism. However, it has been demonstrated that leptin can be produced by cell types other than adipocytes, and that leptin can induce proliferation and migration of cancer cells in culture. Rho GTPases are key regulators of several cellular processes including cell migration. It has been shown that leptin can induce activation of Rho GTPases in several cell types. We investigated the possible role of the GTPases Rac1 and RhoA as regulators of migration of breast cancer cell lines in response to leptin. We used two breast cancer cell lines MCF7 (low migratory potential) and MDA-MB-231 (high migratory potential). Both cell lines were stimulated with leptin and cellular migration was assessed using scratch assays and Boyden chamber assays. Rac1 and RhoA activation was measured using Pulldown assays. To investigate the role of PI3K and MAPK pathways in Rac1 and RhoA activation and cell migration, activation of PI3K and MAPK pathways was evaluated by Western blot using phosphor-Akt and phosphor-Erk antibodies, andwe used chemical inhibitors for PI3K and Erk. **Results**: Leptin induces migration of both MCF7 and MDA-MB-231 cells. In both cell lines leptin induces activation of Rac1 and RhoA, however Rac1 inactivation impairs leptin-induced migration of MCF7 cell but enhances migration of MDA-MB-231 cells. Inhibition of RhoA impairs migration of both cell lines. We also found that leptin activates PI3K and MAPK pathways in MCF7 cells, and that inhibition of these pathways impairs leptin-induced migration. Finally, we demonstrated that leptin-induced activation of Rac1 in MCF7 cells is dependent on Erk activation but not of PI3K activation

Compromised Pak1 activity sensitizes FA/BRCA-proficient breast cancer cells to PARP inhibition.

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Paks are effectors for the small GTPases Cdc42 and Rac that play fundamental roles in several cellular processes, including cell morphology, motility, survival, gene transcription, apoptosis and hormone signaling. These enzymes are widely expressed in numerous tissues and are activated by extracellular signals through GTPase-dependent and -independent mechanisms. In addition, it has been shown that one member of the Pak family, Pak1 which is located on human chromosome 11q13, is amplified and/or overexpressed in several human cancer types, including 25-30% of breast tumor samples and cancer cell lines.

In this work, we performed a comparative gene profiling study in order to identify differentially regulated genes between wild-type and Pak1 deficient mouse and human breast cancer cells. As expected, a considerable number of differentially expressed genes between wild-type and Pak1 deficient human breast cancer cells were also found differentially expressed in mouse breast cancer cells. Surprisingly, several genes involved in the Fanconi anemia (FA)/BRCA pathway, a DNA-damage response signaling pathway which is essential for the repair of DNA interstrand cross-links induced by DNA-damaging agents like cisplatin and doxorubicin, were down-regulated in Pak1 deficient cells. The expression of two FA genes, FANCD2 and FANCI, was confirmed by qPCR and western blot in Pak1 depleted human breast cancer cells with or without 11q13 amplification. Interestingly, the depletion or chemical inhibition of Pak1 in 11q13 amplified breast cancer cells treated with cisplatin, compromised the ability of these cells to repair DNA by homologous recombination, induced cell cycle arrest, promoted apoptosis and resulted in reduced colony formation. In contrast, the inhibition or depletion of Pak1 had little effect on these cellular processes in Pak1-non-amplified breast cancer cells. Finally, we showed that combined inhibition of Pak and PARP had a synergistic effect in 11q13 amplified breast cancer cells, were the dual inhibition of these molecules totally abrogated colony formation and enhanced apoptosis. These findings indicate that depletion or inhibition of Pak1 creates a state of “FA/BRCAness” in transformed cells and represents a rational approach for expanding the efficacy of PARP inhibitors to FA/BRCA-proficient cancer populations.

Akt inhibits β-catenin signaling.

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Abstract

The proinflammatory cytokine IFNγ influences intestinal epithelial cell (IEC) homeostasis in a biphasic manner by acutely stimulating proliferation that is followed by sustained inhibition of proliferation despite continued mucosal injury. β-catenin transactivation has been associated with augmented cell proliferation. However, our results show that IFNγ inhibits IEC proliferation in colonic epithelial cell models despite hyperactivation of Akt/β-catenin signaling. Here we demonstrated that inhibition of β-catenin-mediated cell proliferation by IFNγ requires the formation of a protein complex containing phosphorylated β-catenin 552 (pβ-cat552) and 14.3.3ζ. Akt1 acted as a bimodal switch that could promote or inhibitβ-catenin transactivation in response to IFNγ stimulation. Aktpromotesβ-catenin transactivation via phosphorylation of β-catenin c-terminaldomain, this process promotes its association with 14.3.3ζ and induces nuclear accumulation of β-catenin. Augmented β-catenin transactivation facilitates Akt1 protein increase and lead to the accumulationof Akt1 in the nucleus. Once in the nuclear compartment Akt is activated and phosphorylates 14.3.3ζ,which in turns results in the expulsion of the complex 14.3.3ζ/β-catenin from the nucleus thereby inhibiting β-catenin transactivation and IEC proliferation. These results outline a dual function of Akt1 that suppresses IEC proliferation during intestinal inflammation.

This authorequallycontributedtothiswork. *Correspondingauthor

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Akt-induced apoptosis during inflammation is regulated by 14.3.3 proteins.

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Summary.
Akt activation has been associated with proliferation, differentiation and survival in epithelial cells. Akt hyperactivation in contrast, contributes to epithelial cell damage a hallmark of Inflammatory Bowel Diseases. Full Akt activation requires phosphorylation at Serine 473 and Threonine 308. Despite the relevance of Akt in inducing apoptosis in intestinal epithelial cells the mechanisms guiding its activation during this process remain unclear. Here, we reported that 14.3.3 proteins control Akt activation during intestinal inflammation in order to regulate apoptosis and cell survival. Mechanistically we found that 14.3.3ζ homo and hetero dimmers with 14.3.3ε prevented Akt phosphorylation at T308 by PDK1, but facilitated S473 phosphorylation by mTORC2. In contrast phosphorylation of 14.3.3ζ at S58 disrupts dimmer formation and allows the association of 14.3.3ζ with 14.3.3η that result in PDK1 activation and full activation of Akt, its accumulation in the nucleus and cell apoptosis. Notably inhibition of 14.3.3 function during inflammation also results in uncontrolled Akt activation and increased cell death. Taken togetherour results show that 14.3.3 tampers Akt activation to regulate its physiological functions, thereby providing a new mechanistic link between aberrant cell cycle progression and apoptosis phenotype on intestinal epithelial cells during inflammation.

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NOVEL RACK1 LIGANDS FROM THE DINOFLAGELLATE *Symbiodinium microadriaticum* IDENTIFIED BY THE YEAST TWO-HYBRID SYSTEM

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The interest in understanding the biological mechanisms of symbiosis between photosynthetic dinoflagellates of the genus *Symbiodinium* and cnidarians such as corals, anemones or jellyfish, has increased in the last decades due to the rise in frequency and severity of coral bleaching events. Coral bleaching is the sign of loss of symbiosis, which could lead to coral death. Mechanisms underlying both, symbiont acquisition and its loss leading to bleaching are still not fully understood. We have focused on identifying signal-transduction pathways involved in the symbiosis establishment and maintenance in a *bona fide* model of symbiosis. *Symbiodinium microadriaticum* and the jellyfish *Cassiopea xamachana* represent a model of *Symbiodinium*-cnidarian symbiosis easily manipulated under controlled laboratory conditions. The Receptor for Activated C Kinase (RACK1), which has the ability to bind multiple ligands in several signaling pathways, was used as bait to detect signal transduction components. In this work, we screened a yeast two-hybrid cDNA library from *S. microadriaticum*, which was synthesized using the DINO Spliced Leader sequence. After the first screen, we identified two ligands of RACK1 with molecular weights of 30.8 and 18.6 kDa, both containing the DINO SL sequence at the 5' UTR. These ligands have not been previously reported as RACK1 binding patterns, and it will be necessary to identify their particular functions to better understand their role in the RACK1-mediated signal transduction pathway (s) involved.

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Real time monitoring of the acrosomal reaction in human sperm

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Fertilization is an essential event for organisms that depend on sexual reproduction. This process is highly synchronized and involves a complex series of interactions between sperm and egg, culminating in the fusion of these gametes.
It has been shown that Ca^{2+} is essential in regulating processes required after sperm ejaculation that lead to fertilization, including the acrosome reaction (AR), which consists on the acrosomal granule exocytosis characterized by the formation of multiple fusion points between the plasma membrane and the outer acrosomal membrane in the anterior region of the sperm head. This process is necessary for sperm to traverse the egg envelope and to expose a fusogenic membrane that allows egg-sperm fusion.
Although one of the glycoproteins from the external egg matrix, ZP3, has been regarded as the natural inducer of the AR, other studies have shown that several compounds are able to induce this reaction with the consequent Ca^{2+} mobilization in the sperm. The physiological relevance of these inducers has been strengthened by recent results showing that at least in mouse, sperm that succeed to fertilize an egg are those that underwent the AR before reaching the egg. Therefore, it is important to further characterize other ligands encountered by sperm during its transit through the female reproductive tract known to induce AR.

The classical test to determine the acrosomal status is performed in fixed sperm using lectins coupled to fluorophores, which recognize glycosylated proteins present in the acrosome. However to characterize the real time dynamics of this process and to correlate it with Ca^{2+} mobilization, assays in living cells that allow simultaneous monitoring of AR and intracellular Ca^{2+} changes are required. Fluorescent dyes from the FM family display properties suitable to track plasma membrane dynamics during exocytosis and endocytosis in somatic cells. A previous work from our laboratory showed that FM4-64 successfully reports the occurrence of the AR in real time and that the associated intracellular Ca^{2+} changes can be followed with a Ca^{2+} sensitive dye at the same time.

In this study we monitor in single cell experiments the AR and the associated intracellular Ca^{2+} changes mediated by GABA, progesterone and PGE1 in human sperm.

We observed that progesterone and PGE1 induce AR with an associated Ca^{2+} increase while GABA elicit Ca^{2+} oscillations levels that do not lead to AR.

Functional characterization of *AtGRDP1* gene during the growth and development of *Arabidopsis thaliana*

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AtGRDP1 is a novel gene that belongs to a new gene family that presents a domain of unknown function 1399 (DUF 1399) in the N-terminal. Additionally *AtGRDP1* and its paralog*AtGRDP2* contains a putative RNA binding motif, and in the C-terminal a glycine rich motif. It has been demonstrated that the over-expression on of *AtGRDP1* in *Arabidopsis thaliana* plants provide stress tolerance to salt (NaCl orLiCl) and osmotic (mannitol or sorbitol) treatments compared with the WT Col-0 and the knock-out *Atgrdp1* line. Presumably, AtGRDP1 is involved in ABA signaling pathway since their overexpression caused an ABA-insensitive phenotype (ABI) in seedlings of Arabidopsis through modulation of gene expression of *ABI3* and *ABI5* genes. Here, we show different interesting phenotypes for AtGRDP1 overexpression and mutant lines, as the seeds of different size and shape; and also the overexpression lines present accelerated growth in comparison with Col-0 and *Atgrdp1*-null mutant lines. Therefore, we aimed to analyze the expression of *AtGRDP1* in different tissues of Arabidopsis, finding a high expression in cauline leaves and siliques in 45 days old plants. Furthermore, transcriptional fusion of the *AtGRDP1*promotor with GUS gene reporter shows expression in siliques, trichomes and anters in adult plants.On the other hand, fusion of *AtGRDP1* with *GFP* shows a nuclear localization in onion monolayers. In conclusion the *AtGRDP1* gene shows specific expression patterns during the plant development and could have its functional activity in the nucleus.

Futile Hog1p phosphorylation in response to hiperosmotic stress in *Saccharomyces cerevisiae*

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In response to high extracellular osmolarity, *Saccharomyces cerevisiae* activates the HOG signaling pathway (High Osmolarity Glicerol), which regulates the glycerol synthesis. This signaling system has two separate branches that converge at the MAPKK Pbs2p level. As MAPKKK the SHO1 branch uses Ste11p, while the SLN1 (Sln1p-Ypd1p-Ssk1p) phosphorelay branch uses the redundant Ssk2p and Ssk22. Once active, Pbs2p phosphorylates threonine and tyrosine residues in Hog1p. The absence of components that inactivate both branches leads high sensitivity to hiperosmotic stress. This is the case of the Δ*ssk1*Δ*ste11* mutant, which shows sensitivity to high salt and high sorbitol concentrations.

We have observed that event though, the *ssk1 ste11* fails to growth in medium containing 1M NaCl, Hog1p becomes phosphorylated. We determined that this phosphorylation depends on the presence of Pbs2p and one of the MAPKKK, either Ste11p or Ssk2p/Ssk22p. Additionally, we observed that in response to high osmotic conditions, Hog1p is imported and retained (longer than in the wild type strain) inside the nucleus in the *ssk1 ste11* mutant, but fails to activate transcription of target genes. Finally we determined that the de-phosphorylation of Hog1p in the double *ssk1 ste11* mutant is faster than in wild type cells, suggesting that its regulation follows a different mechanism.

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Dynamic characterization of the native state of amiloidogenic protein 6aJL2 and the mutant R24G

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ABSTRACT

In order to function correctly, most proteins have to adopt a stable three-dimensional structure. However, in the cellular environment the proteins may be subject to conditions that may lead to partial or total loss of their native conformation, promoting the formation of aggregates or fibers. Primary amyloidosis (AL) is a disease characterized by the amyloid fibril formation of immunoglobulin light chains proteins (LCs).

We have used Nuclear Magnetic Resonance to characterize the LCs lambda 6a germline protein (6aJL2) and its single point mutation at position 24 (R24G). This mutation decreases by 1.7 kcal/mol the stability of the protein and whereas structurally both proteins are very similar, they have very different amyloid fibril formation propensities. In order to get a better understanding of the correlation between movements and the amyloid fibers processes, we have investigated the dynamics behavior for these two proteins at different time scales.

At fast time scales, both proteins have very similar dynamics (Model free analysis), nevertheless, we have found that at slower time scales (relaxation dispersion experiments) 6aJL2 has a higher mobility in strands F and C, whereas R24G has a higher mobility in strands C". At even slower times scales (H/D exchange experiments) 6aJL2 exchange around 6.5 magnitude orders slower than R24G. And the ΔG of 6aJL2 is 0.76 kcal/mol higher than for R24G. According to all dynamic results, we found that the most stable regions of both proteins are the strands D, E, B, C, F, G1 and G2. These stable strands could be acting as native like template to promote the polymerization process for the fiber formation.

Molecular cloning, expression and transport analysis of the first SWEET transporter from Zea mays.

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In plants, sucrose is synthesized in photosynthetic cells, but not all is used, and/or accumulated in the mesophyll cells;the excess is distributed to the non-photosynthetic tissues through the plant's vascular system. In 2010,a new class of sugar transporters was identified, the SWEET transporters,they are the unique facilitate bidirectional sugar transporter in plants.The sugar efflux by SWEET transportersis essential to remove the sucrose from the mesophyll cell and load with sugar the apoplast of the phloem parenchyma cells. It is also important in pollen development, nectar production and pollen tube growth.Interestingly, phytopathogens manipulate the expression of some AtSWEETsby introducing transcription factors to the plant'cell[1,2]. Despite of its importance, the SWEET transporters have been characterized in only three species, Arabidopsis, rice and *M. truncatula*[2], and there are many questionsto solve about the remarkable strategy that the microorganisms exert to obtain nutrients from the plants.
The aim of this work was to identify and characterize SWEET transporters in *Zea mays,* specifically those that arealtered when the plant interacts withdifferent microorganisms. In order to accomplish the goal, analysis of the EnsemblPlantdatabase was done,the nucleotide sequences for the putative SWEET transporters in maize werealigned with arecognized nucleotide SWEET sequence from*Arabidopsis thaliana, AtSWEET12*.One sequence obtained from this analysis was selected and named ZmSWEETα. Analysis *in silico* of ZmSWEETαpredicted a protein with seven transmembrane helices, as expected for the members of the SWEET transporter family[1]. ZmSWEETα was cloned using the Gateway® technology and theprotein expression and transport characterization were made by heterologous expression in HEK293 cells. The protein was found in the cell membrane with a molecular weight around 33 kDa. Radiotracer experiments demonstrated sucrose transport activity pH-independent.Additionally, expression analysis ofZmSWEETαby PCR and qPCRon leaves from plants infected was made.We found an induction of ZmSWEETαafter 9 days of infection with*Trichodermaasperellum,*alone or following by the infection of*Fusariumverticillioides. The* sole plant infection with *F. verticillioides* does not induce the SWEET expression. Both fungi are common in soil and in maize but have different life styles, *T. asperellum* is beneficial to the plant and *F. verticillioides* is pathogen.
In conclusion, we cloned one SWEET gene from maize which is induced during the *T. asperellum*plant infection; the protein localizes in the membrane and is able to transport sucrose independent of the proton gradient.

[1]Chen LQ, et al.2010.Nature 468: 527–532.[2]Chen LQ. 2014. New Phytologist 201: 1150–1155.

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Variable and new electron transport pathways in *Candida albicans*, and other surprises.

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It is already known that *C. albicans* possesses a main mitochondrial electron transport chain, as well as an alternative oxidase. Our experiments have shown first that this latter oxidase, sensitive to octylgallate has a very low capacity that can be observed only in starved cells in the absence of a substrate; practically all oxygen consumption can be inhibited with either cyanide or antimycin A, and only a very small additional inhibition was observed with octylgallate. Also a strong catalase activity could be detected, but the free oxygen levels resulting from the addition of H_2O_2 appear to depend on another activity that reduces hydrogen peroxide to water. The analysis of the NADH levels by its fluorescence also revealed that, as expected, the addition of H_2O_2 produced its rapid oxidation, but in the presence of cyanide, this was followed, at a slower rate by its further reduction. However, in the presence of antimycin A, the NADH oxidation was observed to a much lower extent. This indicates the presence of what appears to be a cytochrome *c* peroxidase that oxidizes H_2O_2 to H_2O. In fact, one gene for that activity can be found in the genome of this yeast. The different pathways appear to interact with one another, depending on the possible flux through each one.
Another interesting finding with this yeast was that K^+, and also H^+ transport, are inhibited with cyanide when ethanol is the substrate, but also when glucose is the substrate. This is an interesting puzzle indicating a strong interaction of mitochondria and glycolysis in this yeast.

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Free amino acids profile and consumption during early development of the Pacific red snapper *Lutjanusperu* larvae.

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Recent interest in developing a culture strategy for L peru has led to induced spawning of broodstock in culture conditions. However, intensive larviculture of this species has been characterized by variable survival during hatching and first feeding partially due to variable egg quality. The free amino acids (FAA) in the yolk contribute to protein synthesis as well as providing a source of energy. Additionally, the profile of free amino acids has been proposed as a good indicator of the amino acids requirements of fish larvae and to egg quality.

The objective of the present study was to describe the profile of amino acids in developing embryo and yolk-sac larvae of the Pacific red snapper. This will provide more information in order to evaluate the biochemical determination of egg quality in this species.

Eight Pacific red snapper spawns were obtained from wild-captured broodstock, final maturation and spawning was induced by hormonal injection. Samples were taken from each spawn at several developmental stages: starting at fertilized eggs and during early cleavage (8-32 cells stage), blastula, gastrula, newly hatch, 24 h and 48 h after hatching. Dead eggs were also sampled.The concentration of a total of 15 free amino acids, 9 essential and 6 nonessential was analyzed.

All the amino acids tested were found throughout the embryonic development and yolk-sac larvae of the Pacific red snapper. The mean total FAA content increased from the egg stage and during the embryo development and decreased after hatching. The total content of the essential FAA was higher than the nonessential FAA content throughout development. At the egg stage, glutamate, glycine and alanine were the most abundant non-essential FAA, while leucine, valine, isoleucine and lysine were the most abundant essential FAA.

Live (floating) and dead (non-floating) eggs differ in their composition of FAA. With the exception of serine, leucine and isoleucine, the content of the other FAA was higher in the live eggs when compared with the dead eggs. However, no significant difference was detected in the content of non-essential FAA between dead and live eggs. On the other hand, significantly higher content of histidine, threonine, arginine, methionine, valine and phenylalanine were detected in the live eggs when compared to the dead eggs.

The folding mechanism of the lysine, arginine, ornitine binding protein (LAO). Jesús Renan Vergara Gutiérrez[1], Haven A. López Sánchez[1], Nancy O. Pulido-Mayoral[1], Alejandro Sosa Peinado[1], Rogelio Rodríguez-Sotres[2], D. Alejandro Fernández Velasco[1].[1]Laboratorio de Fisicoquímica y Diseño de Proteínas, Departamento de Bioquímica. Facultad de Medicina. [2]Departamento de Bioquímica. Facultad de Química. Universidad Nacional Autónoma de México.

Periplasmicbindingproteins (PBPs) are involved in thetransport of several substrates inside the cytoplasm of gram negative bacteria. All PBPs share a common structure, constituted by two α/β folds joined by a hinge region. Thermodynamic studies on the folding of PBPs show that the histidine binding protein (HisJ),a 26.1 KDa type II PBP, follows a three-state process, while the maltose binding protein (MBP),a 40.7 KDa type I PBP, folds in a two-state way. Thus, PBPs with the same topology can show different folding behavior. In order to explore the physicochemical consequences of sequence variations within the same topology, we studied the folding process of the lysine, arginine, ornitine, binding protein (LAO) which shares 70% identity with HisJ.

The temperature and urea-induced unfolding of LAO, followed by fluorescence intensity (FI) and circular dichroism (CD), as well as differential scanning calorimetry (DSC) suggest that the temperature and urea-induced unfolding of LAO is as a two state process.On the contrary, results from kinetic studies were more complex. The chevron plot obtained from unfolding and refolding experiments presents two branches at low urea concentrations, both with curvature.This behavior can be determined by proline isomerization and/or the population of an intermediate state.To test the participation of proline isomerization,refolding experiments in presence of a prolylisomerase (CyP18) were performed. The results showed that the slow branch from the chevron plot is due to this process. However, the curvature on the fast branch is still present, indicating the existence of a high energy intermediate.There are three possible models with a single intermediate: 1) Sequential or“on-pathway”model. 2) Non-productive or“off-pathway” modeland 3) Triangular or “parallel pathways” model.An interrupted refolding experiment was carried out to determine the model that best fits the data. The simplest model that described the time-dependent evolution of chemical species in this experiment; and fitted the chevron plot is the sequential mechanism. LAO contains a cys-proline at position 16; the identity of this residue is conserved among PBPs that bind positive amino acids. The slow refolding phase is absent in P16A, indicating that P16 is responsible for the proline isomerization observed in the folding of wtLAO. P16A showed an altered CD spectra and a decreased stability in urea-induced unfolding experiments, Furthermore, isothermal titration calorimetry(ITC) experiments showed a decreasedaffinity for Histidine.

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Kinetic characterization of glycogen synthesis and degradation pathways in tumor cells

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Glycogen is the main cellular energy reserve. It has been reported that tumor cells increase the amount of glycogen that provide the bulk of glucose equivalents to reach a high glycolytic flux. In consequence, the glycogen degradation exerts a significant control (50-60%) over glycolytic flux in tumor cells[1]. Therefore, the inhibition of this pathway would reduce the tumor glycolysis[2].

In this work, the kinetic characterization of enzymes that participate in glycogen metabolism (phosphoglucomutase, PGM; UDP-glucose pyrophosphorylase, UGP; glycogen synthase and GS; glycogen phosphorylase, GP) were carried out in cytosolic cell extracts from normal (rat hepatocytes) and tumor cells (AS-30D, rat hepatoma) under physiological conditions of pH and temperature.

The preliminary results indicated that the kinetic parameters (*Vm* and *Km*) were similar between enzymes from hepatocytes and AS-30D tumor cells. But in tumor cellular extracts the presence of two isoforms of GS with $Km_{UDP\text{-}glucose}$ values of 60 ± 8 nM and 200 ± 20 µM were determined. In contrast, in hepatocytes only one isoform was identified ($Km_{UDP\text{-}glucose}$=2±0.4µM). In addition, it was determined that AMP was essential activator in GP of tumor cells but not for enzyme in hepatocytes. Based in these results, we suggested that mechanisms of regulation of glycogen metabolism are different between normal and tumor cells, which it may be an advantage in the searching for therapeutic targets in cancer cells.

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Purification and characterization of Alt1 and Alt2 of *Saccharomyces cerevisiae*: Functional divergence of the enzymes involved in alanine metabolism

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*Saccharomyces cerevisiae*genome arose from a Whole Genome Duplication (WGD) which occurred approximately 100 millions years ago at the same time in which angiosperms appeared. After WGD there was a massive gene loss, and approximately 90% of genes were lost. It has been proposedthat the selective retention of the two copies of certain duplicated genes favored the development of facultative metabolism; constituting a determinant factor to conquerthe new ecological niche provided by angiosperms fruits.

An important number ofthe duplicated genes that were retained are related to carbon and nitrogen metabolism. *ALT1* and *ALT2*paralogous genes were generatedas a result of the WGD. These genes show striking differences, in their expression patterns and subcellular localization. *ALT1* has a catabolic expression profile, and is located in mitochondria, while *ALT2* displays a biosynthetic expression pattern and the protein is located in cytosol.

Previous work from our laboratory demonstrated that Alt1 has alanine aminotransferase activity, and it constitutes the sole catabolic pathway for alanine utilization, and the major pathway for alanine biosynthesis. Contrastingly, the function for Alt2 remains unknown, and it does not show alanine aminotransferase activity.

The aim of this project is topurify Alt1 and Alt2, to perform a bioinformaticcomparison of their sequence in order to determine their structural differences and identify those amino acids residues, whose presence couldhave impaired Alt2 enzymatic activity as alanine amino transferase. So far we have purified and made the enzymatic characterization for Alt1 and defined by bioinformatic approaches the amino acids changes in Alt2 which probably are the responsible for the loss of Alt2 alanine amino transferase activity, during the process of functional diversification.
Since “sensustrictu” species all show the presence of *ALT1* and *ALT2* it can be presumed that Alt2 has acquired a function which has been selected for. Further analysis will to be carried out in order to address this possibility.

Pseudohysteresis in an enzyme following a ping pong bi bi kinetic mechanism. The case of Thioredoxin-glutathionereductase (TGR).

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The existence of complex behavior in enzyme kinetics is well known. Outstanding between them is the observation of a delay time (lag) in the progress curves before the achievement of the steady-state segment. Such kinetic behavior is known as hysteresis and has been considered as a potential regulatory mechanism of the enzyme activity. In a majority of the cases, the magnitude of the lag time is in the scale of seconds or minutes. The molecular basis of such unusual behavior can be found in a slow equilibrium between alternative conformations or oligomers with different activities. In the present work a model characterized by significant lag times which involve no conformational or oligomer slow transition is presented. The model is developed for a two-site enzyme following a ping pong bi bi kinetic mechanism. The main characteristics of the model are as follows: a) the existence of two active sites with very different catalytic abilities; b) binding of the second substrate at the modified form of the enzyme resulting in inhibition of the enzyme; c) full overlap of the low efficiency catalytic site with the inhibitory binding site for the second substrate; c) the ability of the inhibited enzyme to catalyze the conversion of the substrate bound at the alternative catalytic site, leading into a wrong arrangement of catalytic residues; d) the ability by one of the products of the reaction to revert the substrate inhibition. By using the corresponding differential equations with a suitable set of rate constants, full time courses under a variety of initial conditions were simulated The model predictsatypical hysteresis-like progress curvescharacterized by an initial phase of rapid consumption of the substrates, followed by a temporary inhibition of the reaction and a final stage at which an apparent steady-state segment is reached. The amplitude of the initial fast stage of the reaction will be shortened by increasing the concentration of both substrates, concomitant with an increase in the magnitude of the apparent lag time. By contrast, an increment in enzyme concentration results in a shortening of the apparent lag time.The atypical full time courses will be the result of a continuous competence between the two alternate catalytic pathways, whose relative importance will be dependent on the concentration of substrates and a product. All the features predicted by the model are matchedby the kinetic behavior ofthioredoxin-glutathione reductase (TGR) from *Taeniacrassiceps*.

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A *Burkholderia cenocepacia* gene encoding a non-functional tyrosine phosphatase is required for the delayed maturation of the bacteria-containing vacuoles in macrophages

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Burkholderia cepaciacomplex (*Bcc*) is a group of at least 17 closely related species that are ubiquitously present in the environment.*Bcc* members are causal agents of opportunistic chronic infections in patients with cystic fibrosis (CF). Infections by *Bcc* are difficult to eradicate due to their high levels of antibiotic and antimicrobial peptides resistance.We have previously shown that *B. cenocepacia*(a *Bcc* member) can survive in macrophages within membrane vacuoles (BcCVs) that preclude fusion with the lysosome. The bacterial factors involved in the BcCV arrest are not fully elucidated. We report here that deletion of BCAM0628, encoding a predicted low-molecular weight protein tyrosine phosphatase (LMW-PTP) that is restricted to a few *B. cenocepacia* strains, accelerates the maturation of the BcCVs. Compared to parental strain and deletion mutants in other LMW-PTPs that are widely conserved in *Burkholderia* species, a greater proportion of BcCVs containing Δ*BCAM0628* were targeted to the lysosome. Accelerated BcCV maturation was not due to reduced viability of Δ*BCAM0628* since the mutant strain survived and replicated in macrophages similarly to the parental strain. Therefore, BCAM0628 was referred to as *dpm* (delayed phagosome maturation). We provide evidence that the Dpm protein is secreted during growth in vitro and upon macrophage infection.Heterologous expression of Dpm in *B. multivorans* confers to this bacterium a similar phagosomal maturation delay as found with *B. cenocepacia*. We demonstrate that Dpm is an inactive phosphatase, suggesting that its contribution to phagosomal maturation arrest must be unrelated to tyrosine phosphatase activity.

Characterization of lignocellulolytic activities from a halophile strain of *Aspergillus caesiellus* isolated from a sugarcane bagasse fermentation.

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A halophile thermotolerant fungal strain was isolated from a sugarcane bagasse fermentation in the presence of 2M NaCl that was set in the laboratory. This strain was identified by polyphasic criteria (using molecular market: 18S ribosomal DNA, 28S large sub-unit RNA and Internal transcribes spacers 1 and micromorphological aspects) as *Aspergillus caesiellus*. The fungus showed an optimal growth rate in media containing 1 M NaCl at 28ºC and could grow in media added with up to 2M NaCl. This strain was able to grow at 37 and 42ºC, with or without NaCl. *A. caesiellus* H1 produced cellulases, xylanases, manganese peroxidase and esterases. No laccase activity was detected in the conditions we tested. The cellulase activity was thermostable, halostable, and no differential expression of cellulases was observed in media with different salt concentrations. However, differential band patterns for xylanase activities were detected in zymograms when the fungus was grown in different lignocellulosic substrates such as wheat straw, maize stover, agave fibres, sugarcane bagasse and sawdust. *Aspergillus caesiellus* is a fungus poorly studied. This is a first report of lignocellulosic activity and the first halophile strain for this specie. Optimal temperature and pH were similar to other cellulases previously described. These results support the potential of this fungus to degrade lignocellulosic materials and its possible use in biotechnological applications.

The nonphosphorylated IIA^{Ntr} protein induces RpoS degradation by ClpAP protease in *Azotobacter vinelandii*

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Azotobacter vinelandii is a Gram-negative bacterium able to synthesize polyhydroxybutirate (PHB), a biodegradable plastic of industrial interest. The enzymes necessary for PHB synthesis are encoded by *phbBAC* operon, and its transcription is activated by PhbR. *A. vinelandii* can undergo a differentiation process to form desiccation-resistant cysts, which consist of a central body surrounded by two layers (intine and exine). During the encystment, the bacterium synthesizes the phenolic lipids alkylresorcinols (ARs) which replace the membrane phospholipids and are components of the exine layer. The *arsABCD* operon encodes the enzymes for AR synthesis and its transcription is activated by ArpR. The alternative sigma factor RpoS positively controls the transcription of *phbBAC, phbR* and *arpR*.

The nitrogen-related phosphotransferase system (PTS^{Ntr}) is a global regulatory system in bacteria. It is comprised by EI^{Ntr} (*ptsP*), NPr (*ptsO*) and IIA^{Ntr} (*ptsN*) proteins that participate in a phosphoryl transfer chain from phosphoenolpyruvate, where IIA^{Ntr} appears to be the terminal phosphoryl acceptor. The mutations on *ptsP* and *ptsO* abrogate the PHB and AR syntheses in *A. vinelandii,* while a *ptsN* mutation increased them. The nonphosphorylated IIA^{Ntr} was shown to decrease the expression of PHB and AR genes by an unknown mechanism. Here we characterized the pathway by which PTS^{Ntr} controls the PHB and AR synthesis in *A. vinelandii*

A mini-Tn5 random mutagenesis of the *ptsP*$^{-}$ strain, revealed that mutations on *clpP* and *clpA* genes, that encode protease ClpP and clpP-chaperone ClpA, respectively; restored the PHB and AR syntheses in the *ptsP*$^{-}$ mutant. By western-blot assays we determined that RpoS levels were diminished by mutations on *ptsP* and *ptsO*. The RpoS levels were restored in the *ptsP*$^{-}$ mutant by mutations on *ptsN*, *clpA* or *clpP*. A strain that harbors a *ptsN* H68A, which produces a nonphosphorylatable IIA^{Ntr}, exhibited lower levels of RpoS than these in the wild type strain and the *clpP* mutation restored the levels of protein and the PHB and AR levels in the *ptsN* H68A strain. We conclude that nonphosphorylated IIA^{Ntr} induces the degradation of RpoS by ClpAP protease, reducing the transcription of PHB and AR genes in *A. vinelandii.*

Inhibitory effect of *Parthenium hysterophorus* on *Helicobacter pylori* adherence *in vitro.*

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Helicobacter pylori is a Gram-negative polymorphic bacterium that inhabits the gastric mucosa. Fifty percent of the world population is infected, but this value increases in developing countries. It has been recognized as the main factor for the development of chronic gastritis, peptic ulcers and gastric cancer. Although current therapies are successful, they inherit several problems such as the increment in antibiotic resistance, its high cost, and its side effects.

We are looking for a new therapy through a different approach, by hindering the interaction between bacterial adhesins and the host cell receptors to decrease infection rates. The ethnomedical approach is useful to search bioactive compounds. *Parthenium hysterophorus* is an herb widely used in Mexico for the treatment of digestive illnesses such as stomachache and gastritis (1).

The aim of this study is to find bioactive compounds from *P. hysterophorus* that inhibit *in vitro H. pylori* adhesion to adenocarcinoma cell line (AGS).

Organic and aqueous extracts were obtained from aerial parts of the plant. To determine adhesion to epithelial cells, FITC-labeled bacteria were co-cultured with AGS cells as described (2), in the presence or absence of the herbal extract. After one hour of incubation, the cell monolayers were washed and the adherence was quantified by its fluorescence. Most of the tested extracts partially inhibit the adherence of *H. pylori* to cells (~40%). The dichloromethane-methanol extract (DMPA) has an outstanding effect by inhibiting adhesion in a concentration-dependent manner, reaching 70% of inhibition at 1 mg/ml.

DMPA was fractionated by liquid-liquid partition and subsequent chromatography, obtaining bioactive fractions that can inhibit nearly 90% of bacterial adhesion so they are promising sources for the isolation of bioactive compounds.

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The recombinant enzyme 3-hydroxy-3-methyl glutaryl coenzyme A reductase from *Candida glabrata*(rec-HMGRCg) as a model for studying synthetic inhibitors

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INTRODUCTION: The enzyme 3-hydroxy-3- methyl glutaryl coenzyme A reductase (HMGR) is a glycoprotein involved in triggering the route of biosynthesis of cholesterol in humans and of ergosterol in fungi. A current strategy for controlling risk factors that lead to alterations in the metabolism of lipids is the development of HMGR inhibitors such as statins. These compounds have also been proposed as new antifungal agents. *Candida glabrata* is a yeast that has inherent resistance to azoles, which are inhibitors of sterol synthesis at another level in the route of synthesis. Therefore, new compounds are needed to combat this pathogen. **BACKGROUND:** Our working group has shown that a series of α-asarone analogues (synthesized by Dr. Joaquín Tamariz Mascarúa) inhibit ergosterol synthesis and viability in *C. glabrata* and that these compounds specifically inhibit HMGR *C. glabrata* (HMGRCg) in enriched enzyme extracts. However, the purification of HMGRCg from membrane fractions has proved laborious and the resulting enzyme is very unstable. On the other hand, human HMGR has already been cloned and expressed in *E. coli* to test its *in vitro* activity. Thus we cloned and expressed HMGRCg in the heterologous system of *P. pastoris* and *E. coli*, tested the activity of this enzyme in a cell extract, and explored its inhibition by simvastatin and synthetic compounds. **JUSTIFICATION:** Because the catalytic domain of HMGRs in eukaryotic organisms is highly conserved, we decided to use the HMGR enzyme of *Candida glabrata* as a model for studying different lipid-lowering compounds with potential use in humans, as well as for developing new antifungal agentsfor this opportunistic pathogen. **OBJECTIVE**: Clone the soluble fraction of the HMGRCg in the heterologous expression vectors pPICZαB and pPICZB for overexpression in *P. pastoris*, and in the pET-15b vector for expression in *E. coli*. **METHODS:** 1.-Design of primers to amplify the sequence encoding the soluble fraction of HMGRCg. 2.-Amplify the HMGRCg gene by PCR. 3.-Clone the HMGRCg gene in cloning vector pJET 1.2/blunt. 4.-Subclone the HMGRCg gene into the expression vectors pPICZB, pPICZαB and pET-15b. 5.-Transformation of the genetic constructs pPICZB-HMGRCg and pPICZαB-HMGRCg in *P. pastoris.* 6.-Induce the expression of rec- HMGRCg. 7.-Measure the enzyme activity of rec-HMGRCg in crude extracts. 8.-Inhibit rec-HMGRCg with statins and synthetic compounds. **RESULTS:** It was possible to obtain an enzymatic extract of HMGR from *C. glabrata* and to show its expression in a protein gel, as well as its activity and its inhibition with a statin (simvastatin) and a number of synthetic compounds considered inhibitors of the HMGR. **CONCLUSIONS:** We were able to clone and express the HMGR protein from *C. glabrata* in the heterologous system of *P. pastoris*, and then to demonstrate the activity and inhibition of this protein in an enzyme extract. The results suggest that HMGR can serve as a model for the study of the antifungal activity of synthetic compounds.

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Isolation and partial characterization of TATA binding protein (TBP) gene of *Taeniasolium*.

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Little is known about the transcriptional system in cestodes, therefore we study it, with the goal to found the differences with the human and pig (host) transcription system. The aim of this study was the isolation and characterization of TATA Binding Protein of *Taenia solium* (TsTBP) gene and the identification of the elements present in the core promoter.

We have cloned and characterized the gene and cDNA that encodes TsTBP. The amino acid sequence reveal a 238-residues protein with an expected weight of 26.7 kDa. Southern and northern blot suggests the presence of one gene and two mRNA for TsTBP. *In sillico* modeling reveals a classical ribbon structure for TBP. We produced antibodies anti-TsTBP and identified endogenous protein in nuclear extracts from cysts. The TsTBPproximal promoter analysis reveals this is a TATA-less gene. The Transription Start Site was determined by 5´-RACE, where first transcribed nucleotide correspond to an A_{+1}. Transcript expression levels were measured by Real Time-PCR showed adifferential expression, adult express the gene two times more than larval stage. Finally, EMSA and supershift with nuclear extracts from *T. solium* and TsTBP antibodies showed a specific interaction between the TATA box of different promoters.

Our results indicate that we have isolated the gen encode to TBP andshowed its interaction with TATA box from different genes. In addition, we identified an INR and putative DPE in some genes from *Taenidae* family.This is the first study of transcription factors and core promoter elements in *Taenia solium*.

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Membrane topology and identification of functional amino acids in the ArnT protein from *Burkholderia cenocepacia*

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Burkholderia cenocepacia is a Gram-negative bacterium that requires the presence of *4-amino-4-deoxy-L-arabinose* (L-Ara4N) in its lipopolysaccharide (LPS) for viability. Previous work in our laboratory demonstrated that L-Ara4N is necessary for the export of LPS to the outer membrane and it is also the major determinant for resistance to killing by cationic antimicrobial peptides. ArnT is a membrane protein that catalyzes the transfer of L-Ara4N to the LPS molecule, but its topology and function has not been well characterized. Here, we elucidate the topology of ArnT and identify key amino acids that likely contribute to its enzymatic function. Based on PEGylation assays using a cysteineless version of ArnT we conclude that this protein has 13 transmembrane domains and the C-terminal region oriented towards the periplasmic space. The same topological configuration is proposed for the ArnT homolog in *Salmonella enterica* serovar Typhimurium. Four highly conserved periplasmic residues in *B. cenocepacia* ArnT, tyrosine-43, lysine-69, arginine-254 and glutamic acid-493, were critical for the activity of the protein as determined by *in vivo* restoration of polymyxin B resistance in a Δ*arnT* mutant. Moreover, tyrosine-43 and lysine-69 span two highly conserved motifs ^{42}RYA^{44} and ^{66}YFEKP^{70} that are present in ArnT homologues from other species. Further analysis revealed that the same residues in ArnT from *S. enterica* are also crucial for enzyme activity. We propose these aromatic and charged residues play an essential role in undecaprenyl phosphate-L-Ara4N recognition or to the transfer of the L-Ara4N moiety to the LPS.

Synthesis of acetate in *Entamoeba histolytica*

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Entamoeba histolytica is the causal agent of human amebiasis. Our workgroup is focused in the study of the energy metabolism in this parasite to determine its mechanisms of control. The principal pathway of ATP production in the parasite is glycolysis whose end-products are acetate and ethanol. Acetate is produced in two ways, i) acetate thiokinase (AcTK) converts the acetyl-CoA (produced by glucose degradation) *plus* Pi and ADP into acetate, CoA and ATP; ii) alternatively, acetate kinase (AcK) transforms acetyl-phosphate (acetyl-P) and Pi to produced acetate and PPi. So far, the enzymes that generate acetyl-P in amoeba have not been identified; nevertheless, the parasite can phagocytize bacteria which have high concentrations of this metabolite [2].
The aim of this work is to determine the contribution of AcTK and AcK to the acetate production in axenic *Entamoeba histolytica.*

RESULTS. The AcTK and AcK genes were cloned, the respective proteins over-expressed in *E. coli* cells and the recombinant enzymes purified using standard methodologies. Kinetic characterization was done by spectrophotometic methods. The *Vmax* of AcTK was 15- 22 µmoles/min x mg of protein at pH 6-7; the *Km* values were: acetil-CoA 127 µM, ADP 0.9 mM and Pi 1.8 mM. The concentration of these metabolites in amebal trophozoites were 0.9 mM and 3.4 mM and 5.4mM respectively; these concentrations are 10 folds *the* Km value, therefore the enzyme could be partially saturared by its substrates in this direction of ATP synthesis (forward reaction). The kinetic values in the direction of acetyl-CoA synthesis (reverse reaction) were: *Vmax* 16-20 µmoles/min x mg protein at pH 6- 7; Km_{CoA} 0.2 mM, Km_{ATP} 0.3 mM and $Km_{acetate}$ 0.8 mM. The concentrations of the metabolites in trophozoites were CoA = 0.05 mM, ATP= 3.1mM and acetate was not detected. This suggests that the reaction in this direction would be less favored *in vivo*. In amebal cytosolic fractions, the *Vmax* values for the Fw and Rw reactions at physiological pH interval were very similar.

On the other hand the AcK kinetic parameters determined at pH 7 and 6 were *Vmax* 26 and 14 µmol/min x mg protein, $Km_{acetil\text{-}P}$ 2 and 7 µM and Km_{Pi} 2.2 and 2.6 mM, respectively. The activity in the reverse reaction was not detected, which correlated with previous reports [1]. Moreover, the activity in cytosolic fraction of the parasites was 48 nmoles/min x mg of protein. Nevertheless, it was not possible to detect acetil-P in amebal axenic extracts. A possible explanation for the presence of the AcK in amebal axenic cultures would be that the enzyme has alternative substrates or that it consumes the intracellular acetil-P in a very efficient way.

CONCLUSIONS: AcTK is the enzyme that mainly contributes to the synthesis of acetate in axenic *Entamoeba* parasites. An interesting finding was that AcK was present in axenic amebas even when its substrate seems to be absent or in a very low concentration. This opens the possibility that AcK may have another function in the cell, a hypothesis that remains to be evaluated.

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"Silencing of the mitochondrial calcium uniporter improves post-ischemic cardiac dysfunction and attenuates mitochondrial Ca^{2+} overload and apoptosis in rat myoblast and cardiomyocytes".

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Ischemic heart disease is characterized by the reduction or absence of oxygen supply to the myocardium primarily by occlusion of a coronary artery. Acute ischemia induces cell death during myocardial infarction. However, restoration of coronary flow or reperfusion, precipitates cell death during the first minutes of reperfusion. This ischemia-reperfusioninjuryis producedby multiple interrelated mechanisms. Then,is well characterized that ionic imbalance initiated during ischemia, promotes a significant increase in $[Ca^{2+}]_i$on reperfusion that induces a mitochondrial Ca^{2+} overload, being a cause of the phenomenon called mitochondrial permeability transition (mPTP), which dissipates membrane potential ($\Delta \Psi_m$) and leads to cellular energy collapse. Therefore, the mPTP opening is one of the causes of reperfusion injury, inducing myocyte cell death by necrosis and apoptosis. The aim of this work is to study the role of mitochondrial calcium uniporter channel (MCU) in post-ischemic injury as a regulator of $[Ca^{2+}]_i$overload -induces cell death in myoblast and cardiomyocytes. Specific small interfering RNA (siRNA) targeting MCU was used to silence MCU expression. The messenger RNA (mRNA) level of MCU was measured using quantitative real time PCR, and the protein levels of MCU and regulatory proteins were determined using Western blot analysis. MCU knockdown decrease 70% with a consequent decrease in mitochondrial Ca^{2+}transport. At first, MCU knockdown attenuated cell death ofouabain-induced increase in $[Ca^{2+}]$. MCU silencing effects against hypoxia/re-oxygenation injury in cardiomyocytes will be determined.These cardioprotectiveeffects were reversed with MCU antagonist. These results indicates that MCU have a main role in post-ischemic cardiac dysfunction, thus, the chemical inhibition of MCU or MCU knockdown could be an approach used to prevent $[Ca^{2+}]_i$ overload - induces injury in several pathologies such ischemia/reperfusion, cardiac arrhythmias or heart failure.

Energy metabolism in human triple-negative breast carcinoma

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Introduction. The infiltrating ductal breast carcinoma triple negative subtype (TN, i.e., lacking HR and Her-2 expression)is the most aggressive breast cancer with poor survival prognosis. It has been determined that the TN aggressiveness is associated with the lack of specific therapy. Our group showed that 2OGDH, a Krebs cycle enzyme is 2-20 times over-expressed in TN biopsies of Mexican patients compared to triple negative, Her2+/HR- and Her2-/HR+ indicating that perhaps oxidative phosphorylation (OxPhos) flux could be also increased. In order to demonstrate whether TN cells are depending on mitochondrial metabolism, the protein contents, activities and fluxes of OxPhos (oligomycin-sensitive respiration and mitochondrial membrane potential, $\Delta\Psi m$) as well as glycolysis were determined in the TN tumor line MDA-MB-231 and compared to MCF-7 a typical Her2+ tumor line and under physiological tumor microenvironment (i.e., chronic hypoxic conditions, 0.1 % O2, 24 h).

Results. Chronic hypoxia increased the HIF1-a content by 100-times in both MCF7 and MDA-MB-231 cells correlating with a significant increment in glycolysis flux (1.7-times), but no significant changes in the content of several glycolytic proteins (GLUT1, HKI and II; and LDHA) were observed *versus*normoxia. On the contrary, hypoxia promoted a diminution of 50-75% in the OxPhos flux of both tumor cells (from 8-10 to 2-5ngAtO/min/mg protein*vs.*normoxia) although mitochondrial proteins were without change (2OGDH, ND1, COX IV, and ANT), except for the ATP synthase in MDA whose protein content was significantly diminished. In according with the low respiration flux induced by hypoxia, $\Delta\Psi m$was also abolished in both tumor cells (60-70%) *versus*normoxia.

In normoxic conditions, OxPhos was the principal ATP supplier (>60%) in both tumor cells. However, prolonged oxygen limitation (i.e., physiological condition in solid tumors) promoted a metabolic shift where glycolysis predominates in the ATP production (>70-80%). Our observations clearly indicate that a potential treatment to render TN proliferation is the anti-glycolytic therapy.

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Membrane fluidity of kidney microsomes during development of diabetes.

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Introduction: Type 2 Diabetes (T2D) represents a major complication of obesity and is associated to metabolic disorders (metabolic syndrome). Nutritional and epidemiological studies indicate that consumption of polyunsaturated fatty acids (PUFAs) omega-3 (ω-3) counteract the effects of diabetes. However, there are insufficient evidence of a beneficial effect of alpha linolenic acid (ALA) of the development of diabetes and the membrane fatty acid composition.

Objective: It was evaluated the effect of alpha-linolenic acid on the development of diabetes and lipid metabolism as well as on the physicochemical properties of kidney microsomal membranes.

Methodology: T2D was induced by intraperitoneal injection of streptozotocin (STZ) in 48 hours-old, newborn male rats. The control group was injected only with citrate buffer. Rats were weaned at 4 weeks-old and each group was divided into two: one of them was given ALA and the other does not. Thus, there were four groups: Wistar, Wistar-ALA, Wistar-STZ and Wistar-STZ-ALA. Blood glucose was determined weekly with glucose reactive strips. The animals were killed at 1, 3, and 6 months of age. Kidney microsomes were obtained and lipids were extracted. Fatty acid composition was determined by gas cromatography and the membrane fluidity was measured with the fluorescent monitor dipyrenilpropane (DPyP).

Results and conclusion: Wistar-STZ had glucose concentrations ranged between 150 and 350 mg/dl during the 6 months of life, whereas in the Wistar-STZ-ALA glycaemia decreased at 4 months (3 months of treatment). In kidney microsomes it was observed that the membrane fluidity reported by DPyP reflects a pattern similar to the unsaturation fatty acids ratio, being sensitive to changes in fatty acid composition of these microsomes. In the case of Wistar-STZ-ALA it was found that the percentage of arachidonic acid and ω-3 PUFAs decreased at 6 months of life, as well as membrane fluidity. In Wistar-STZ-ALA increased the ω-3 PUFAs increasing unsaturation ratio U/S and membrane fluidity. With the development of diabetes the membrane fluidity kidney microsomes decreased. ALA supplementation modifies the content of ω-3 PUFAs in microsomes increasing membrane fluidity.

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"GENETIC DETERMINANTS ASSOCIATED WITH ANTIBIOTICS RESISTANCE IN *Mycobacterium tuberculosis*STRAINS FROM MICHOACÁN"

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TheActinobacteria *Mycobacterium tuberculosis*is thecausal agentof tuberculosis (TB).Based on the antibiotic resistance of *Mycobacterium tuberculosis,* TB strains have been classified as multidrug-resistant (MDR-TB) and extensively drug-resistant(XDR-TB). The six-month treatmentagainst TB involvesrifampin, isoniazid, pyrazinamide, ethambutol and streptomycin. Associations between mutations in specific genes and resistance to antibioticshave been reported (*rpo*B and rifampicin; *kat*Gand promoter *mab*Aand isoniazid; *pnc*A andpyrazinamide; *emb*B and ethambutol; *rrs*and*rps*Land streptomycin).The frequency of these mutations varies in each country and region within a country so in order to design diagnostics tools, the relationship between mutations and first line resistance patterns in each country must be known. We recognized the susceptibility patterns(using BACTEC-MGIT equipment) and detected mutations (by PCR amplification, sequencing of genes of interest, alignment with Clustal X 2.0 software, and comparison with the TB Drug Resistance Mutation Database) in 33 strains of *Mycobacterium tuberculosis* collected in the state of Michoacán from 2009 to 2011.Wefound 21.2% of the studied strains were resistant to rifampin.Genes*kat*G and *rpo*Bshowed the highestmutationpercentage within the studied collection of strains (30.3% and 27.2%, respectively).54.5% of the strains presents at least one mutation in any of the analyzed genes. Ouranalysis of resistant strains found both new and previously reported in the literaturemutations such as the *kat*G gene Ser315Thr.As reported for other regions of the world, the four analyzed strainsisolatedfrom patients with meningeal tuberculosis showed extensive drug susceptibility of first line antibiotics. Only two of the latter strains had mutations in the *kat*G gene: strain 049with synonymous Ala411Ala mutation having no influence on antibiotics sensitivity, and 040 with Arg463Leu mutation reportedas producing resistance in some cases. 62.5% of the strains resistant to drugs showed some resistance mutation.

Expression and clinical relevance of heat shock proteins of 90 kDa, Hsp90α and Hsp90β, in patients with renal carcinoma.

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Abstract

In recent years the renal carcinoma (RC) has had pour results to chemotherapy and immunotherapy, were the development of drugs designed to inhibit signaling pathways that stimulate angiogenesis and cell proliferation offers new hope in clinical therapy. In this regard, we have seen that several of the oncogenic-proteins involved in these signaling pathways are proteins “client” of Hsp90. Hsp90 is responsible of the stability, activity and/or degradation of its more than 100 proteins “client” reported so far. In clinic practice, the over-expression of the Hsp90 protein has been related with poor prognosis in a wide variability of carcinomas, denoting several oncogenic-proteins as the main protagonist of the signaling pathways regulated by Hsp90; among which are: kinases (eg. Akt, mTOR), transcription factors (eg. HIF-1α), and growth factor receptors (eg. VEGFR). In a recent study, we demonstrate that de mainly isoforms of Hsp90, Hsp90α and Hsp90β, can differentially and even opposite modulate the activity of the oncoproteinAkt, through change their activation state. In renal carcinoma, the expression of the different isoforms of Hsp90 has not been explored; and even less its functional involvement in the regulation of its oncogenic “client” proteins.

In the present study, we studied the possible association between the Hsp90α and Hsp90βprotein expressionin patients with RC, as well with the activation of the signaling pathway mediate by Akt/mTOR/HIF-1α with cancer progression. For this, we determinate the protein expression of the Hsp90isoforms, Hsp90α and Hsp90β, as well as their “client” proteins involved in the proliferation pathway:Akt, $mTOR_{Ser2448}$ and HIF-1α, by Western blot (WB) assays. We included two patients groups:ccRCC (clear cell renal cell carcinoma)with and without metastasis (n=19).

The results obtained by WB are able to identify an expression pattern between Hsp90α and Hsp90β that is capable of differentiate patients of ccRCC in clinic metastatic stage from those without metastasis. While the expression pattern of Hsp90α allow to define a group of patients with ccRCC on metastatic stage, characterized by its over-expression in FG3(Fuhrman Grade 3), which dictates its progression. Hsp90βexpression pattern of allow to define patients with ccRCCwithout metastasis, characterized by a supported minimum over-expression in any clinical stage of RC determinate by its FG.

Finally, over-expression Hsp90 protein used as a progression marker in other types of cancer, doesn’t allow defining the metastatic clinical stage in patient with ccRCC, as obtained with the expression pattern obtained of their isoforms Hsp90α and Hsp90β.

MicroRNA amplification from neonatal screening samples

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Background. MicroRNAs (miRNAs) are non-coding RNA molecules that act as post-transcriptional regulators. They have been proposed as biomarkers since they appear to be stable across tissues and biological fluids [1,2], have tissue specificity and are distinctive for disease stage [3]. Neonatal screening, a widely used routine test around the world, is applied to 90.38% of Mexican newborns during first week of life [4]. For the test, a blood sample is taken from a heel prick and stored as dried blood spots (DBS) inGuthrie cards.DBS could be a useful sampling method for nucleic acids [5] as they are low cost, easy to transport and stable in long-term storage.However, methodological studies on the isolation of miRNA from DBS are so far scarce [6].
Aims. To develop a reliable, reproducible and affordable protocol for optimal extraction and amplification of miRNAsfrom Guthrie cards used in neonatal screening.
Methods. Several miRNAs isolation protocols from clinical samples of Guthrie cards were examined. Quantitative and qualitative analysis of total isolated miRNAs wasperformed using state-of-the-art technology, namely Small RNA Assay for the 2100 Bioanalyzer. Specific amplification of three different miRNAs was assesed by a LNA-probe based stem-loop RT-qPCR targeted to mature miRNAs only.
Results. The isolation protocol resulted in high total miRNA yields. Results from miRNA amplification assays revealed the usefulness of LNA-probe based stem-loop RT-qPCR for analysis of miRNAs obtained from clinical samples of Guthrie cards.
Conclusions. This work is the first to report a reliable and affordable method for miRNA expression analysis using Guthrie cards from neonatal screening.The ease of this method along with the comprehensive coverage of neonatal screening, opens new possibilities for basic research and diagnostics.

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Combination of cell therapy and gene therapy reduces experimental liver fibrosis

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BACKGROUND: Gene therapy using Ad-huPA has diminished liver fibrosis and increased hepatocyte regeneration. On the other hand, administration of adipose derived stromal cells (ADSCs) has improved liver function and has regenerated liver tissue in experimental models.The aim of this study was to evaluate the combination of this therapeutics in fibrosis and fibrogenic molecules.

METHODS: hADSCs were isolated from fat tissue, expanded and characterized. Ad-huPA vector was generated previously by recombination of pMH4-HuPA and pJM17ΔE1Ad-v backbone. CCl_4 cirrhotic rats were administered with hADSCs ($2X10^6$ cells/rat) or Ad-huPA ($3x10^{11}$vp/rat) orboth hADSCs/ Ad-huPA via ileac vein. All groups were immunosuppressed with 10mg/kg of Cyclosporine A.Percentage of fibrotic tissue and mRNA levels of TGF-β1 and collagen α1were evaluated. Also, biodistribution of hADSCs was examined.

RESULTS: hADSCs, Ad-huPA and Ad-huPA/hADSCs administration reducessignificantly the percentage of fibrotic liver in 78.9%, 65.2% and 72%, respectively, compared to controls ($P>0.05$). Ad-huPA and hADSCs administration decreases TGF-β1 mRNA levels ($P>0.05$), while mRNA of collagen α1 diminishes ($P<0.05$)in Ad-huPA, hADSCs and Ad-huPA/hADSCs groups compared to cirrhotic group.

CONCLUSION: Xenogenic hADSCs, Ad-huPA and Ad-huPA/hADSCs therapies decrease liver fibrosis andprofibrogenic molecules. However, cell therapy seems to reduce liver fibrosis in a major proportion ($P>0.05$).

Drug resistance promoted by hypoxia in human medulloblastoma cells involves modulation of extracellular pH control proteins, cytochrome P450 enzymes and cell cycle arrest

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Introduction: Hypoxia is a common condition observed in solid tumors, characterized by reductions in the O_2 levels. This phenomenon contributes to overregulate diverse adaptive cell responses, including additive features of drug resistance which are poorly understood and may involve mechanisms of drug metabolizing enzymes, such as cytochrome P450 (CYP), drug transport, extracellular pH control and alterations in the cell cycle. The aim of the present study was to determine the resistance to cyclophosphamide, ifosfamide or vincristine of human meduloblastoma cells (Daoy®) under hypoxia, and its association with the expression of proteins of extracellular pH control (NHE-1, MCT-4, CA-IX), CYP isoforms (2B6, 3A4, 3A5) and cell cycle distribution. **Material and methods:**Monolayer cells were growninEMEM media and placed in hypoxia for 24 h to 1% and 0.1% O_2 in a Modular Incubator Chamber®(Billups-Rothenberg, Inc.). Cytotoxicity was evaluated with MTT, mRNA byqRT-PCR and protein by Westernblotting; cell cycle was analyzed using propidium iodide staining and flow cytometry. **Results:** Inhibitory concentrations 50 (IC_{50}) for cyclophosphamide were 11.8 ± 1.6, 23.5 ± 2.4 and 16.7 ± 3.1 mM; ifosfamide 12.5 ± 1.5, 27.5 ± 3.8 and 31.8 ± 5.0 mM; vincristine 48.5 ± 5.4 nM, >100 nM, in normoxia and 1% or 0.1% O_2, respectively. Additionally, hypoxia increased protein levels: CYP2B6 by 200% (1% O_2), MCT-4, 50% (0.1% O_2) and CA-IX, 1,300% (in 1% and 0.1% O_2). *CYP2B6* mRNA levels, increased by 300% in 1% O_2 and 500%, in 0.1% O_2. Whereas levels of NHE-1, CYP3A4 and CYP3A5 were not modified. Finally, hypoxic conditions of 1% and 0.1% O_2, increased 39.7% and 36.5% the number of cells in G0-G1 phase; diminishing 55.4% and 46.3% those in S phase, respectively. No variations in phase G2-M were observed, comparing to normoxia. **Conclusions:** Two experimental hypoxia conditions, 1% and 0.1% O_2, promoted a greater resistanceto cyclophosphamide, ifosfamide and vincristine in human medulloblastoma cells. This behavior was associated to overexpression of MCT-4, CA-IX and CYP2B6, and changes in the cell cycle progression, suggesting an important role of hypoxia in modifying processes of drug mechanisms of action, transport and metabolism.Funding CONACyT 152919 and INP 39/2010.

Lipid droplet accumulation in *Ustilago maydis* depends on the nitrogen source.

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Ustilago maydis is the fungus that produces the corn smut. The availability of the whole genome sequence has facilitated the use of this organism to study other cell biology processes, including intermediary metabolism. In this sense, glucose is the main carbon and energy source for *U. maydis,*an organism that carries out no fermentation and is fully respiratory.
Depending on the culture conditions, glucose can be used by cells to synthesize lipids, which in turn are stored in intracellular organelles called Lipid Droplets (LDs). These structures are organelles implicated in the storage of fat and its mobilization. As expected, these two processes are tightly regulated. In this research the main objective was to study the dynamics of lipid droplets in the fungus *Ustilago maydis*. We used an assay based on the recovery of quenched BODIPY-fluorescence that allows the analysis of lipid droplets dynamics in presence of several types of stimuli. The results indicated that the amount of LDs in the cell changed with the growth phase and the culture media composition. In rich medium (YPD) the greatest accumulation of LDs was observed in the exponential phase, while in the stationary phase the LDs were consumed. In contrast, in minimal medium with glucose as carbon source and nitrate or urea as nitrogen sources, there was an increase in the LDs when cells reached the stationary phase. Growing the cells in the absence of a nitrogen source induced a steady increase of LDs in the cells.Inhibition of the acetyl-CoA carboxylase activity with soraphen A, indicates that *de novo* fatty acid synthesis is the main pathway for LDs synthesis, while inhibition by rapamacynshows the possible participation of TOR protein in the accumulation of LDs when cells are growing in nitrogen source.

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Kinetic and thermodynamic control in the thermal unfolding of bacterial Triosephosphate Isomerases.

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Triosephosphate isomerase (TIM) is a ubiquitous glycolytic enzyme that catalyzes the isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. All the wild type TIMs so far studied are homooligomers, where each monomer folds into a $(\beta/\alpha)_8$ barrel. The structure of the TIM barrel is conserved among species, whereas the folding mechanism seems to be sequence dependent. The unfolding of several TIMs, mainly of eukaryotic organisms, has been extensively studied. In most cases the temperature-induced unfolding is an irreversible process and/or out of chemical equilibrium; this has prevented a detailed energetic-structural characterization.

Here, we study TIMs belonging to diverse bacterial phyla: *Deinococcus radiodurans* (*Dr*TIM), *Nostoc punctiforme* (*Np*TIM), *Gemmata obscuriglobus* (*Go*TIM), *Clostridium perfringens* (*Cp*TIM) and *Streptomyces coelicolor* (*Sco*TIM). All these TIMs were expressed in *E. coli* and purified to homogeneity. Enzymatic activity showed Michaelian kinetics; catalytic efficiency was similar to that reported for other TIMs. Size exclusion chromatography indicated that all the TIMs are dimeric. Circular Dichroism (CD) and intrinsic fluorescence spectra indicate that secondary and tertiary structure are formed.

Thermal unfolding was then followed by Circular Dichroism (CD) and Differential Scanning Calorimetry (DSC). Unfolding of *Np*TIM and *Go*TIM was irreversible and T_m was scan rate-dependent, indicating kinetic control; a behavior observed in all eukaryotic TIMs. The transition state for both TIMs is dimeric and native-like in terms of solvent exposure. Interestingly, the thermal unfolding of *Dr*TIM, *Cp*TIM and *Sco*TIM, was reversible with a hysteresis <1 K between unfolding and refolding traces. The change in T_m among different scan rates tested was <1.5 K; all these evidences are indicative of a reversible equilibrium process under thermodynamic control; a behavior not observed previously for others TIMs. CD and DSC experiments were well fitted to a two state dimer dissociation/unfolding model. At 298 K the total unfolding ΔG is very similar to the dissociation ΔG, and approximately half of that reported for other TIMs unfolded by urea or guanidinium hydrochloride, a result that suggests a physicochemical "fee" that these enzymes pay for their reversible thermal unfolding. The high resolution 3D structures of *Cp*TIM and *Dr*TIM were obtained. The only significant difference so far observed reversible and irreversible TIMs is the lower amount of hydrophobic volume present in the cavities of the protein and a higher number of salt bridges. The results presented in this work indicate that for a particular topology the aminoacid sequence determines if the unfolding is under thermodynamic or kinetic control. In addition, the reversibility in thermal unfolding seems to have multifactorial origin and may also be encoded in the folding pathway.

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Using X-rays to describe electronic fluxes in REDOX enzymes.

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X-ray radiation induces two main effects at metal centres contained in protein crystals: radiation-induced reduction and radiolysis and a resulting decrease in metal occupancy. In blue multicopper oxidases (BMCOs), the geometry of the active centres and the metal-to-ligand distances change depending on the oxidation states of the Cu atoms, suggesting that these alterations are catalytically relevant to the binding, activation and reduction of O_2. In this work, the X-ray-determined three-dimensional structure of laccase from bacterial and fungus, are described. By combining spectroscopic techniques (UV-Vis, EPR and XAS) and X-ray crystallography, structural changes at and around the active copper centres were related to pH and absorbed X-ray dose (energy deposited per unit mass). Depletion of two of the four active Cu atoms as well as low occupancies of the remaining Cu atoms, together with different conformations of the metal centres, were observed at both acidic pH and high absorbed dose, correlating with more reduced states of the active coppers. These observations provide additional evidence to support the role of flexibility of copper sites during O_2 reduction. This study supports previous observations indicating that interpretations regarding redox state and metal coordination need to take radiation effects explicitly into account.

"Different expression of the inhibitory ζsubunit of the F_1F_O-ATPase nanomotoramongfree-living, symbiotic, and parasiticα-proteobacteria"

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The ζsubunit is a novel natural inhibitor ofthe F_1F_O-ATPase nanomotorofα-proteobacteriathat we discovered originally in*Paracoccusdenitrificans*[1,2].The structure of this protein is different to the other two canonical F_1-ATPase inhibitors of eubacteria(subunit ε) and mitochondria(inhibitor protein ,IF_1) [1,2];thus ζ represents a new control mechanism of the intrinsic rotation of the F_1F_O-ATP nanomotor. The inhibitory domain of ζ lies on the N-terminal side of the protein [2] and shows a limited similarity to the analogous N-terminal inhibitory domain of mitochondrial IF_1 [2], suggesting independent and convergent evolution of ζ and IF_1 inhibitors.The ORF of ζis exclusive ofα-proteobacteria, and the expression of ζ bound to the F_1F_O-ATP synthase has been confirmed only in *P. denitrificans* and *Rhodobactersphaeroides* [1]. Therefore, in order to analyze the expression of ζall along the α-protobacteria class, we purified the F_1 and/or F_1F_O complexes from several α-proteobacteria including free-living and symbiotic species.As shown by Coomassie staining after SDS-PAGEand/or by Western-blot, the ζ subunit was expressed in all α-protobacteria analyzed, but its expression, relative to the catalytic β subunit, varied among the different bacterial species.Furthermore, the lower the expression of ζ, the higher the F_1-ATPase and F_1F_O-ATPase activities;for instance in symbiotic*Rhizobiumetli* and *Sinorhizobiummeliloti*, as compared to the same ATPase activities of free-living *P. denitrificans*and *Rhodobactercapsulatus.* Thus, the data confirm the role of ζ as inhibitor of the F_1F_O-ATPase of α-protobacteria and show that the expression of ζ is higher in free-livingthan in symbiotic α-protoebacteria. In addition, the ζ ORF was absent in the order of *Rikettsiales*, which are obligate intracellular parasites.Therefore, the data suggest that the expression of ζ declined progressively through evolution from free-living to symbiotic α-protobacteria, until the ζ ORF became dispensable and lost in parasitic α-protobacteria, just before the endosymbiotic event that raised mitochondria. This confirms that ζ is not a predecessor of mitochondrial IF_1 but a totally novel mechanism to control rotation of the α-proteobacterial F_1-ATPase nanomotor.

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Evolutive and structural correlation of reconstructed eukaryotic ancestors of the enzyme *triosephosphate isomerase.* Mariana Schulte-Sasse Jiménez[1], Nancy O. Pulido Mayoral[1], Miguel Costas Basín[2], Enrique García Hernández[3], Adela Rodríguez Romero[3] and D. Alejandro Fernández-Velasco[1].
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The glycolytic enzyme *triosephosphate isomerase* (TIM) is an oligomeric $(beta/alpha)_8$ barrel that catalyses the interconversion of D-Glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP) in a diffusion-limited reaction. Although each subunit has its own active site, naturally occurring monomeric TIMs have notbeen reported; in fact, monomer association is very tight. TIM topology is well conserved among the three domains of life. Nevertheless, their folding mechanism and inhibition properties vary across species. Comparative studies of proteins have proved to be very useful in understanding the relationship between sequence and physicochemical properties, however, they lack the capacity to give a more integrative and evolutive correlation. In order to elucidate how the catalytic properties, the oligomerization state and the stability of extant TIMs arose, in this work we examined the molecular history of eukaryotic TIM through ancestral protein reconstruction methods and the subsequent physicochemical characterization of the enzymes. We characterized two ancestral eukaryotic TIMs, inferred by Maximum Likelihood methods, corresponding to the last common ancestor of animals and fungi (TIM63) and that of animals, fungi and plants (TIM55). Both proteins were successfully expressed and purified. Their CD and fluorescence spectra indicate the presence of stable secondary and tertiary structure in a wide range of temperatures. Dilution-dependent inactivation shows that only the dimer is active. Dilution experiments carried out in an isothermal titration calorimeter indicate that the dissociation enthalpy is small and the heat capacity change observed suggests partial unfolding upon dissociation. Although the catalytic efficiency of the ancestral proteins is reduced 10-fold compared to extant TIMs, the binding thermodynamics of the inhibitor PGH, a transition-state analogue, show a similar binding enthalpy as that observed for extant TIMs, thereby suggesting a stereochemically correct conformation of the catalytic residues in the active site. Crystals of TIM63 have been obtained and X-ray diffracted at 1.9 Å resolution. The data so far obtained suggest that although monomer association may have been less tight in ancestral TIMs, catalysis has been always linked to oligomerization. Further analysis of the structure of our ancestral protein and comparison with the structure of extant TIMs will shed light on the molecular basis of this behavior.

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Functionalizationof Multi-Walled Carbon Nanotubes with*AmaranthusLeucocarpus*Lectin

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Carbon nanotubes are new structures artificially made which present unusual physical properties. Nowadays, these features are better used in the biology field. However, these nanomaterials need to incorporate some functional groups, that's why several methodologies have been developed, these consist in modifying its superficial properties bysubmitting the nanotubes to acid medias (covalent interaction) or to hydrophobic interactions (non covalent interaction), improving in this way its compatibility in aqueous media and organic solvents. This process is known as functionalization and it can be present on the edges, the cylindricalwall or the inside of the nanoparticle. The functionalized carbon nanotubes have particular potential for carrying, releasing and delivering biologically active molecules, and for the conjugation with proteins, such as the lectins, which have at the same time a great capability to recognize carbohydrates on cell surface and thus they interact with other cells. The *Amaranthusleucocarpus*lectin (ALL) is a glycoproteinhomodimeric with a molecular weight of 35 kDA for subunit, and a sugar content of 8%. The monosaccharide analysis indicates that the glycans are type N-glycosidic with the galactose, mannose, N-acetylglucosamine; and the xylose, in the molar relation 4:3:5:1, is specific to agglutinate type ABO blood. This lectin was marked with fluoresceinisothiocyanate (FITC) and it was conjugated through the covalent method with multilayers carbon nanotubes that were previously oxidized with an acid treatment. For the analysis and characterization of conjugate, fluorescence optical microscopy was made, which facilitate the observation of lectins in the carbon nanotubes, also Raman spectroscopy, UV-Visspectroscopy and Kaiser Test were used.

Key words: carbon nanotubes, lectina, ALL, FITC, functionalization.

Disentangling the tertiary-quaternary coupling mechanism of allosteric transition of Glucosamine-6-phosphate deaminase of *Escherichia coli*.

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Many models have been postulated with the aim to understand allosteric transitions and hemoglobinhas been the most studied model for this purpose. In one hand, Monod-Wyman-Changeux model considers a conformational selection ofligand-binding between two preexistent quaternary states in equilibrium. On the other hand, the model by Koshland-Nemethy-Filmerconsiders allosteric transitionsoriginated in an induced-fit process,started by ligand binding.This model considers only tertiaryconformational changes, occurring sequentially in each hemoglobin subunit. New experimental approaches in hemoglobin researchrevealed that allosteric effects relies on tertiary structural changes and does not depend on the quaternary changes. This has been shown linked to another classic “allosteric” function: homotropic cooperativity. It was also shown that the ligand binding to the T quaternary conformer of hemoglobin (the lowest affinity one), occurs without quaternary change. The subunits undergo a tertiary transition (*t-r*) and ligands are bound by a conformational selection mechanism.
The glucosamine-6-phosphate deaminase of *Escherichia coli* (EC 5.3.99.6, EcGNPDA) is alsoan interesting model for studying allosteric mechanisms.
The catalysis of its reaction, thedeamination-isomerization of glucosamine-6-P, is cooperative and allosterically-activatedby *N*-acetyglucosamine-6-phosphate. EcGNPDA T state wasnanoencapsulated in wet porous silica to fix it in this quaternary conformation. We have demonstrated that the allosteric activation of this T-trapped enzyme is produced bya sequence of tertiary transitions. Substrate or allosteric activator binding triggers this subunit conformational change by an induced-fit mechanism. In contrast, the ligand binding to hemoglobin, occurs by a conformational selection mechanism.
We are currently using nanoencapsulation to study the mechanism of tertiary structural changes linked to allosteric activation of the R (higher affinity) quaternary conformer. Our preliminary results indicate that the allosteric activator binds trough a conformational selection process, one order of magnitude faster than binding to the T conformer.
These findings may help us to describe thermodynamically the mechanism responsible of the tertiary-quaternary coupling in the allosteric transition of the EcGNPDA.

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Structural plasticity of the LAO protein in response to the binding of different ligands

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Proteins that bind severalligands can assume a wide variety of structural conformations;thiscapacity has beendefined as plasticity of the binding proteins. The periplasmic lysine-arginine-ornithine binding protein, LAO, is areceptor for the histidine permease of *Salmonella typhimurium*.This protein is component of prokaryotic transport systems and is required for chemotaxis and quorum-sensing. LAOiscomposed by two lobes connected by a deep cleft. The bi-lobular structure is in an“open” conformation in theunliganded crystal structure, when the ligand is bound, the lobes suffer a large conformational changepresenting a“closed” conformation.It is known that LAO binds with high affinity basic amino acids ($K_d$between 1-2700 nM).In this work we used fluorescence to study the binding of no polar amino acids to LAO.We found that LAO binds alanine with low affinity (K_d=0.3 mM) demonstrating an unexpected plasticity of this protein.

Global changes in expression of microRNAs in *Caenorhabditiselegans* worms subjected to fasting.

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Feeding is a fundamental aspect in the life of any organism, important for obtaining energy for processes such as development, reproduction, ability to contend with various types of stress and longevity. Changes in feeding frequency affect the health condition of organisms in beneficial or harmful ways. In this respect, there are many reports that document the effects of fasting at the metabolic level in different experimental models, but our knowledge of the mechanisms involvedat the molecular level is still at a very early stage.

MicroRNAs are small non-coding RNA molecules of 19-24 bases in length that regulate gene expression at the post transcriptional level, participating in the control of different processes such as cell cycling, programmed cell death, differentiation, tumor development and the response to a wide variety of stresses. We chose to study the changes in expression of the microRNAs of *C. elegans* when the worm is subjected to a 12 hr fasting. The genome of *C. elegans*contains approximately 250 microRNAs, of which half of them are homologous to those found in human. We found that early L4 larvae subjected to fasting were thinner and shorter in length than well-fed animals. We also observed that fasting, followed by normal feeding, produced an increase in the worm´s lifespan.

Additionally, we performed a deep sequencing analysis of the microRNAs differentially expressed in early L4 larvae fasted for 12 hr. We were able to recognize that 14 miRNAs (*miR-34-3p*, the family of *miR-35-3p* to *miR-41-3p*, *miR-39-5p*, *miR-41-5p*, *miR-240-5p*, *miR-246-3p* and *miR-4813-5p*, and the miRNA hairpin *mir-359*) were upregulated, while 4 miRNAs (*let-7-3p* and *miR-85-5p*, and the miRNA hairpins *mir-79* and *mir-85*) were downregulated in fasted vs well-fed larvae. As *gld-1* mRNA, that codes for a regulatory protein that controls germline proliferation, has been reported to be a direct target of *miR-35-3p*, we were interested in assessing their abundance in fasted vs well-fed larvae. We found that *miR-35-3p* expression was upregulated in fasted larvae and then decreased when larvae were refed, while that of *gld-1* mRNA was downregulated under fasting conditions and, upon refeeding of the larvae for 3 hrs, its level was increased, suggesting that the observed changes in abundance of *miR-35-3p* and of *gld-1* mRNA are related to the feeding status. Our results point in the direction that some of the morphometric and functional changes brought about by fasting in *C. elegans* could be mediated by changes in the expression of a set of genes that are regulated by a group of microRNAs.*The authors wish to thank the support by PAPIIT-DGAPA-UNAM IN209310 and IN203514*,*FondosFederales Inst NalPediatría and a PhD. fellowship from CONACYT to LGS.*

Systematic identification of subtelomeric silencing pathways in *Saccharomyces cerevisiae*

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The telomere position effect (TPE) or subtelomeric silencing is a kind of negative regulation of genes encoded close to telomeres. In unicellular eukaryotes, the TPE is related to important cellular processes such as ageing, adherence, or virulence. The Sir complex plays a central role in TPE, and to date, more than 100 genes have been reported to affect Sir-mediated silencing levels in different telomeres in *Saccharomyces cerevisiae*. However, the precise function and interactions for all these genes in silencing remains largely unknown.

To characterize theTPE in yeast in a systematic and quantitative manner, we developed an experimental approach using a novel*URA3-GFP*reporter. This reporter was integrated in severalsubtelomeric loci and loss of silencing was tested in Sir complex mutants. We identified the *COS12, YFR057* and *COS8* loci as alternatives to evaluate TPE in a large-scale. The subtelomeric reporter strains were then crossed to the nonessential knock-out collection and were analyzed by flow cytometry to measure the GFP expression level. The results of these screens confirmed the participation of some reported silencing factors and revealed new genes whose activity regulates silencing levels at the subtelomeric loci studied. As expected, some hits included genes intrinsically related to DNA as are the ones of thecomplexes which modulates transcription. We will present a comprehensive catalogue of genes that modulatesubtelomeric silencing in yeast, and a possible outline of how are these genes connected to TPE at these loci.

This study represents the first high-throughput genetic approach intended to screen for silencing factors in a genome-wide scale and to describe the functional associations that determine TPE silencing in yeast.

Epigenetic deregulation of BORIS and CTCF in breast cancer

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CCCTC binding factor, also known as CTCF, has been described as a gene transcription promoting factor. In a genome–scale, this protein is able to mediate long-range chromatin interactions enabling the regulation of the expression of domain genes. In a gene-scale, CTCF is associated to an insulation function that counters propagation of methylation and repressive histone marks, specially in promoters associated to CpG islands of genes such as *BRCA1*, *ER, Rb*, *p16*, *p53*, *and miR-125b1*. For this reason, CTCF dissociation is associated to epigenetic silencing. *CTCF* has a paralog gene, *CTCF-L* or BORIS (*CTCF-Like* or Brother of the Regulator of Imprinted Sites), whose over-expression has been reported in neoplastic tissue such as breast cancer. BORIS is endogenously expressed only in testes, and its gene is epigenetically silenced in all other cells via DNA methylation. While CTCF is distributed throughout the nucleus, BORIS is localized in both nucleolus and nucleus. The main interest of our work was to determine whether BORIS and CTCF were deregulated in breast cancer. First, we analyzed the expression of both genes in three different cell lines (MCF7, MDA-MB-231 and MCF 10A) by RT-PCR. In order to evaluate the sub-cellular localization of BORIS and CTCF we performed immunofluorescent detection of both proteins in the three cell lines. As a way of establishing a correlation between cell lines and cancer/neoplastic tissue, we performed an immunohistochemical staining of BORIS and CTCF in tissue samples from breast cancer patients. Sodium bisulfite assays were carried out in order to elucidate whether the changes in DNA methylation were responsible for the over-expression of BORIS. The results suggest that BORIS is abnormally over-expressed in cancer cell lines. Data showed that BORIS was found within the nucleolus in the non-neoplastic cell line MCF 10A, whereas in MCF7 it was only found in the nucleoplasm. CTCF localization showed no difference between cell lines. In the breast cancer samples, BORIS was found in the cytoplasm while CTCF was not found in the aggressive cancer tissue. These data point out that there might be a deregulation both at protein localization and gene expression levels. We evidenced a loss of methylation in the BORIS promoter in the samples of breast cancer patients in contrast with the normal tissue samples. We also observed a reactivation of BORIS expression in the samples that exhibited a loss of DNA methylation. In conclusion, BORIS and CTCF may be deregulated in a model of breast cancer.

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The role of ADX proteins in chromatin organization

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Mutations in the human gene *hATRX* cause a syndrome that includes alpha thalassemia, profound developmental delay, mental retardation, genital abnormalities and facial dimorphism, among other manifestations (Gibbons *et al*., 1995). These mutations usually generate a change in protein functionality and mostly fall into two highly conserved domains: an helicase-ATPase domain in the carboxyl terminus, which classifies it as a member of the SNF2 family of chromatin remodeling complexes, and an ADD motif(named after the three proteins that carry it, ATRX-DNMT3-DNMT3L), composed of a PHD and a GATA-like zinc fingers,which recognize the H3K9me3 and H3K4 un-methylated combination of histone marks (Argentaro*et al*., 2007).

Xnp/dATRX, the putative ATRX homolog in *Drosophila melanogaster,* has a conserved helicase/ATPase domain but lacks the ADD domain. A bioinformatics search of the *Drosophila* genome using the human ADD sequence allowed us to identify the *CG8290* annotated gene, which encodes four ADD harboring isoforms generated by alternative splicing. We demonstrated these proteins can physically interact with Xnp/dATRX and HP1a and demonstrated through genetic interactions that they cooperate with *xnp/dATRX*in the maintenance of heterochromatin. For these reasonswe named *CG8290,adx* (“amigo de xnp”) (López-Falcón *et al.,* 2014*submitted*).

To address the roles of the four isoforms in heterochromatin, we modified the levels of the proteins in the cells of salivary glands of third instar larvae, and made polytene chromosome preparations. When we over-expressed the proteins, heterochromaticregions such as the chromocenterdisappeared, also the chromosomes became fragile and lost the over-all polytene structure, localization of Hp1a protein was also affected. The opposite happened when we dismissed the levels of the Adx proteins, chromosomes became highly compacted but we could also see that there is an apparent loss of polytenization. We are currently working on lowering the levels of each isoform individually and analyzing if there are other proteins involved in the resulting phenotype.

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Impact of Random Mutagenesis of the Transcriptional Regulator IscR of *Dickeya dadantii* Over Genetics Regulation of Iron-Sulfur Clusters Biogenesis

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Background: *Dickeya dadantii* (Syn. *Erwinia chrysanthemi*) is considered one of the ten plant pathogenic bacteria of great scientific and economic interest [1] and has within its genome the three Fe/S clusters biogenesis systems studied to date (NIF, ISC and SUF) [2]. IscR is encoded by an ORF located immediately upstream of the genes encoding Fe/S clusters assembly proteins organized in the ISC operon (*iscRSUA-hscBA-fdx*). In its dimeric form contains a Fe/S cluster within a highly conserved region endowing with its functions as transcriptional regulator allowing to sense the clusters available in the cell [3]. IscR plays a key role in the virulence of *D. dadantii* on *Arabidopsis thaliana* and maintains a homeostatic level of Fe/S cluster to adapt its metabolism to physiological conditions encountered over infection such as oxidative stress and iron starvation [4, 2]. Studies to date are based on some amino acids residues contained in IscR, notwithstanding to study waste for which there is no prior knowledge is considered the technique of directed evolution in which the main goal is generate protein modifications in a relatively short time period to simulate the natural evolution that operated on genetic variation, reproduction and selection of individual winners [5]. Hence this research will focus on building, evaluating and selecting IscR orthologous proteins impacting the genetic regulation of Fe/S clusters and virulence of *D. dadantii.*

Procedures: IscR orthologous proteins of *D. dadantii* will be generated by using directed evolution through the use of the epPCR technique (error-prone PCR) to evaluate mutations rate of 2, 3.5 and 8.1 mutations per 1000 bp which will be cloned under the arabinose promoter into a pBAD expression vector and the phenotypes are going to be evaluated under oxidative stress, iron depletion and virulence conditions in a *iscR* null mutant of *D. dadantii.* The null mutant will be generated by homologous recombination process using an *iscR* construction containing an insertion of a nonpolar kanamycin resistance cassette (*aphA*-3) and downstream-upstream recombination sites. Orthologous proteins that generated phenotypic differences to wild type will be analyzed by bioinformatics for the establishment of a mechanism of differential genetic regulation. **Results and expected:** The construction to be used for recombination of iscR null mutant was generated and characterized by the selection marker. We standardized the epPCR mutagenesis of *iscR* including 2, 3.5 and 8.1 mutations / 1000 bp. We expect to find differential phenotypes related to oxidative stress, iron starvation and virulence to subsequently characterized them by bioinformatics tools and propose a differential regulatory mechanism.

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Aim25 and its role during aging and oxidative stress in the yeast *Saccharomyces cerevisiae*

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Cellular aging is determined by a large number of genes and proteins involved in conserved pathways.
Due to its easy manipulation, the yeast *Saccharomyces cerevisiae* has been used as a model for studying mechanisms of cell aging and longevity.
In *S. cerevisiae* can be studied two types of cell life span, the chronological life span (CLS) which is defined as the time that a cell remains viable in stationary phase; and replicative life span (RLS), which refers to the number of daughter cells produced before death (Garay, E., et al., 2013).
Studies show that the *RAS2, TOR1* and *SCH9* genes are activated in response to nutrient availability, regulating growth and cell division. Deficiency in any of these genes promotes the extension of the CLS and RLS, as well as protection against oxidative and thermal stress (Wei, M., et al., 2008).

Free radicals that mediate oxidative damage to DNA, lipids and/or proteins are an important -but not the main- cause of cell aging. In addition growth conditions in low concentrations of glucose (calorie restriction) increases cellular respiration, but faster and more efficient electron transport promotes decreased production of mitochondrial reactive oxygen species (ROS). Process that has been associated with increased CLS (Barros, M.,et al., 2004).

In *S. cerevisiae*, the protein encoded by the gene *AIM25* (YJR100c) has been identified in purified mitochondria and has been involved in biogenesis, organization and mitochondrial inheritance (Hess, Myers et al. 2009).

In this work we analyze the relevance of Aim25 during cell longevity and cell response to oxidative stress.

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Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichodermaatroviride*

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RNA silencing is a process based on the recognition sequences specific for small RNA molecules to regulate gene expression and cellular processes highly conserved in plants, animals and fungi. In the latter our knowledge about this mechanism of regulation is limited. Therefore, in order to improve our understanding of the role of small RNAs in processes such as development and conidiation, we generated mutants in all components of the small RNAs synthesis machinery in the fungus *Trichodermaatroviride*.

T.atroviride is a soil saprophytic fungus that has numerous applications in biotechnology. One of these applications makes use of its antagonistic power for the biological control of plant pathogens. *Trichoderma spp*. display different antagonistic strategies, it is recognized as a mycoparasite, but it is also good competitor for space, using antibiotics as a barrier against other microorganisms. The antibiotics produced by *Trichoderma* are volatile and non-volatile compounds, most *Trichoderma* antagonism studies have focused on non-volatiles compounds. Although volatile compounds have been shown to be a source of communication in plants, in fungi little is known about their function. Therefore we wonder if a highly conserved gene regulatory machinery as that mediated by RNAi (RNA interference) modulates antagonism by *Trichoderma*.For this aim we evaluated the antagonistic capacity of a battery of mutants affected in the small RNAs synthesis machinery (*ago1, ago2, ago3, rdr1, rdr2, rdr3, dcr1, dcr2, dcr1dcr2*). The antagonistic effect of the mutants and the parental strain *T. atroviride* (IMI206040) as a control was evaluated in direct and indirect confrontations against a wide range of phytopathogenic fungi. We have found antagonistic differences in direct confrontation, antibiosis assays and in control by volatiles, in all mutants. To clarify these differences, we decided to study the metabolic profiles of volatile and non-volatile compounds in the mutants, independently and during the *T. atroviride*-pathogen interaction by gas chromatography coupled to mass spectrometry. Briefly, the Δ*dcr2* and Δ*dcr1dcr2* mutants produce high levels of important antibiosis compounds including 6 pentyl-α-pyrone, while the Δ*ago1, 2*, and *3*, as well as the Δ*rdr1, 2, 3* mutants poorly produced these metabolites or did not produce them at all, affecting the inhibitory capacity of *T. atroviride* towards certain pathogens. Additionally, we have evaluated the ability of the mutant strains to communicate with plants in dual cultures, using marker lines carrying a reporter gene under the control of promoters (*pLox2:uidA* and *pPR1:uidA*) that are induced during the plant defense response in Arabidopsis thaliana. In these experiments, the Δ*ago1, 2,* and *3*, as well as the Δ*rdr1, 2,* and *3* mutants failed to induce plant defense responses in *A. thaliana*, correlating, apparently, with their deficiency in the production of metabolites, indicating the importance of this regulation process in direct and indirect *Trichoderma*biocontrol capacity.

Global Methylation Profiling in Mexican Patients with Locally Advanced Cervical Cancer

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Abstract

Epigenetic changes are the result of several mechanisms that interact to establish alternate states of the chromatin structures. One of the most representative changes is the hypermethylation of CpG Island, associated with the inactivation of gene expression. On the other hand, DNA hypomethylation contributes to cancer growth, causing chromosomal instability, reactivation of transposable DNA and losing of the imprinting, which leads to mitotic recombination and to deletions and translocations, or to chromosomal rearrangement.

Recent studies have shown that epigenetic changes may have an important role in the development of cervical cancer. Evidence suggests that aberrant DNA methylation is directly involved in pre-cancerous stages that can rapidly progress, causing dysplasia. Epigenetic changes in neoplasic cells provide not only therapy targets, but also offer unique possibilities for early stage cancer diagnose, for response to the treatment and for disease prognosis and progression, even though the survival rate has not improved with current treatments.

In our paper, by using methlylation microarrays, the methylation profile of a group of tumoral samples was compared with dysplasia-free cervix epithelium samples. This comparation allowed us to identify the methylation deregulation, resulting in both a global hypomethylation and a local hypermethylation of the neoplasic cells genome. Our data shown genes previously reported by the scientific literature, whose promoters are hypermethylated, such as genes TIMP3, RASSF2, RASSF7, WNT9A, PAX8 and TERT, all of which are involved in carcinogenic processes.

By comparing the methylation profile of tumoral samples that shown a different response to the conventional treatment, we observed a gain in the local hypermethylation in the cluster with poor prognosis, where we can find genes that so far have not been reported in the scientific literature.

“Digital PCR: Overview and Applications”

Maribel Acosta
Lifetech Technologies

Microscopy as a tool for molecular and subcellular analysis - an overview

Ph.D. Christopher David Wood
Director of Laboratorio Nacional de Microscopía Avanzada (LNMA)
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Epifluorescence microscopy (and more recently confocal microscopy) have transformed our understanding of cell biology. These techniques have provided unprecedented insight into the distribution and dynamic interaction among almost all cellular components, however, a suite of techniques are now available that analyse the properties of the emitted light produced by the fluorophores to derive information about the local environment present in increasing smaller subcellular compartments. By analysing such properties as fluorescence lifetime, light-dark cycles, resonance energy transfer, anisotropy and fluorescence recovery, we can analyse the various properties of subcellular domains, right down to the nanometre scale. Such methods enable us to spatially map protein-protein interaction, protein complex structure and stoichiometry, cellular micro-architecture, component diffusion rates and myriad other parameters with unprecedented spatio-temporal precision. This presentation will give an overview of these methods, most of which are under development and/or available for researchers to access within the Laboratorio Nacional de Microscopía Avanzada, UNAM.

SECUENCE CAPTURE: a simple single-step enrichment method.

Dra.VereniceRamírez Rodríguez

Recent advances in sequencing technologies have contributed to the exponential increase of genomic databases, transcending in many areas of biology. In studies of genetic variation, the discovery of new mutations or the analysis of evolution at the population level, to name a few, sequencing of the entire genome is unnecessary or impracticable, reason why methods of enrichment of specific regions have become an important tool. These methods allow the efficient and relatively inexpensive sequencing of hundreds to thousands of genes or genomic regions of more samples.

Roche-NimbleGen in-solution Sequence Capture is a technology that enables researchers tocapture and efficiently sequence predefined (e.g. the whole exome) or customer-specified regions of interest from genomic DNA. Regions of 100kb to 200Mb of continuous or discontinuous sequences can be captured utilizing biotinylated probes built upon an optimized design algorithm. This algorithm allows having the biggest number (2.1 million) of probes in one single tube compared to any other commercial house.

The process for capture relies on in-solution hybridization of a pool of biotinylated oligonucleotides (length, between 50 to 105-mer) against a sample fragment library. The probes selectively hybridize to targeted regions of interest allowing their capturing and subsequent sequencing.

Roche-NimbleGen has a versatile and flexible portfolio that includes designs for human genome and any other organism genome previously sequenced.Designs to capture sequences from cDNA and a strategy to study DNA methylation are now available. Furthermore sequences captured with Roche-NimbleGen methods can be sequenced either in the platform 454-Roche or illumina.

The talk will focus on fundamentals of the design of the probes, the main applications of sequence capture method and briefly on the general features (workflow, portfolio).

Cell Mutiparameter Analysis using Fluorescence Microscopy
High-content analysis – Automated imaging and analysis of cellular assays

Sandra Rosa da Silva, Msc
Product Group Manager - Latin America
GE Healthcare Life Sciences

What's the future of your cellular research?

Cellular research traditionally involves analysis techniques such as biochemical assays, microscopy, Western blotting, and flow cytometry. While these techniques are highly valuable to the researcher they can have low throughput, involve labor-intensive processes, and destroy the cells being analyzed. This puts constraints on the number and complexity of assays that can be handled and limits the number of questions that can be asked in the research, particularly where a high number of assays are required, for example in RNAi studies. Recent advances in high-throughput automated microscopy, now commonly referred to as high- content analysis (HCA), mean researchers are using these traditional techniques in combination with newer, more informative cellular assays. The automation and throughput provided by HCA allows you to ask questions that you would not get the opportunity to ask with other techniques, and to answer them more quickly.

Why use HCA?

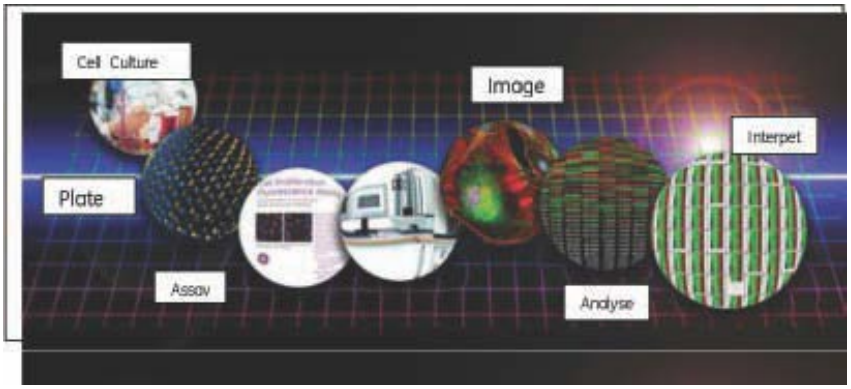
The benefits of HCA apply to a wide variety of cellular assays including cell signaling, toxicology, RNAi knockdown, cell differentiation and morphology, cell cycle, neurology, protein trafficking, and receptor activation. However, with the increased use of HCA in mainstream cellular research, the possibilities of the technology are constantly expanding. Whatever your cellular research involves, HCA can offer that extra dimension:

Increase throughput and productivity:
Assays typically take much less time than traditional techniques such as Western blotting and light microscopy enabling you to generate more data for publication.

Improve data quality:
Single cell analysis in multiple wells enables you to easily quantitate results, run multiple controls for increased data confidence, and produce statistically relevant data in a fraction of the time typically taken manually.

Investigate in breadth:
Test hypotheses rigorously by correlating results from other techniques with effects observed and measured directly in the cell.

Investigate in depth:
Automated imaging of non-destructive multiplexed live cell assays allows you to follow complex signaling events as they happen and also measure proteins, DNA, RNA, and cell morphology in a single assay.



Reporter Bioassays to Assess Therapeutic Antibodies in ADCC and Immunotherapy Programs

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Antibody-dependent cell-mediated cytotoxicity (ADCC) contributes to clinical efficacy of a broad range of therapeutic antibodies. Classic ADCC cytotoxicity assays rely on primary effector cells that are heterogeneous and highly variable. To quantitatively measure antibody activity and evaluate the impact of FcγRIIIa polymorphisms, Promega developed a pair of reporter-based ADCC assays using two engineered effector cell lines in Jurkat that stably express a NFAT-RE driven luciferase reporter and either FcγRIIIa/V158 or FcγRIIIa/F158 polymorphism variant.

In addition to biological drugs using ADCC as MoA, cancer immunotherapy was named the 2013 "Breakthrough of the Year" by *Science*. Immunotherapy aims to stimulate a patient's own immune system to treat cancer.To enable investigation of potency assays for key drugs in the market or clinical trials such as ipilimumab (Yervoy, an anti-Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) antibody), nivolumab and lambrolizumab (anti-Programmed cell Death protein 1 [PD1] antibodies), BiTE such as catumaxomab(Removab), and multiple anti inflammation drugs, Promega has developed a portfolio of bioluminescent reporter gene assays using firefly luciferases. In combination with sensitive luciferase detection reagents, these genetic reporter systems enable interrogation of important cellular responses involved in cancer, inflammation, and CNS disease. Here we demonstrate multiple bioluminescent reporter-based assays that can be used to rapidly measure potencies of multiple biological immunotherapy drugs. We also demonstrate that these bioassays reflect mode of action of each drug, and quantify potencies of on-market monoclonal antibody drugs for cancer.

Flujo de Trabajo para la Optimización de Purificación de Proteínas

La purificación de proteínas es esencial para aplicaciones que van desde la determinación estructural y caracterización bioquímica hasta la producción de anticuerpos. Un método común de purificación de proteínas recombinantes es la adición de una etiqueta de afinidad, sin embargo, esta opción no siempre es viable. La proteína de interés puede ser inestable o perder funcionalidad y los investigadores prefieren tener una proteína con pobre pureza para no involucrarse en un proceso de optimización tedioso. En estos casos, la optimización incluyen la selección de la columna o matriz, el pH óptimo, así como la búsqueda de un gradiente de elución (%B) adecuado.

Objetivo:
Demostrar que con el sistema NGC de Bio-Rad se puede automatizar y facilitar el proceso de optimización de columna, pH y %B para que la obtención de proteínas de alta pureza no etiquetadas pueda estar al alcance de cualquier investigador.

La optimización de un protocolo de purificación requiere probar diferentes condiciones de corrida y resinas. Aún cuando existen resinas diseñadas para un uso similar (en este trabajo, intercambio aniónico), pueden tener diferentes eficiencias para una proteína definida. De ahí la importancia de poder evaluar diferentes resinas/columnas de una manera sencilla y rápida.

Generalmente la evaluación de la pureza de una proteína se realiza siguiendo el rendimiento de la proteína de interés y el de las proteínas contaminantes por SDS-PAGE y la selección del mejor método de purificación se basa en:

- Alto rendimiento de la proteínas de interés
- Bajo rendimiento de las proteínas contaminantes
- Elución de la proteína de interés en un volumen bajo

En este artículo se introdujo un cuarto parámetro, el PQD (Purity Quotient Difference) aprovechando que la proteína Prancer purple absorbe a 525 nm y se puede distinguir de las demás proteínas que absorben a 280 nm. El análisis de PQD es complementario y no reemplaza al SDS-PAGE ya que el primero indica el enriquecimiento con la proteína de interés, pero no excluye la presencia de otras proteínas contaminantes.

Conclusión:
El sistema NGC facilita el proceso de optimización y automatización de procesos de purificación de manera sencilla y rápida

Detección de Proteínas con Fluorescencia: Cuantificación en Westerns y en Células

La detección y toma de imágenes con fluorescencia en el Infrarrojo Cercano (NIR) ofrece grandes beneficios en la detección cuantitativa de proteínas en Western Blots, inmunohistoquímica e incluso en células en cultivo o animales vivos. El uso de anticuerpos secundarios, ligandos o sondas específicas conjugadas con fluoróforos infrarrojos permite la cuantificación precisa y altamente reproducible de las proteínas sobre membranas u otras matrices, además de que ofrece la ventaja de detección directa, no enzimática. Este tipo de detección con fluorescencia ha sido ampliamente utilizada en la detección cuantitativa de niveles de expresión y modificaciones de proteínas, tales como la fosforilación o glicosilación y para estudios de transducción de señales, reciclaje de receptores, y prácticamente todos los aspectos de la biología celular. Además, la baja dispersión y alta penetración de tejidos de la luz infrarroja permiten la sensible detección *in vivo* de sondas específicas inyectadas en animales.
En esta presentación, se discutirán varias de estas aplicaciones y los principios por los cuales fluorescencia NIR se pueden utilizar en la cuantificación de proteínas, basado en teoría y publicaciones científicas.

Fluorescence-based Detection of Proteins: Quantification in Western Blots and in Cells

Near Infrared (NIR) fluorescence detection offers significant benefits for the accurate quantification of proteins in Western Blots and in tissue sections, as well as in cells in culture or even live animals. The use of secondary antibodies or molecular probes conjugated with NIR fluorophores allows for the precise and highly reproducible quantification of proteins on membranes or other matrices, in addition of providing direct, non-enzymatic detection. This type of detection has been successfully used in the quantitative detection of expression levels or post-translational modifications and for the study of signal transduction, receptor recycling and essentially all aspects of cellular biology. In addition, low scattering and high tissue penetration of NIR light allows for the sensitive detection of targets *in vivo*.

In this presentation, we will discuss several of these applications and the principles involved, based on theory and the scientific literature.

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Inferring microRNA functions: regulation beyond direct targets

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MicroRNAs (miRNAs) are small non-coding RNA molecules that post-transcriptionally regulate gene expression by binding to partially complementary sites in 3'UTRs [1]. They are highly abundant in most animals, with the human genome encoding around 2,000 according to the latest annotation. Collectively, they are predicted to target more than 50% of mammalian coding genes [2]. Many of them are highly conserved across bilaterian organisms, and miRNA regulation is increasingly recognized as a key player involved in a variety of biological processes, from development to disease.

Most research on miRNA function has focused on the discovery of direct targets, *i.e.* mRNAs that are directly bound by a miRNA. So far, however, no one has systematically explored genes that are indirectly regulated by miRNAs. Our current work addresses this problem, using computational methods.

We first analyzemRNA profiling experiments (microarrays or RNA-Seq) that study the effect of over-expressing a miRNA. In these experiments we expect to findthe biological processes that are affected by the miRNA. In addition to direct targets being down-regulated, other mRNAs with no miRNA binding site are also down-regulated. We call these indirect targets "co-targets". The experiments also show mRNAs that are up-regulated upon miRNA over-expression, and we call these "anti-targets".

We predict direct targets by using machine-learning algorithms to combinescores from TargetScan [3],microT-CDS [4]withan experimentally derived expression profile. We find thatadding expression information improves prediction scores and makes our resultsspecific to particular cell-types. We predict indirect targets using co-expression networks [5]. In order to find anti-targets we first cluster the whole network and localize the cluster that is enriched in direct targets for a single miRNA. We then predict that the most distant cluster from this point should be enriched in anti-targets.

In order to confirmour resultswe used RNA-Seq data from several human tissues and observe which functions are down/up regulated due to a miRNA effect.From our predictions, down-regulated targets are related tofunctionsthat would cause tissue failure without proper regulation. On the other hand,up-regulated targets (anti-targets)are related to functions conferring tissue specificity.Our results highlight the importance of studying both classes of targetsto understand the global function of microRNAs.

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Evolution of Codon Usage Bias in Bacteria

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Abstract

It is well known that the genetic code is redundant, that is, more than one codon is used to encode the same amino acid. These different codons that encode the same amino acid are called synonymous codons. The use of synonymous codons varies among different organisms and even between genes from the same organism, this differential use of synonymous codons is called codon usage bias. Bacterial organisms have been studied for some time now where it has been found that highly-expressed genes, such as ribosomal proteins, tend to have a more biased codon usage. Although the bias is varied between genomes, organisms where this bias is more pronounced tends to have shorter generation times and the number and type of tRNAs that the genome uses to encode an amino acid are more specific. In this way synonymous codons that are most used are recognized by the most abundant tRNA. This has led to the proposal that the codon usage bias in some organisms is to help enhance the efficiency of the translational process, being selective processes responsible for generating such bias. In this work we have done an in silico analysis in bacterial genomes sequenced to date to assess the importance of selective processes in shaping the codon usage bias.

Adaptive mutations typically increase the performance of metabolism independently from one another

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When organisms adapted to their environment face perturbations such as environmental stress or genetic mutation, they adapt to such unfavorable conditions at the long term with adaptive mutations. The beneficial effect of these mutations may be changed by epistasis in such a way that those genetic interactions decelerate adaptation. Previous reports showed that the adaptive effect of mutations is diminished when the beneficial allele appears in a strain with higher fitness, indicating that epistasis is mostly negative among adaptive mutations. Here, we report a systematic analysis of epistasis among loss-of-function beneficial mutations in *Saccharomyces serevisiae*'s perturbed metabolic network. We used the genome wide metabolic model and two constraint based methods, FBA and MoMA, to simulate beneficial gene-knockouts in response to perturbations in metabolism. The computational modeling of single and double beneficial mutants highlighted a very frequent appearance of independent effects of adaptive mutations even when the most common kind of significant deviations from aditivity are indeed negative epistasis. We confirmed our findings experimentally using a collection of slightly beneficial metabolic knockouts in yeast cultures perturbed with phosphate limitation. Our results show that even when negative epistasis is the most common kind of interaction among beneficial alleles, most of the pairs of slightly beneficial mutations do not interact with one another. This suggests that beneficial mutations with small effects may persist in evolution in the long term because they can pile on each other regardless of the order of appearance until they accumulate mutations to more adapted phenotypes.

Evolutionary codependence as crucial information during LAO protein redesign. Jesús Agustín Banda Vázquez*, Rogelio Rodríguez Sotres**, Karina Marisol Maya Ramírez* y Alejandro Sosa Peinado*. National Autonomous University of Mexico, UNAM. *School of Medicine and **School of Chemistry, UNAM. Facultad de Medicina, UNAM. Apdo. Postal 50-179, Ciudad Universitaria. Mexico DF. 04510. Mexico. E-mail: jabanvaz@gmail.com

INTRODUCTION:

Periplasmic binding proteins (PBP) are capable of transporting different types of ligands from the periplasm to the cytoplasm in bacteria and archea. PBPs present two conformations: an "open" state in absence of ligand, and a "closed" state bound to a ligand.[1] Also, PBPs present low sequence conservation (~20% sequence identity in some cases), but high structure similarity (RMSD ~2Å in many cases).

MODEL OF STUDY:

Salmonella typhimurium LAO protein is 238 residues protein that binds Lysine, Arginine, Ornithine, and Histidine; and transfers the ligand to a membrane transporter. Structural superposition of the four reported structures with ligand shows that residues directly involved in binding adopt the same spatial arrangement at their lateral groups (D11, Y14, F52, D30, S69, S70, S72, R77, L117, T121, D161) except D11[2].

RESULTS:

A BLAST search made using LAO sequence of *Salmonella typhimurium* as query was used to construct a phylogenetic tree using the server PhyML 3, specifying the EX-EHO model for a MAFFT alignment of 379 LAO homologous (Figure 1).

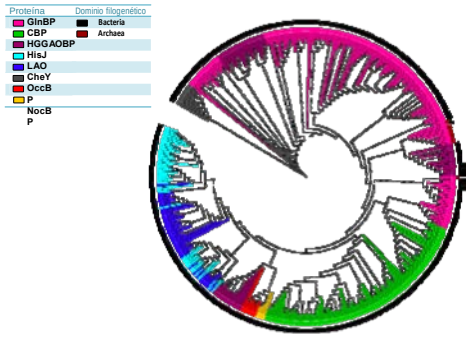
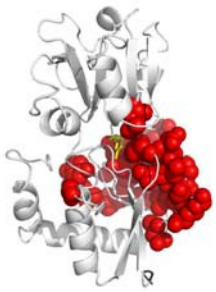


Figure 1. Phylogeny of some PBPs. Mainly, the phylogenetic separation obeys to ligand preference. Glutamine (GlnBP), Cysteine (CBP), His, Gln, Glu, Ala, Ornithine (HGGAOBP), Histidine (HisJ), Octopine

Figure 2. SCA sector 2 in LAO. According to the zone, this sector (in red spheres) seems more involved in ligand recognition. Arginine ligand is shown in yellow sticks. For illustrative purposes, other sectors are not shown.



In order to change the selectivity of any of the three groups for another ligand (Figure 1), protein redesign tools require the analysis of the differences between sets of homologous with different specificity. However sequence searching for LAO, Glutamine and Cysteine groups using BLAST or jack-hmmer tools retrieved too much overlapping hits. To avoid the sequence overlap, we used the Rd.HMM approach, which uses pdb coordinates instead of just sequence as starting point. The idea is to use ROSETTA, which is a program with notable robustness for the assignment of amino acids to a known three-dimensional structure. The resulting sequences are used to build Hidden Markov models, to search the sequence databases, such as UniRef90 database.

Preliminary statistical coupling analysis (SCA)[3] draws three sectors (Figure 2); and positionally, the red one seems to be involved in ligand recognition. Posterior analysis will be used to elucidate the best strategies to generate LAO redesigns changing this portion of the protein to alter ligand preference.

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Core and pan-genome analysis of the genus *Streptococcus*

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The progress in sequencing technologies and the genome sequencing projects have provided enormous amounts of information, such that, there are up to 7,411 completely sequenced bacterial genomes. Genome comparison of related bacteria strains may aid us to make insights into taxonomic problems, such as the bacterial species definition or studying the bacteria functional roles within complex environments. Comparative genomics of bacteria and its largely within group variations of gene content has aroused the pan-genome concept, the universe of genes within a strain. The pan-genome can be divided into a core genome, containing all the common shared features and the accessory genome, where strain specific genes can be found (Tettelin *et al*, 2008).

In this work, we compared and analyzed 108 genomes and its predicted proteomes of the *Streptoccus* genus to get both pan-genome and its core genome. The streptococci comprehends a large number of important human and animal pathogens, as well as some commensal species. To get the core genome we made a search of the shared orthologs by pairs of genomes following the Bidirectional Best Blast Hit strategy, for the pan-genome the 108 proteomes were concatenated and the proteins were clustered into families with the CD-HIT algorithm. The pan-genome is constituted by a large number of 33,039 protein families, comprising 212,348 redundant genes present in the pan-genome. The genus core genome is composed by only 404 proteins which is astoundingly low number of shared common features across the genus. We did a functional analysis of the proteins in both core and pan-genome by means of hierarchical classifications of its gene functions . This analysis revealed the conserved genes among the streptococci are mostly involved in cell division, translation, transduction and cell wall production; meanwhile in the pan-genome, we found that gene functions like defense mechanisms are variable among species and appear underrepresented in the core genome, showing the variability among species. Finally, we used all the pairwise shared orthologs to build a Genomic Similarity Score (GSS) matrix. The GSS was graphed as a Neighbor-Joining tree and compared to a classic 16S rRNA gene phylogeny, where GSS shows promising results to discriminate amongst closely related strains.

MOLECULAR FLEXIBILITY OF A FAMILY OF PERIPLAMIC BINDING PROTEINS FOR BASIC AMINOACIDS: LAO-binding protein. Tania Raquel Berrocal Gama, Andrés Escandón Flores, Alejandro Sosa Peinado.National Autonomous University of Mexico, Depto de Bioquimica. Facultad de Medicina, UNAM. Apdo. Post 50-179, Ciudad Universitaria. Mexico DF. 04510. México email.taniarbg020589@gmail.com.

INTRODUCTION

Permease complexes transport a variety of molecules (amino acids, peptides, sugar, vitamins, ions, etc) from the periplasmic space to the cell in bacteria and archea. The permease system is composed by a protein-membrane complex and periplasmic binding proteins (PBP´s). PBP´s perform two main functions: substrate binding and membrane complex recognition. PBPs share a bilobulated structure with α and β motifs. Our model system is the L-Lysine, L-Arginine, L-Ornithine binding protein (LAO) which contains 231 amino acids and it binds arginine, lysine and ornithine with nanomolar affinity. This protein presents two structures given that the protein change conformation upon ligand binding: the open structure in absence of ligand and a closed structure in presence of ligand.

The role of molecular flexibility in the ligand binding and protein stability is not perfectly understood. Our first approach to this problem was the use of computational strategy to predict the protein flexibility. The Constraint Network Analysis (CNAnalysis) which is a graph theory-based rigidity analysis approach that allows to analyze the global and local flexibility and rigidity characteristics from the structure of a protein. Also, this analysis applied different level of energy to the system. Which permit to analyze the molecular flexibility at different temperatures. In another word is a simulation of protein denaturation of a protein according to the Fig 1.

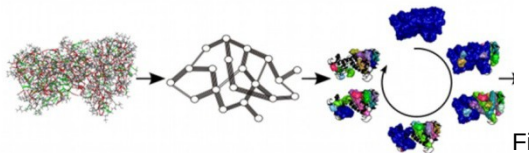

Fig 1.Analyis of rigidity/flexibility at different temperatures.

CONCLUSION

The CNA analysis was carried out for a family of periplasmic binding proteins that bind basic amino acid. This family includes the mesophilic and thermophilic version. The first conclusion is that residues which may start the unfolding process of the protein (unfolding nuclei) are similar but not identical in all proteins. This nucleus involves residues in the discontinuous lobule. Is interesting to note that β-sheets are predominant in this region.

Several pathways of unfolding process are suggested, that present variations depending on absence or presence of ligand. Also was observed that thermophilic proteins are more rigid that mesophilic proteins. A general mechanism of unfolding is discussed in this work

Evaluation of parameters and adequate sampling to measure sea urchin sperm motility

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Sea urchin sperm are model cells for studying signal transduction events underlying flagellar motility. However, evaluation of motility in the presence of modulating drugs for components of the signaling pathway has been left aside. Although simple parameters have been evaluated for different species of sperm, there is no consensus on how to estimate them and neither the importance of the observation interval has been considered. We explored different parameters of sea urchin sperm movement that allow the assessment in a large number of cells in 2D, without a complicated experimental set. Eight videos were acquired at
1000 frames per second (fps) and analogically subsampled for different temporal resolutions (from 20 to 1000 fps). Swimming trajectories (130 sperm) were followed by sperm head tracking, using an algorithm based on particle tracking. High temporal resolution revealed that sperm trajectory has two components: a sinusoidal function (a zigzag line) wrapped by a quasi-circular function, which we verified by a theoretical study. The zigzag component, observed only at temporal resolutions > 70 fps, correspond to the flagellum beating; then, it is possible to measure effects on flagellar motility just by following the sperm head. We report the analysis of the different parameters of sea urchin sperm motility observed in
2D, emphasizing adequate sampling for the analysis of the trajectory.

This work was supported by CINVESTAV and CONACyT grant No. 82831 to BEG.

High-resolution genome-wide aging screens reveal novel molecular mechanisms of lifespan extension by dietary restriction

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The study of chronological lifespan (CLS) in *Saccharomyces cerevisiae* has provided important insights into the genetic regulation of lifespan in organisms ranging from yeasts to mammals. In recent years, the identification of new lifespan factors has been accelerated with the aid of high-throughput phenotyping and genotyping technologies. Here, taking advantage of a new genome-wide screening methodology developed by our group, we characterized the CLS of ~4,000 single knockout mutants in nutrient-rich and dietary-restricted (DR) growth media. We found that over 500 gene knockouts have deleterious or beneficial effects on yeast CLS in at least one of the tested conditions. Furthermore, we carried out statistical tests to evaluate the differential effects that the conditions tested had in the CLS of each gene knockout. These analyses allowed us to pinpoint two specific groups of genes, namely positive and negative regulators of DR response. Noteworthy, DR treatment buffers both the beneficial and deleterious effects that some gene knockouts exhibit when aged in rich medium. Autophagic and mitochondrial activities are among the positive regulators of DR response, confirming their role in lifespan regulation through nutrient signaling. Interestingly, we found that FAR genes, required for cell-cycle arrest in response to pheromone, are important positive regulators of DR. This result indicates that cell-cycle arrest is necessary for longevity enhancement in yeast when facing a low nutrient environment. Our study contributes to the identification of gene-lifespan regulators through environmental interactions and sheds light on the mechanisms of lifespan extension by dietary restriction.

Evolutionary and physiological correlation of redundant gene dosage within the *Debaryomyces hansenii* genome, a preliminary approach.

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The long-term purposeof this project is to determine whether there is a correlation between the redundant gene dosage in the extremophilic yeast *Debaryomyceshansenii*and the proteins involved in different physiological roles including tolerance to extreme conditions such ashigh osmolarity.
The genome sequence and gene annotation of *D. hansenii* strain CBS767 are freely available on page Genolevures Consortium (http://genolevures.org/). Studies of this consortium have suggested that this genome stands out for its increased redundancy compared to other yeasts genomes. Previously in other organisms it has been reported that many components of essential metabolic pathways often have functionally redundant copies; sowe expect that certain coding genesof *D.hansenii* involved in chemo stress tolerancecould have multiple copies.

In order to determine the association among the redundant genes of *D. hansenii* with its adaptive phenotype against extreme conditions, we generated a database that includes theBlastpcomparisonsof all proteins of *D. hanseniivs.*themselves. The output was refined to specify only the groupsof proteins that fulfill the conditions of≥ 70% coverage and ≥30%identity. Numerous candidate proteins were detected and further associated tothe KOG database (clusters of orthologous groups of Eukaryotes).The KOG classification assigns functional annotationsenabling the analysisof the distribution of redundant proteins through all the functional groups.

Subsequently, we carried out a second analysis that revealed the number of repeats of each redundant gene, with the aim of finding out any correlation between gene reiteration andthe proteins involved in various physiologicaladaptive responses.Analysis of the results, will allow the identification of proteins that might be involved in the extremophile lifestyle of *D.hansenii.*The relevance of these new candidates in stress tolerance must be confirmed byfunctional genomics.

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Microbial evolution: a systems biology approach

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Microbes, collectively speaking archaea, bacteria and unicellular eukaryotes are the most ancient and diverse organisms on Earth. They have been the only inhabitants for at least the first ¾ parts of life history.However, in comparison with plants and animals, relatively little is known regarding the evolutionary process that led to their huge diversity.

On the other side, the overflow of DNA molecular sequences, paired with the development of powerful computational techniques, is revolutionizing biological sciences. Here we propose the use of methods derived from the systems biology discipline to study the evolution of microbial symbiosis.

In particular, we show how an *in silico* analysis of the amino acid metabolism of the symbiotic bacteria *Chlorobium chlorochromatii*CaD3 led to the proposal of an alternative nitrogen assimilation pathway *via*alanine dehydrogenase.*C. chlorochromatii* CaD3 is a photolithoautotrophic green sulfur bacteria that lives in symbiotic association with the heterotrophic Beta proteobacteria "*Candidatus* Symbiobacter mobilis". Together conform the phototrophic consortium known as "*Chlorochromatium aggregatum*". We also show how our analysis ledto a hypothesis about the origin of this symbiosis.

In addition, we also present ongoing research on the evolution of the chromatophore of *Paulinella chromatophora*. The chromatophore is a plastid-like structure that originated from a free-living cyanobacterium*via* primary endosymbiosis with a unicellular protist from the phylum cercozoa.This symbiosis led to *P. chromatophora*, an obligate autotrophic protist.Notably, this symbiosis is independent and more recent than the symbiotic event that led to plastids from plants. Herewe propose two *in silico* evolution experiments to: *i*)identify the nutritional basis of the symbiosis; and *ii*) to assess the relative importance of natural selection versus genetic drift on the evolution chromatophore metabolism.

Dendroctonus rhizophagus Thomas Bright cytochrome *CYP6DG1V1* structure prediction and interaction analysis with α-pinene

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Bark beetles [genus *Dendroctonus* (Curculionidae:Scolytinae)] perform an important ecological cleaning role in softwood forests since they colonize damaged, weakened and/or old trees facilitating forest renewal. However, if forest undergoes imbalance: bark beetles become a pest causing significant economic losses to the forestry sector as well as irreversible ecological damages, for example, *Dendroctonus rhizophagus* is a species that colonizes young or small pines (<3 m in height, 11 species) throughout their distribution area but, for successful beetle colonization of trees, their survival and reproduction needs to detoxicate diterpenes, monoterpenes and complex sesquiterpenes present in the resin of host tree. Multigene enzyme families such as cytochromes P450 (CYP), esterases and glutathione-S-transferases among others metabolize such terpenes. Due to CYPs are the majority of proteins that metabolize small molecules elsewhere, their CYP genes have been induce to increase the protein expression of the families 4, 6 and 9 using insects which have been stimulated in the laboratory with different monoterpenes present in the resin of their host trees as well as after being exposed to the resin of the pine trees during the early stages of colonization. In particular, the *CYP6DG1V1* gene presents a significant over-expression with monoterpenes α-pinene, *S*-(-)-α-pinene, *R*-(-)-α-pinene, *S*-(-)-β-pinene and 3-carene, suggesting that this enzyme is involved in these monoterpene biotransformation. Therefore, the aim of this investigation was to generate a tridimensional (3D) structural homology model of *CYP6DG1V1* by using the SWISSMODEL server. We assessed its stereochemistry and structural quality with the ERRAT and SAVE servers. Then, the heme group was coupled to the globin moiety by using Autodock 2.6; then, the model obtained was structurally refined by molecular dynamics (MD) simulations of 500ns at 310 K, CHARMM 27. In addition, the *CY6DG1V1* and α- pinene iterations were analyzed by PDBsum server and suggested possible conformations of the enzyme-substrate complex. This model will be very useful to understand the bark beetle metabolism of monoterpenes by *CYP6DG1V1*, its role in the detoxification processes and relevance in the host-parasite relationship. These tools will be useful for designing new bio specific chemical to control these organisms. (We thank: Cluster Híbrido de Supercómputo - C H S C CGSTIC - Coordinación General de Sistemas de Tecnologías de la Información y las Comunicaciones for providing us access to installations and for its logistic support. SSA/IMSS/CONACyT grant 162391; Postdoctoral research CONACyT grant to M.F.L.G 175839).

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Improving sequence capture design for genetically modified organisms identification by next generation sequencing

Roberto Galindo Ramírez, Salvador Ángel Romero Martínez
y Abraham Itzcóatl Acatzi Silva

In recent years Mexico's government has been approving experimental release of Genetically Modified Organisms (GMO) plantations. Because of that it is necessary that Mexico can verify and monitorGMO crops in order to regulate the activities from enterprises that have legal rights to release their products. Technics such as PCR have been widely used for detection, identification and quantification of GMO events; nevertheless they are gettinginsufficient compared to the knowledge and rapid research in GMO's field. Furthermore, there is a lack of detection power when there are stacked events that arise from traditional mating improvement from both, farmers and enterprises. For those reasons it is needed a high throughputtool to determine whether an organism has genetic modifications due to biotechnology. Sequence capture is a technic that allows picking specific molecules of DNA that have a particular sequence. One of the most used systems to capture sequences is the one made by RNA probes such as SureSelect system developed by Agilent Technologies. This system has been extensively used in clinical research in complementation with next generation sequencing (NGS). Furthermore,ServicioNacional de Sanidad, Inocuidad y CalidadAgroalimentaria (Senasica) proved this technique for GMO detection previously. Our groupdeveloped aprobe design usingSureSelect in order to enrich sequences with known transgenic elements and events from matrix samples of crops, and then those sequences were sequenced by Roche 454sequencing. Additionally it was developed a bioinformatics pipeline, which maps reads to references, assembles and return statistics about the probability of having each event per sample.This work builds upon that previouscapture design,now improved byusing a refinement of the information that had been compiled for the previous design. Database with GMO's events was enriched using sequences from several sources included GMDD, CERA and Patent Lents databases, Bats-Center report and private information given to Senasica. Afterwards it was designed a pipeline to reduce redundancy in the new database, this was based on a BLAT implementation between sequences within the database. Sequences in the database served as input for the Agilent Genome Workbench software (AGWB), this program generates the theoretical probes for RNA sequences that capture DNA sequences of interest. The improvements in this new designalso included increasing the number of sequences of events of GMO events that were used as input for the AGWB and decreasing redundancy between the probe that were obtained as output of the AGWB.With this new design Senasica is able to detect and identify a wide range of GMO events, this is a significant step for the regulation of GMO's in Mexico.

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BAC-end sequencing of a BAC-based genomic library of *Saccharomyces pastorianus* for genome sequence assembly improvement

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The next-generation sequencing technologies (NGS) allowed the generation of the genome sequence of a lager beer yeast strain (*Saccharomyces pastorianus*) relatively quickly and at a reduced cost. However, the short read size of these technologies hinders the genome sequence assembly process resulting in a fragmented genome draft. One option to improve the draft sequence is by incorporating information from alternative sources. To this end, we intend to obtain the BAC-end sequences (BES) of a BAC -based genomic library of *S. pastorianus* in order to compare the result with the existing genome sequence assembly, identify conflict regions for further analysis and ultimately improve the assembly by scaffolding.

This project is funded by Cervecería Cuauhtémoc Moctezuma, S.A. de C.V.

Evolution of global gene expression from fermentative to glycerol-based respiratory growth in *Escherichia coli* and *Schizosaccharomycespombe*: A comparative transcriptome analysis using RNA-seq.

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Summary

The Next Generation Sequencing has determined a new era in biological sciences, especially in genomic researches. Currently, RNA-seq has increased the knowledge about the RNA world through a deeper transcriptome characterization and gene differential expression studies, as well as with the continuous discovery of non-coding regulatory transcripts like the small RNAs (sRNAs). The sRNAs are important elements of gene regulatory networks and participate actively in some metabolic responses (e.g., the central carbon metabolism) in eukaryotic and prokaryotic organisms. The aim of the present project is to infer how the gene regulatory circuits have been evolved in two model organisms, *Escherichia coli* and *Schizosaccharomycespombe*, in responses to grown-up infermentable (glucose) and non-fermentable carbon source (glycerol/acetate) and if they are partially regulated by sRNAs. The total RNA and an enriched fraction of sRNAshas been isolated from both organisms growing in the same culture media (Yeast extract) supplemented with its correspond carbon source and are beingprocessed for RNA-seq. The obtained data will be analyzed by different bioinformatics tools and systems biology approaches. The acquired results will allow us to infer underlying common features in regulatory networks in two different domains of life (Eubacteria and Eukaryota) and devise new hypotheses about the cluster of genes differentially expressed in response toa change from fermentative to respiratory metabolism. Finally, we also wantto understand whether there are similarities in the regulation of orthologous genes subject to control by sRNAs in both organisms.

ABSTRACT SUBMISSION

Study *In silico*of protein-protein interaction of T-type voltage-gated Ca^{2+} channels and auxiliary subunits.Teresa Hernández-Segura[1], Nidia-Beltrán[1], Jacaranda Rosendo-Pineda[1]& Heriberto Manuel Rivera[1]*.

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Voltage-activated calcium channels (CaV) can be divided into two subgroups low-voltage-activated (LVA) and high-voltage-activated (HVA). Low-voltage gated (T-type) calcium channels (CaV3) have been shown to modulate important cellular functions like cell excitability, secretion, contraction, growth, differentiation, proliferation and fertilization. They have been implicated to pathophysiological conditions such cancer, epilepsy, neuropathic pain development, cardiac hypertrophy, etc. The physiological role of CaV3 is determined by its biophysical properties and the interaction with regulatory proteins and other cellular components. A few studies have suggested that the β and α2δ subunits might modulate CaV3, whereas other studies found that β subunits do not regulate CaV3 current density and expression. However, an understanding of the structure and function of the CaV3 is limited by the absence of structural models and potent antagonists that inhibit CaV3 currents with high specificity. The study of protein-protein interactions is crucial for understanding the physiological, pathological mechanisms based on information on their structure and function. In this study, we investigated the interaction between CaVα and CaVβ. We have obtained structural models of CaV2.1 and CaV3.3 of human by homology modeling.We confirm the interaction sites of CaV2.1 and β subunit, AID (the alpha-interaction domain) and BID (the beta-interaction domain) domains as control; and we described the interaction between CaV3.3 and β1 subunit. Interaction sites between CaVs and auxiliary subunits will help us to understand of the molecular basis of functional effects of the CaVs in the disease mechanism where are involved.

Phylogenetic analysis of Zinc-dependent alcohol dehydrogenases and aldehyde dehydrogenases in animals

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In vertebrates, ethanol metabolism is performed through the concerted action of two enzymes: a Zn-dependent alcohol dehydrogenase (Zn-ADH) that oxidizes ethanol to acetaldehyde, and aldehyde dehydrogenase (ALDH) that oxidizes acetaldehyde to acetate. The presence of these different ADHs in animals has been assumed to be consequence of chronic exposure to ethanol. By far the most common natural source of ethanol is fermentation of fruit sugars by yeast, and available data support that this fruit trait evolved in concert with the characteristics of their frugivorous seed dispersers. Therefore, if the presence of ADHs in animals evolved as an adaptive response to dietary ethanol exposure, then it can be expected that the enzymogenesis of these enzymes began after the appearance of angiosperms with fleshy fruits, because substrate availability must precede enzyme selection. Furthermore, because several whole genome duplications occurred through animals evolution, the identification of orthologous groups of proteins is not convincingly resolved for ADHs and ALDHs in animals. Thus, a phylogenetic analysis of these enzymes was performed to obtain insights about the enzymogenesis of this group of proteins.

Zn-ADH and ALDH amino acid sequences were retrieved by Blast searches at the NCBI site (http://blast.ncbi.nlm.nih.gov/Blast.cgi): 695 non-redundant Zn-ADH, and 2113 ALDH protein sequence were identified in animals. Progressive multiple amino acid sequence alignments were performed with ClustalX version 2 (http://www.clustal.org/clustal2/) using as a guide a structural alignment constructed with the VAST algorithm that included all non-redundant Zn-ADH or ALDH protein structures deposited in the PDB. Phylogenetic analyses were conducted using the MEGA6 software (http://www.megasoftware.net).

Results of phylogenetic analyses, shows that class III ADH give rise in first place to the class I ADH, which is present in all animals. Later, class II and VII emerged by duplication of class III ADH before the amphibian-amniota split, but interestingly, class VII ADH was lost in mammals. Finally, classes IV to VI have a more recent origin, because the duplication that gave rise to these three classes occurred just after the appearance of mammals. Class VIII ADH is restricted only to amphibian lineage, and possesses divergent characteristics in comparison to the rest of ADH classes and appeared after the amphibian-amniota split. Thus, all different Zn-ADHs (seven classes) in animals appeared before angiosperms and land plants, independently of ethanol availability. With respect to aldehyde dehydrogenases (ALDH), phylogenetic analyses showed 12 different ALDH families in animals, and all they appeared also before land plants. Because these enzymes are not induced by ethanol and all they possess a high activity with non-ethanol endogenous substrates. Thus, it can be concluded that Zn-ADH and ALDH participation in ethanol metabolism can be considered as incidental, and not adaptive.

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Characterization of pathogenic organisms using next generation sequencing and bioinformatic

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The globalization of markets has accelerated he food trade, as a result governments require the implementation of strategies that contribute to the assurance of food safety. Food borne diseases represent a significant impact on health human, health animal, vegetable and economic sector. **SENASICA** is responsible to ensure food safety and fort his its implementing state of the art molecular biology methods such as next generation sequencing (NGS). NGS allow stoget sequences of complete genomes in short time coupled to appropriate bioinformatics tools that can assess, process and select the information necessary to characterize epathogenic organisms and to generate enough information that allows to build genomic data bases wich are of high importance be cause they facilitate the production of biological meaning from DNA information. For these reasons **SENASICA** has sequenced forty genomes of *Salmonella enterica,* all this in order to find differences between Mexican strains and the foreign strains, all this information is being stored in a data base wich will be used for later epidemiological analysis.Briefly ,the bioinformatics process for the characterization of the sequenced strains began with the selection of reference sequeces for the*rrs*gene from the NCBI and RDP databases, then these sequences we reused as references for mapping and alignment analysis, then we get a consensus from the previously mapped reads which is compared in databases such as the ribosomal data project database(www.rdp.org). Afterwards a Local Multi Locus Sequence Typing (MLST) search is done using BLAST, as implemented in CLC Genomics Workbench 6.5 (CLCGWB), against a local database (based up on the MLST database,www.mlst.org) using as queries the consensus sequences obtained from genome wide read mapping processes, where the regions of the commonly used house-keeping genes were previously identified in the reference genomes to extract the consensus sequences. The allele ID of the best Identity Score in the BLAST MLST search was selected. The consensus sequences for each allele we reused to build concatenated alignments using both CLCGWB to build Neighbour Joining Trees and Bionumerix v7.1 to build Minimum spanning tree. As a complementary tool we are developing the implementation of a bioinformatics tool called Phylomark so that we can exploit the whole genome information obtained in our sequencing as says to develop DNA markers addition alto the consens us markers used traditionally and to obtain better detection assays for regional Salmonella strains. The strains sequenced we found differences we will analyses significantly. The power of next generation sequencing where coupled with appropriate bioinformatics tools we can obtain phylogenies of the different studies which have its own advantages disadvantages these results depen do n information which are present in databases. We found differences between the data generated and the data wich are in database as NCBI, RDP, MLST, we lack evaluate whether these differences have a meaning who allow usindicating which are Mexican strains.

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Study of reductive genomic evolution in prokaryotes

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Intracellular organisms, driven by the symbioses with their hosts, have lost part of their genetic content giving rise to some of the smallest genomes known. During the reductive process, the prokaryotes and their genomes develop a series of characteristics that in the sum conform what we know as the syndrome of genomic reduction. In particular, mutualistic endosymbiotic prokaryotes in extreme stages of reduction contain genomes that challenge our understanding of required cellular functions and genes, fading the boundaries of concepts like organisms and organelle and providing us with a unique opportunity to study life of “simplified” organisms.

In this work we are interested in study the process of reductive genomic evolution in symbiotic prokaryotes. In particular we will identify the genes that tend to be conserved in these microorganismsand compare them to the genes known to be essential in model organisms as well as those that are believed to be present in the last universal common ancestor. This will contribute to the understanding of the mechanisms of their conservation. The study will include all organisms with less than 1,300 genes, which is an empirical limit that separates obligated symbionts from facultative ones.

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Genome assembly of *Burkholderia* sp. and analysis of the functional annotation of genes involved in the degradation of organophosphorus compounds

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Structural genomics has been used as a tool to identify genes involved in several biological processes, for example, the degradation of xenobiotic compounds such as pesticides. Methyl parathion (MP) is an organophosphate pesticide which irreversibly inhibits acetylcholinesterase, a key enzyme in the central nervous system of insects and non-target organisms, including mammals. Hydrolysis of MP generates two intermediate compounds: dimethylthiophosphoric acid and *p*-nitrophenol (PNP). PNP is a toxic environmental pollutant and is among the most common nitrophenolic compounds can be used for manufacturing explosives, drugs, pharmaceuticals, dyes and plasticizers. Biodegradation of PNP proceeds via two distinct pathways: the hydroquinone (HQ) pathway in Gram- negative bacteria and the benzenetriol (BT) pathway in Gram-positive bacteria. However, Vikram *et al*. (2013) reported a gene cluster in the genome of *Burkholderia* sp. SJ98 involved in both pathways of degradation of PNP. In 2012, Popoca-Ursino reported three bacterial strains of the genus *Burkholderia* that were isolated from agricultural soils of the state of Morelos, Mexico, which hydrolyze MP and use it as a carbon source. *Burkholderia* sp. strain S4-3 completely degraded to PNP in 15 hours; while the *Burkholderia* sp. strains S5-1 and S5-2 hydrolyze MP more efficiently than the strain S4-3, and also completely degraded to PNP in 21 hours. These results are relevant because there are few reports of microorganisms capable of simultaneously hydrolyze MP and degrade PNP. This work aims to identify genes involved in the degradation of PNP by the structural analysis of the genome of *Burkholderia* sp. strain S4-3 and to propose if it uses one of the two PNP degradation pathways reported (HQ or BT), or it uses both PNP degradation pathways simultaneously or even if it has an alternative degradation pathway.

In this study, we reported the draft genome of *Burkholderia* sp. strain S4-3 wich consists of 7.6 Mb. In total, 507 contigs were produced in 169 scaffolds through *de novo* assembly. Phylogenetic analyses showed that *Burkholderia* sp. strain S4-3 clusters with *Burkholderia zhejiangensis* species. We have detected and mapped a methyl parathion degrading (*mpd*) gene and two PNP catabolic gene clusters (*pnpABA'E2E1FDC* and *pnpE2E1FDC*) in the genome of *Burkholderia* sp. strain S4-3 using the Blast tool. This included the detection of open reading frames (ORFs) 5 Kb up- and downstream to catabolic genes. Based on these genetic evidences we propose that PNP degradation in *Burkholderia* sp. strain S4-3 proceed via both BT and HQ branches of catabolic pathway.

Metagenomic analysis of root-associated bacteria in bean plants

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Land plants associate with a specific root microbiota that is selected from the complex microbial diversity present in the surrounding soil [1]. Colonization of the root occurs in spite of the plant immune system, suggesting a finely tuned discrimination mechanism, segregating mutualists and commensals from pathogens [2]. The microbiota present in the rhizosphere (soil immediately surrounding the root) and the endophytic compartment (within the root) contribute to plant growth, productivity, carbon sequestration and phytoremediation [3]. Nevertheless, the role of bacterial endophytes and soil microbial communities remains largely unexplored, mainly due to difficulties in growing most prokaryotes in the lab [4] Nowadays, with current high-throughput sequencing technologies, metagenomic approaches involving the direct extraction of DNA from soil and roots can greatly improve our understanding of these communities [5].

In this work we will perform high-throughput sequencing of 16S rDNA amplicons as well as total bacterial DNA extracted from three different growth stages (first trifoliated leaf, flowering and pod filling) of *Phaseolus vulgaris* L. Using the 16S results, we will investigate the composition of the rhizospheric and endophytic bacterial communities associated with bean roots. With this, we will assess how bacterial communities inside and outside the roots change according to the growth stage of the plants. In addition to studying changes in composition throughout the bean plant lifetime, we will select the growth stage at which the endophytes are most different from the soil communities to perform sequencing of total bacterial DNA, in order to identify the full list of genes encoded by the bacterial community. With this, we aim to find out if the bean plant discriminates bacteria from the rhizosphere compartment using taxonomical or functional criteria.

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A dynamical model of the regulatory network that controls terminal differentiation of B lymphocytes.

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Adaptive immune response in vertebrates depends on the rapid maturation and differentiation of B lymphocytes which are responsible for the humoral response of the organism by the production of high-affinity specific antibodies. B cells develop in the bone marrow from hematopoietic progenitors and migrate as mature B cells (Naive) to highly specialized environments of the secondary lymphoid organs, the germinal centers (GC), where they undergo diversification of the B cell antigen receptor genes (BCR). After the encounter with antigen, Naive and GC cells become activated and differentiate into antibody-producing plasma cells (PC) and memory cells (Mem), which are able to respond to foreign agents. Terminal differentiation of B cells is controlled by the concerted action of transcription factors that responds to multiple physiologic signals thus creating a complex regulatory network. It is not completely understood how this network ensure cell differentiation from a precursor Naive B cell to GC cells, and effector Mem or PC cells. Moreover, there is not a general consensus about the architecture and dynamical properties of the regulatory network and the way it integrates multiple external signals that direct the differentiation process.

In this work we aim to study from a computational biology perspective, the dynamical behavior of the regulatory network that controls the terminal differentiation of B cells. First, we inferred the network architecture by integrating published data referring to the key molecular regulators of the differentiation process into a single computational model. We then constructed two dynamical systems, one discrete and one continuous, to analyze the dynamical properties of the inferred regulatory network. Specifically, we found the stationary states of the models and compared them against the known stable molecular patterns observed in Naive, GC, Mem, and PC cells, under wild type and mutant background. We show that both, the discrete and the continuous models, are able to qualitatively describe the cellular differentiation pattern from a precursor Naive and GC cell fates to terminally differentiated Mem or PC under a variety of external signals. Also there are differences between the discrete and continuous models in terms of the effect of strength and duration of the external signals over the dynamical behavior of the regulatory network. Finally, we propose four interactions among the regulatory factors PAX5, BCL6 and IRF4 in order for the network to attain the four stable expression patterns characteristic of each B cell type.

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The Regulatory Network of Lymphopoiesis in Mammals

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The hematopoietic system is one of the most extensively studied systems in mammals. As a result, there is a large body of experimental data regarding the pattern of differentiation of the cell lines that belong to this system, as well as a set of molecules known to intervene during this process. Due to the large number of diseases associated to a malfunction of the hematopoietic system, there is an interest in knowing the molecular and cellular mechanisms controlling the differentiation process of blood cells.

The creation of models in the form of regulatory networks has become a *de facto* standard to understand the molecular mechanism controlling the generation and differentiation of blood cells. I present in this work a regulatory network containing 81 nodes, representing several types of molecules, that regulate each other during the process of lymphopoiesis. Such regulatory interactions were inferred mostly from published experimental data, while a handful of interactions are predictions arising from the present study. The network is modeled as a continuous dynamical system, in the form of a coupled set of differential equations. The dynamical behavior of the model describes the differentiation process from the common lymphocyte precursor (CLP) to several mature B and T cell types; namely, PC, CTL, Th1, Th2, Th17, and Treg cells.

The network model recovers the pattern of differentiation in lymphocytes, describes a large set of gain- and loss-of-function mutants, provides a unified framework to interpret the role of intra- and extracellular signals during lymphopoiesis, and joins the molecular pathways described for T and B cell differentiation. Finally, the model predicts some missing regulatory interactions and constitutes the largest regulatory network to date modeled as a dynamical system to describe any differentiation process in mammals.

Meta-analysis of DNA microarrays for generate a progression model of melanoma

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Introduction. Cancer research, at this time, focuses on early detection strategies, better and less aggressive treatments and assessment of recurrence risk. In this work, we focus on the study of melanoma cancer, the most lethal type of skin cancer because of its resistance and metastatic ability [1]. Moreover, in the last 30 years, the incidence of this cancer increased by 400 % worldwide [2, 3]. This research focus on to identify a specific set of genes useful as biomarkers to susceptibility for develop metastasis. Also we will identify those genes that define a model of progression through the different tumor stages. We propose to re-analyze microarray datasets to extract new information about the melanoma.

Methods. We use a total of 89 microarray file from 11 dataset stored in the public database GEO [4]. The files were classified into 5 groups: melanocytes (normal cells), melanoma stage II-IV and metastasis. The differential expression were generated in all possible comparisons.

Results. Differential gene expression was analyzed in all possible comparisons among the 5 groups, 10 contrast in total. We considered two statistics of interest for obtaining differentially expressed genes, the level of change in log2 scale: >0.27 and the level of confidence in these values or log odd rates: >3 (> 95% of confidence).

Table 1. Numbers of differential expressed genes resulting for each contrast.

Contrast	Genes downregulated	Genes upregulated
Melanoma stage I and II vs Melanocyte	58	79
Melanoma stage III vs Melanoma stage I and II	0	0
Melanoma stage IV vs Melanoma stage III	533	270
Metastatic melanoma vs Melanoma stage IV	477	1444
Melanoma stage III vs Melanocyte	153	199
Melanoma stage IV vs Melanocyte	433	84
Metastatic melanoma vs Melanocyte	236	120
Melanoma stage IV vs Melanoma stage I and II	283	80
Metastatic melanoma vs Melanoma stage I and II	2702	1555
Metastatic melanoma vs Melanoma stage III	3284	2406

Conclusions. A major challenge in melanoma cancer research has been the characterization of molecular events associated with progression. In this study, a large number of differential expression genes in each contrasts outlines a molecular progression model. Interestinlgy, our results shown that gene expression differences between metastatic melanoma and melanocytes is less that among metastatic melanoma and any melanoma stage.

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MOLECULAR DYNAMICS SIMULATION OF EhCFIm25 PROTEIN AND ANALYSIS OF ITS ABILITY TO BIND RNA

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INTRODUCTION: The mRNA polyadenylation process is an important event in gene expression regulation in eukaryotes. A fundamental factor in this process is the CFIm25 protein, which interacts with RNA 3'UTR to regulate the recruitment of other polyadenylation factors and the selection of alternative poly(A) sites. Analysis of the crystallographic structure of the CFIm25-RNA complex allowed the identification of amino acids interacting with the RNA molecule. Interestingly, multiple alignments revealed that these residues are conserved among homologous proteins of different species. Notably, the EhCFIm25 protein of *Entamoeba histolytica,* the causal agent of human amoebiasis, conserves both Leu135 and Tyr236 residues. Site direct mutagenesis and REMSA experiments revealed that the recombinant EhCFIm25 totally loses its RNA binding capacity when these residues were independently changed to Ala residue. However, the nature of the interactions between L135 and Y236 residues, and the RNA is still unclear.

METHODS: Through SPDBV 4.10 software, punctual mutations were made in the EhCFIm25 protein sequence to change Leu135 and Tyr236 residues to Ala. Then, molecular dynamics simulations of 20 ns were performed at distinct temperatures using the 4.5.5 Gromacs software in order to evaluate the effect of each mutation on the 3D structure and stability of EhCFIm25, and identify the changes that could influence the RNA binding capacity of the protein.

RESULTS: Both L135A and Y236A mutations did not induce important changes in the EhCFIm25 3D structure at 300ºK. When the temperature increased to 400ºK and 500ºK, the mutated proteins appeared to be less stable; the structure seemed to expand, the alpha helices began to disappear but not the beta sheets.

CONCLUSIONS: The loss of RNA binding capacity of mutated EhCFIm25 proteins (L135A and Y236A) may be related to a reduction of protein stability.

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Proteomic analysis of Entamoeba histolytica in vivo assembled pre-mRNA splicing complexes.

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The genome of the human intestinal parasite Entamoeba histolytica contains nearly 3000 introns and bioinformatic predictions indicate that major and minor spliceosomes occur in Entamoeba. However, except for the U2-, U4-, U5- and U6 snRNAs, no other splicing factor has been cloned and characterized. Here, we HA-tagged cloned the snRNP component U1A and assessed its expression and nuclear localization. Because the snRNP-free U1A form interacts with polyadenylate-binding protein, HA-U1A immunoprecipitates could identify early and late splicing complexes. To avoiding Entamoeba's endonucleases and ensure the precipitation of RNA-binding proteins, parasite cultures were UV cross-linked prior nuclear fractions immunoprecipitations with HA antibodies. Precipitates were subjected to tandem mass spectrometry (MS/MS) analyses. To discriminate their nuclear roles (chromatin-, co-transcriptional-, splicing- related), MS/MS analyses were carried out with proteins eluted with MS2-GST- sepharose from nuclear extracts of an MS2 aptamer-tagged Rabx13 intron amoeba transformants. Thus, we probed thirty-six Entamoeba proteins corresponding to 32 cognate splicing-specific factors, including 13 DExH/D helicases required for all stages of splicing, and 12 different splicing-related helicases were identified also. Furthermore 50 additional proteins, possibly involved in co-transcriptional processes were identified, revealing the complexity of co-transcriptional splicing in Entamoeba. Some of these later factors were not previously found in splicing complex analyses.

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The role of molecular hinges in protein stability and folding

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The mobilityoflarge portionswithinthree-dimensional structuresof proteinsis attributed to thepresence of molecular"hinges". Molecular motionsallowproteinsto perform theircatalytic functionsoptimally andto recognizeparticularmetabolitesandtags madeofcarbohydrates. The hingescan be formed bya set ofamino acids,orbysecondary structure elements, which allowconcertedmovements evenofentire domains.

Protein foldingis an eventthat occurs in themillisecondorderboth incellsand *invitro*. Theinvolvement of molecular hingesto facilitatethe folding process couldbean explanation for thehigh speedshowed toachievethe appropriatethree-dimensional structure of these macromolecules. It has been proposedthat somesupersecondarystructures, such as helix-helix orbeta-hairpin loops,needto bestabilizedto beusedasascaffoldtobuildthe restof themolecule. Thesestructurescontainsmallaminoacids(proline, alanine, glycineorserine) alternated withmorestericorchargedgroups (lysine, glutamateoreventryptophan)in their connecting regions, thatcouldconstituterealhinges.

In this work the flexibility of these linker regions has been studied by modifying the sequence with site directed mutagenesis *in silico* and performing molecular dynamics simulations both *in vacuo* and with the presence of explicit water molecules to observe their behavior.

Structural modelling of the N463D, R275G mutations in FKRP

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Abstract

Fukutin related protein (FKRP) gene encodes a protein which istargeted to the medial Golgi apparatus and is necessary forposttranslational modification of dystroglycan. Mutations in this genehave been associated with different pathologic phenotypes includingthe autosomal recessive limb-girdle type 2I phenotype (LGMD2I). Inthis study, we determined two heterozygous missense mutations N463Dand R275G on the FKRP gene in a Mexican patient and analyzed thestructural implications caused by these substitutions. Homologous FKRPprotein sequences where obtained from a PSI-BLAST search according todiverse phylogenetic depth and aligned with T-COFFE. The alignment andsequence conservation were visualized using Bioedit software andWebLogo Server. In order to elucidate structural changes caused by thespecific amino acid substitutions we performed structure modeling of mutated and wild-native proteins. Structure protein models of mutated(N463D, R275G) and wild-native FKRP proteins were performed throughthreading methods using I-TASSER server. Since FKRP homologousproteins with >40% identity on I-TASSER server were initially notdetected and there were not structures for template homology modelingall predicted models were resolved by means of threading methods. Thismight successfully resolve important fold changes. The models obtainedwere structurally aligned to the wild-native model by a carbon-alfaiterative magic fit using SwissPDB Viewer software. This is asuperposition of the best fragment matchings in the sequencecomparison between mutated and native-wild models using a PAM-200matrix. The root mean square values (RMS-values) for the whole finalalignments and for the similar and dissimilar atoms were calculated.Then we apply a colouring scheme by alignment diversity to showstructural discrepancies between mutated vs native-wild proteins.Alignment of homologous FKRP proteins shows that mutated sites arehighly conserved across the phylogeny. N463D is a phylogeneticallyconserved site from human to insects where all residues correspond toasparagine (N). R275G site has a preference for arginine (R) or lysine(K) which are both positive charged residues at physiological pH.There were local secondary structure changes observed in bothmutations affecting B-strand, coil and alpha-helices, modifying also3D conformation of some local and central regions. Although mutationsare not proximal, both substitutions show changes over the samecentral region that might act as a dynamic structural part of theprotein with an important function. The effect of these structuralchanges in the function of the protein warrants further study.

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Effect of pH on the Affinity of Chymopapain to Chicken Cystatin.An enzyme-inhibitorassociation modulated by electrostatics.

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Abstract:

Cysteine proteases are one the most important family of proteases andthose from the papain superfamily are most widely distributed in nature,having been found in viruses, bacteria, protozoa, fungi, plants and mammals.The main features of the binding and inhibition of papain-like cysteine proteases by the cystatin inhibitorshave been well studied and established. However, slight structural differences make theses proteases distinct with respect to their substrate specificityand regulation. In this work, we present a fluorometric study of the binding of chymopapain, one representative papain-like proteinase, with chicken cystatins. We determined the dissociation equilibrium constant (K_d) at 14 different pH values in the range between 6.0 and 10.0.The far-UV Circular Dichroism spectra of individual proteins in the studied pH range evidenced no significant changes in protein secondary structure. Neither a one-proton nor a two-proton linkage model coulddescribeproperlythe experimental results. Nevertheless, a proton transfer model with three ionizable groups qualitatively explains the variation of the binding constant (K_b) with pH.

In order to better understand the binding mechanism, an atomic model was constructed by docking the3D structures of single proteins, followed by a refinement step of 100 ns molecular dynamics simulation in explicit water. The distribution of charged residues on the interface of the chymopapain-cystatin complex (at ≤ 4.5 Å between chains)was analyzed in this study. Eight ionizable residues (D158, H159, Y61, Y67, K64, K139, K145, K156) on chymopapain and five ionizable residues (E19, Y100, K59, R52, G9 (N-terminus))on cystatins were found.The theoretical calculation of the electrostatic component of the binding free energy ($\Delta G_{b,elec}$) of the enzyme-inhibitor complex at the different assayed pHs, using an implicit solvent model based on the numerical resolution of the Poisson-Boltzmann equation, qualitatively describes the experimentally observed behavior of the binding energy as a function of pH.Besides, the calculation of $\Delta G_{b,elec}$ varying the charged state of each one of the ionizable interfacial residues, revealed the importance of each residue in the energetics of binding.

In conclusion, the pH dependence of chymopapain-cystatin complex affinity is grossly explained bythe protonation/deprotonation of their interfacial ionizing residues.

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Transcriptional regulation of coding and non-coding RNAs by the Cyclin Dependent Kinase 8 (CDK8) module of Arabidopsis Mediator

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Mediator is an evolutionarily conserved, multiprotein complex that is a key transcriptional regulator of both protein-coding and non-coding genes[*1*]. The complex consists of the three modules of Core Mediator(Head, Middle and the Tail);plus a fourth, detachable, moduleis calledthe Cyclin Dependent Kinase 8 (CDK8) Module. This Kinase module is composed of four proteins (Med12, Med13, Cyclin C and CDK8), and is mainly involved in negative regulation of transcription[*2*]. The CDK8 module is known to regulate transcription by steric inhibition of the interaction between Core Mediator and RNA pol II[*3*]. In *Arabidopsis thaliana* the CDK8 module participates in several key developmental programs, including embryogenesis[*4*], vegetative development [*5,6*] and flowering[*7*].

In this work we performed high-throughput sequencing of small and messenger RNA transcriptomesof wild-type, *med12*, *med13*, *cdk8*, and *med12;cdk8* plants at different stages of development, to investigate the general role of these Kinase module subunits.Results from the differential expression analysis of the small RNA data suggest a role for the CDK8 module in the regulation of microRNAs that are important for the control of spatial and temporal aspects of development in Arabidopsis.Deregulated microRNAs include members of the miR166 family, which participate in the regulation of abaxial/adaxial identity of leaves by spatially restricting their HD-ZIPIII targets,and miR164 that targets the *CUC2* gene to controlleaf serrations during vegetative development. Taken together with previous results from our laboratory demonstrating that the CDK8 module regulates miR156 and its *SPL* target genes [*6,7*], our genome level analysis of microRNA and mRNA transcription points to a role for the CDK8 module of Mediator as an integrator of the temporal inputs and patterning outputs, resulting in the developmental timing of leaf traits characteristic of Arabidopsis development.

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Phylogenetic study of sperm-specific Na+/H+ exchanger.

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Sodium proton exchangers (NHEs) are membrane proteins that catalyze Na^+/H^+ exchange across biological membranes. Therefore, NHEs play a crucial role in intracellular pH (pH_i) regulation. Mammals have eleven NHE orthologues expressed in a ubiquitous manner. It has been demonstrated that the pH_i plays a fundamental role in the regulation of sperm motility. Indeed, sperm-specific Na^+/H^+ exchanger (sNHE) is essential for male fertility since sNHE-null male mice are infertile due to severe defects in sperm motility. Here, we report a comparative evolutionary analysis of sNHE based on genome DNA sequences available from diverse species of metazoans. Interestingly, the distribution of species which possess a gene encoding the entire sNHE was revealed as mosaic in metazoans, which is almost same as the distribution of CatSper (sperm specific Ca^{2+} channel)-related genes in metazoans as previously demonstrated. It is supposed that the sNHE underwent rapid evolution and functional divergence, while distinct evolutionary constraints appear to have acted on different domains and specific sites of sNHE. These results reveal interesting evolutionary characteristics of sNHE and their adaptation to sperm biology through metazoan evolution. Since CatSper channel is known to be regulated by pHi, our results strongly suggest a physiological coupling between sNHE and CatSper in spermatozoa.
This study was supported by PAPIIT (IN203513) y CONACYT (177138).

GMOseq: A Next Generation System for GMO characterization

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The increase in diversification and modifications ofGMO constructions is surpassing the diversification in screening methods availablefor their detection and characterization, often being limited to qPCR, end point PCR or even just strip tests. These methods also lack the capability of showing thegenomic characterization of GMOs since they do not offer nucleotide sequence level information of the genetic elements being tested. These limitations cause the regulatory and monitoring agencies worldwide to strive to get adequate and enough information andtools to cope with GMO developments, legislation and commercialization. To cope with these limitations and to incorporate state of the art molecular biology methodologies to the SENASICA laboratory infrastructure we developedexperimental and bioinformatic methods to screen every publicly known transgenic elementin a GMO sample at a genome wide and high throughput level that allowed us to detect and characterizeat the nucleotide sequence level GMO modifications to provide the regulatory parties with enough information to create better regulations and to develop wider and more accurate GMO testing assays.

The developed method is based on three main components, first a bioinformatic design of 120-mers thatcover every transgenic element DNA sequence reported on commercially available events as well as other transgenic and control DNA sequences relevant to GMO detection, second a Next Generation Sequencing assay coupled to target-enrichment by RNA probes and third a custombioinformatic pipeline that handles the high-throughputsequencing data to filter,process, analyze and summarize the transgenic elements present on the sequenced sample. Briefly, the bioinformatic design of 120-mers is based on a local DNA database developed by SENASICA compiled from the databases of NCBI, GMDD,CERA,Patent Lens and the BATS report, which contains theDNA sequence information for every commercially available event, many experimental transgenic elements and all the endogenous genes used as controls in GMO detection assays.The previously described database was used as input for a series of manual and programmatic curation processes that allowed taking the DNA sequences in the database and transforming them in a set of 120-mers that covered every region in the DNA sequences in the database. The target-enrichment process to capture the transgenic elements present in a sample of DNA was based on the set of 120-mers described which was sent to Agilent to generate a set of SureSelect RNA probes for such enrichment.The captured DNA was sequenced using the Roche 454 GSFLX Titanium platform. Finally the sequencing reads were processed using an in-house developed pipeline that checks, trims and filters the reads using CLC Genomics Workbench, align all the filtered read using a BLAST-like algorithm with BLAT, classify the aligned reads and summarize the representation of the transgenic elements in the sequenced samples with custom Perl scripts. The resulting summaries can be checked against internal controls included in the target- enrichment system.

Área: Biología de Sistemas y Bioinformática

Phylogenetic and biogeographic inference from six cpDNA loci reveals Neotropical origin of grammitid ferns

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Grammitid ferns include 34 described genera, grouped into a monophyletic clade inside the Polypodiaceae family. Half of these genera are restricted to Asia and Australia, 8 genera are exclusively Neotropical, and the rest include distribution in Neotropics, Africa, Madagascar and Polynesia. Molecular markers from cpDNA have a wide use in phylogenetic reconstructions for ferns, due to reduced levels of horizontal gene transfer (usual in plant mtDNA) and absence of influence of allelic polymorphism within diploid or polyploid individuals.

In the analysis of grammitid ferns six cpDNA loci, two of which are coding (*rbcL*, *atpB*) and four non-coding (*trnL-trnF*, *trnG-trnR*, *rps4-trnS*, *rbcL-atpB*), were used to perform phylogenetic reconstruction with Bayesian inference and Maximum Likelihood. An incomplete character matrix, with only part of sequences available for two thirds of terminal nodes, was used to include insufficiently sequenced taxa. Bayesian inference was used, as well, for determining time of divergence between lineages, with a relaxed log-normal molecular clock algorithm. Biogeographical analysis included reconstruction of ancestral areas for each phylogenetic tree node, using Statistical Dispersal-Vicariance Analysis (S-DIVA) and Bayesian Binary Method (BBM).

The phylogenetic reconstruction from the incomplete matrix was proved to be robust, comparing it with the framework topology for less taxa, obtained from complete dataset of three cpDNA loci. The results of phylogenetic and biogeographical reconstructions suggest basal position of most Neotropical clades and derived position of 16 genera from South-Eastern Asia, Australia and New Zealand. The origin of grammitid ferns in the Lower Oligocene (~31–34 Ma) was
followed by a long evolution in the Neotropics, that appears as center of origin for modern Neotropical, African and Madagascan genera. The dispersal of the Asian clade ancestor happened in Lower Miocene (~20–23 Ma), when it was established in South-East Asia. The dispersal to Australia and New Zealand happened much later, in Upper Miocene (~10–12 Ma).

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Systematic identification of signal integration by Protein Kinase A

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Cellular processes and homeostasis control in eukaryotic cells is achieved by the action of master regulators such as Protein kinase A (PKA). While the out bound signals from PKA directed to processes such as metabolism, growth and aging have been well charted, what regulates this conserved master regulator remains to be systematically identified to understand how it coordinates biological processes. Using a yeast PKA reporterassay, we identified genes that influence PKA activity by measuring the protein-protein interactions between the regulatory and the two catalytic subunits of the PKA complex in 3726 yeast genetic deletion back grounds grown on a fermentable and a non-fermentable carbon source. Overall, nearly 500 genes were found to be connected directly or indirectly to PKA regulation, including 80 core regulators, denoting an unsuspected diversity of signals regulating PKA, within and beyond the described upstream linear pathways. PKA regulators span multiple processes, including the antagonistic autophagy and methioninebiosynthesispathways. Weidentified a mechanism of PKA regulation by posttranslationally sineacetylation, which is conserved between yeast and humans and regulates both carbohydrate storage and aging. Altogether these results show that theextent of PKA regulation was largely under appreciated, as this master regulator receives information from upstream and downstream processes and highligh thow biological processes are interconnected and coordinatedby PKA. Our work stresses the need for a paradigms hift from a pathway-centricview of cell regulation to a network-centricview for a better understanding of the functionalo rganization of the cell and the maintenance of homeostasis.

Cleavage of recombinant Proenkephalin A overexpressed in Chinese hamster ovary cells

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Proenkephalin A is an opioid neuropeptide precursor, which under cleavage produce the opioid peptides Met- and Leu-enkephalin. The intact precursor molecule is released by activated human mononuclear cells (Padrós 1989). To analyze the role of full-length PENK we purify it from the conditioned medium of CHO-DL1 cells, a derivate cell line that express high levels of recombinant rat PENK using the dihydrofolate reductase (DHFR) system (Lindberg 1991).

Western blot and radioimmunoassay analyses showed that, besides the already described 32-34 kDa full-length PENK CHO-DL1 cells secrete an 8.6 kDa PENK-derived molecule. This molecule is recognized by the antibody against Syn-enkephalin but not by the antibody against the C-terminal heptapeptide indicating that it belongs to the N-terminal domain of PENK. A full characterization of the 8.6 kDa PENK-derived molecule was conducted with reverse phase (C4) high performance liquid chromatography (HPLC).The elution profile was determined by radioimmunoassay, and the fractions containing proenkephalin-derived peptides were submitted to mass spectrometry and Edman degradation. All these analyses confirmed that CHO-DL1 cells produce and secrete the 8.6 kDa PENK-derived molecule, previously described by group of Udenfriend (Jones 1982) in adrenal bovine medulla. Additionally, our data suggested that 32-34 kDa and the 8.6 kDa PENK-derived molecules are present in the conditioned medium of CHO-DL1 as a complex.

Overall our data reveal that recombinant PENK is processed in CHO-DL1 cells. Proprotein convertases SKPC1 or SKPC2 may be present in this cell line. These data indicate that, under the experimental conditions used, CHO-DL1 cells are not adequate to produce intact recombinant PENK.

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Factors regulating the expression of heterologous proteins in *E.coli*; using seven homologous triosephosphateisomerases (TPI) from different sources.

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Background:Heterologous expression of proteins in *Eschericha coli* is affected by different factors that act during mRNA translation in the ribosome when producing a functional native protein. A frequent factor is the presence of the rare arginine codons(RAC) (AGA and AGG) for which the tRNAsnecessary for protein synthesis are scarce in *E. coli* strain Bl21 (Bl21) and plentiful instrain Bl21 Codon Plus (CP). We evaluated the effect of rare codons(RC) in TPIsfrom different species. These were selected to have increasing numbers of RAC (from none in *Trypanosomabrucei*TPI to 8 in *Saccharomyces cerevisiae* TPI). Besides, the RC had variations in their position among the first 25 amino acids, and/or they had two adjacent positions in the sequence, both factors being known to produce pauses during translation of mRNA. Pauses in translation can cause low protein production, errors in the amino acid sequence, changes in function, stability or solubility of the synthesized protein.**Results**: Our study included seven TPIs from the following species: *Homo sapiens, Boophilusmicroplus, T. brucei, T. cruzi, Plasmodium falciparum, Giardia lamblia*and*S. cerevisiae.*The homology of the TPIs in our study varied between 41 to 69%. Some of them had RC among their first 25 amino acids, e.g. *B.microplus* TPI, *Homo sapiens* TPI, *P. falciparum* TPI and *S. cerevisiae* TPI. Additionally they can also have rare adjacent codons (RAdC), e.g. *H. sapiens* TPI, *P. falciparum* TPI and *S. cerevisiae* TPI. To evaluate if the number of RC was important for protein synthesis we measured the relative quantity of protein produced by strain CP as compared to that produced by strain Bl21. The ratio of protein produced had a linear relationship to the number of RC. The position ofthe RC also influenced protein synthesis; e.g. *G.lamblia*TPI, which lacks RC in the first 25 amino acids and also lacks RAdC, had a lower CP/Bl21 ratio than *B. microplus* TPI, which has the same number of RC, but has one in position 5. *S. cerevisiae* TPI had a very high CP/Bl21 ratio which shows its dependence on the abundance of tRNAs to achieve maximum expression.Since we had previously studied*H. sapiens* TPI, which has 4 RC(among which are one RC in position 5 and a pair ofRAdC) we changed the position of the first codon by adding a his-tag at the amino-terminal end. This change optimized the ratio CP/Bl21. We subsequently studied if the velocity of synthesis was dependent on the induction or non-induction of *E. coli* cultures with Isopropyl $\beta-\Delta-1-$ thiogalactopyranoside. Our results showed that the CP/BL21 ratio is better in the case of non-induction, and has no relationship to the position of the RC.

Our study demonstrates the influence of the number and position of RC on the synthesis of heterologous proteins in *E. coli*.

Insights in the structure and association of the capsomer of Parvovirus B19.

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Virus had several forms and sizes with distinct stabilities and properties. A virus consists essentially of genetic material surrounded and protected by a protein coat, the capsid. Many virus show icosahedral structure with multiple copies of one or more proteins arranged in dimeric, trimeric, pentameric or hexameric structural subunits called capsomers. To understand the formation process of capsids is necessary determinate the structural, energetic and functional role of the individual amino acids into the interactions protein-protein presented at the interfaces for the association and stability of capsids. The structural information gives the bases for the design of vaccines, antiviral agents and viral vectors favoring the assembly or disassembly of capsids.

Human parvovirus B19, a member of the *Parvoviridae* family, is the causative agent of the common childhood illness *erythema infectiosum*, as well as a number of other less common conditions including fetal hydrops, miscarriage, transient aplastic crises, transient postinfection arthropathy and chronic anemia in immunocompromised patients. Diverse studies of capsids into the *Parvoviridae* family suggest that their capsomers are trimers. In the present study we prove the presence of trimers in the assembly process of B19 virus-like particles (VLPs), through the construction of mutants that allow the formation of stable capsomers with impaired ability to form capsids. We determined the association state of the mutant proteins as well as its association energy landscape to propose an assembly mechanism for the capsomer.

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Characterization of two cysteine-less wild-type triosephosphate isomerases and a cysteine containing mutant enzyme from lactic acid bacteria

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We characterized the triosephosphate isomerases from the lactic acid bacteria *Leuconostoc mesenteroides* (LmesTIM) and *Oenococcus oeni* (OoeniTIM), and the mutant LmesTIM-D132C. The wild type enzymes of both bacteria lack cysteine in their amino acid sequences. Scientific bibliography and sequence alignments of available TIMs in the common databases, indicate that position 126 in the sequence has a strictly conserved cysteine in practically all known organisms. But LmesTIM and OoeniTIM have an aspartic acid in that position (corresponding to D132 in LmesTIM and D126 in OoeniTIM). Initially we determined the kinetic constants and the stability of the three enzymes. Using glyceraldehyde-3-phosphate as substrate the Km and k_{cat} at 15 ºC for LmesTIM, OoeniTIM and LmesTIM-D132C were 0.8 ± 0.13 mM and 2.6 x 10^3 min^{-1}, 0.2 ± 0.06 mM and 3.9 x 10^3 min^{-1}, and 0.6 ± 0.13 mM and 1.1 x 10^4 min^{-1}, respectively. And, at 25 ºC, the three TIMs, in the same order as above, had specific activities of 40 ± 8, 340 ± 25 and 380 ± 17 µmol-product/min-mg. Molecular exclusion experiments showed that the three enzymes elute in a volume corresponding to a homodimer, that is, they have the same degree of oligomerization as TIMs from mesophilic organisms. Circular dichroism spectra indicated that at 25 ºC LmesTIM and LmesTIM-D132C are appropriately folded. After previous incubation for two hours, the stability of the three enzymes depended on protein concentration. Activity diminished by 50% at concentrations of 4.8, 0.3 and 1.2 µM for LmesTIM, OoeniTIM and LmesTIM-D132C, respectively. Thermal stability using ellipticity was studied for LmesTIM and LmesTIM-D132C at concentrations of 10 and 20 µg/mL. The transitions had little cooperativity, were reversible, and showed no hysteresis. Calorimetric analysis at 90 ºC /h indicated that the D132C mutation increases the apparent Tm by 12 ºC. In summary, the mutant enzyme LmesTIM-D132C has ten times more activity at 25 ºC, is four times less stable to dilution and is also less thermally stable than wild type LmesTIM.

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Identificationof lectin from teosinte coleoptile (*Zea diploperennis, Zea mexicana* y *Zea parviglumis)* by RT-PCR

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Teosinte is the closest wild relative of maize, both species exhibit differences in their adult morphologies but their genomes are so similar that some species of teosinte can cross hybridization with corn [(1)].

We have identified a lectin from teosinte coleoptile (*Zea diploperennis*)that is similar in amino acid composition to corn BGAF. These lectins agglutinate erythrocytes of humans and animals, are specific for galactose, its activity is inhibited by lactose and N-acetilactosamina [(2, 3, 5)] and are presented as a molecular complex with β-glucosidase [(3,4)]. Of these, only BGAF has been studied at the level of molecular biology.

BGAF belongs to a small group of lectins identified in the family Poaceae (gramineae) known as monocot chimeric jacalins. This lectins consisting of a dirigent domain and a jacalin-related lectin domain. Recent studies have shown that these proteins play important roles in plant stress responses and development.

Based on the above information (evolutionary relationship between maize and teosinte and similar biochemical properties betweencorn BGAF and teosinte lectin), weused primers that amplified BGAF to obtain cDNA from teosinte coleoptiles (*Zea diploperennis, Zea Mexicana, Zea parviglumis*) by RT-PCR to determine whether the gene oflectin is retained in maize and teosinte along the process of evolution of both species.

We observed in the agarose gel the presence of a well-defined band with molecular weight of about 1100 bp (molecular weight of corn BGAF is 1118 bp) which is very similar in all samples analyzed.

The cDNA was sequenced, partial sequences showed 90% identity with corn BGAF (GenBank accession AF232008). Particularly we observe that the sequence of teosinte *Zea diploperennis* showed a small region in the 3 'end that does not exist in corn, which is consistent with their phylogenetic relationship furthestcompared to the other studied teosintes. This result suggests that the lectin gene from corn and teosinte coleoptile (*Zea diploperennis, Zea mexicana* and *Zea parviglumis*) has been preserved in these species along the evolutionary process.

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Analysis of Enzyme Kinetic Aspects of PhenanthreneDihydrodiol Dehydrogenasein *Mucorcircinelloides* YR-1

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Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic compounds which are formed of two or more benzene rings combined in linear, angular shape or cluster arrangements. They are ubiquitousin the environment and as byproducts of human activities. There are over 100 compounds of PAHs, and most of them remain in the ecosystem for many years for its water solubility and absorptionlow. The presence of these contaminated soils and sediments is a great risk, because many PAHs are toxic, mutagenic, and carcinogenic in some cases.

The YR-1 strain of *Mucorcircinelloides*has been isolated from soil contaminated with oil in Salamanca, Guanajuato. We have demonstrated the ability of this strain to use naphthalene, phenanthrene, anthracene and pyrenePAHs as the sole source of carbon and energy. It has been proposed this strain has a complex enzymatic system gives the ability to degrade aromatic hydrocarbons.

In this work, different kinetic aspects of onephenanthrenedihydrodioldehydrogenase (PDD) enzyme are analyzed, this PDD is induced when phenanthreneused as a sole carbon source for growth of YR-1 strain of *M. circinelloides*.The goals were: (1) purification of onePDDenzyme and (2) determination of kinetic parameters of the PDD enzymeofYR-1 strain of *M. circinelloides*.

NADP(+)-dependent dihydrodiol dehydrogenase activity was detected in a cell-free extract from YR-1, after high-speed centrifugation. We purified onePDD enzyme to homogeneity and estimate its isoelectric point. Also was calculated the molecular weight for the native protein and for the monomer that conforms it.

We analyzed the enzymatic activity of purified PDD enzyme by spectrophotometry with variations in its substratum naphthalene-diolcis and trans isomers, and cofactorNADP(+). With these results we obtained the Km, Vmax y Kcat, for the cofactor and its substrata.

PepGMVinfection benefit the interaction of the host plant with the withefly*T. vaporariorum*bychanging the defense responses

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Plant viruses as other pathogens modify the physiology and metabolism of their host plants, and also, enhance the defense responses mediated by the hormones jasmonic acid (JA) and/or salicylic acid (SA). These changes affect the interaction of the host plant with other organisms.In this work we are studyingthe changes in the JA and SAlevels after virus infection in host plants and the implications in the performance of an herbivore insect. As a model system, we are usingchilli plants (*Capsicum annuum*), whitefly (*Trialeurodesvaporariorum*) and a begomovirus (*Pepper Golden Mosaic Virus*, PepGMV). We observed that more nymphs developed on PepGMV-infected (with 20 days post inoculation, dpi) than on healthy plantsafter performing an oviposition assays, especially in leaves that show severe symptoms of the infection. Thus, *T. vaporariorum*whiteflies benefit themselves from oviposition on PepGMV-infected plants. The quantification of JA and SA in all leaves of PepGMV-infected plants and healthy plantsbefore the whitefly oviposition did not show important differences between them. This result indicated that the JA and SA responses are not activated at this point of time (20 dpi)which helps the whitefly performance.However, we cannot discard the activation (or suppression) of JA and SA-depended responses during the development of the whitefly in PepGMV-infected plants. Until now, these results indicate that thePepGMV-infected plant is a better host than healthy plants, because the JA and SA-dependent defense are suppressed by the virus infection. The results of JA and SA quantification, as the expression of some defense genes during the whitefly development in PepGMV-infected plants and healthy plantswill be presented and discussed.

Development and evaluation of a recombinant sperm-activating peptide tagged with fluorescent proteins

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Long-range cellularcommunication between egg and sperm is essentialfor external fertilization. Sperm-activating peptides (SAPs) promotethe sperm encounter with the egg. Speract (a decapeptide) is a SAP from the outer layer ofsea urchin eggs that binds to its receptor in the sperm flagellum causing physiological changes, modifying the flagellum beating. The Speract-induced sperm responses have been studied with purified or chemically synthesized peptide. However, the utility of recombinant Speractproduced in *E. coli*has not been explored.In this work, using molecular biology techniques, we prepared Speract fused to the C-terminal of three fluorescent proteins (FPs; 28 kDa), Ametrine (Yellow Fluorescent Protein which has a long Stokes shift), seCFP (super enhanced Cyan Fluorescent Protein) and Venus (improvedEnhanced Yellow Fluorescent Protein)using a GGGSGGG peptideas a linker.As we added a 6His tag to the N-terminal of the recombinant protein, all FP-tagged Speract proteins were efficientlypurified using affinity chromatography in a nickel column. Subsequently, using fluorescent indicators, we tested the effectiveness of seCFP-Speract to induce the typicalchanges caused by Speract in membrane potential(Em) andintracellular pH (pH_i) and Ca^{2+}($[Ca^{2+}]_i$)in*Strongylocentrotuspurpuratus*sperm. In addition, we observed binding of Venus-Speract to its receptor in *Lytechinuspictus* spermusing confocal microscopy. Finally, performing competitive binding assays with a Speract fluorescent analogue (F-Speract; that quenchesits fluorescencewhen it binds to the sperm), we measured the affinity of Ametrine-Speract to its receptor on *L. pictus* sperm. Our results indicate that seCFP-Speract and Speract induce similar changesin Em,pH_i and $[Ca^{2+}]_i$, and that Venus-Speract binds specifically to the flagellum of the sperm.Our quantitative analysis ofthe affinity of Ametrine-Speract to its receptor indicates that FP-tagged Speract has a 3.5-foldless affinity thanSperact, probably due to the large size of the fluorescent protein incomparison with the Speract without label (only 10 amino acids).Currently, there are full color fluorescent proteins with further interesting functions such as photo-activation and photo-conversion. Therefore, we conclude that FP-tagged Speract can be a useful toolfor basic sciences and also for education.

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Identification of structural determinants involved in the difference of conformational change in EF-hand motifs

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Calcium signals are regulated by several proteins, most of which belong to the EF-hand superfamily. The EF-hand is an helix-loop-helix motifthat binds calcium through its loop. The EF-hand motifs occur in adjacent pairs,forming a single globular domain which is the basic structural and functional binding Ca^{2+}unit.This family of proteins are classified into calcium sensors and modulators. The first group undergoesconformational change upon calcium binding while the modulators remain practically unchanged. In both classes, the calcium-binding loop binds calcium by a pentagonal bipyramid configuration. To explain the biophysical and structural differences between the two groups,we have sought to identify important structural determinants for these features, especially for the difference in the conformational change

We analyzed the effect of the helical elements in the function of modules EF-hand. We found in both proteins a patch of conserved amino acids in helix H1 and H2 in the EF-hand of the troponin C (SCIII; EF-hand sensor) and bovine ClbN motif (EF-hand modulator). The main differences between the EF-hand motifs are thebinding Ca^{2+}loopand charged group residues present at the H2 EF-hand modulator. We have constructed chimeric EF-hand motifs containing the helix H1 or H1/H2 from ClbN in the SCIII sensor motif (H1ClbNSCIII and H1H2ClbNSCIII).

These constructs were analyzed using a reporter system that discriminates EF-hand-sensor motifs from signal-modulators. The results in the context of the fusion protein indicated that the substitution of the connector of 14 amino acids that binds Ca^{2+} in modulator motif by the connector of 12 residues of EF-hand motif sensor SCIII is sufficient to confer a Ca^{2+}dependent conformational change. In addition we analyzedthe conformational change of the H1H2ClbNSCIII and H1ClbNSCIII isolatedmodules by spectroscopic techniques.

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Engineering the reaction media to enhance the enzymatic synthesis of a sugar-based surfactant

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Surfactants are important components in food, pharmaceutic and cosmetic products, which keep water-oil mixtures in one phase. Alkyl glycosides are very attractive alternative for these purposes due to their low toxicity and modularity, which allow to adjust their properties modifying either their sugar moiety or alkyl chain. However, the traditional chemical route to synthetize them requires many steps to ensure the formation of a single product. When alcohols are present, some -amylases can produce them in a single step from the readily available starch through an alcoholyisis reaction. Among these enzymes AmyA from *Thermotoga maritima* has the best performance for this reaction. This proficiency has been improved through amino acid modifications. Nonetheless, the improvement of the reaction through changes in the composition of the reaction media has not been persued. In this work we explored the effect of three cosolvents on the production of butyl glucosides.

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"Electrophoretic analysis of maize stressed with*A. parasiticus*"

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One of the most important basic growing plants in the world is maize, which, under certain conditions, is susceptible to the attack of *Aspergillus*gender fungus. The maize is exposed to different stress factors given by the presence of pathogens, temperature changes and dryness. Several authors have reported the presence of proteins when *Aspergillus* exists. In this research, the protein profile of a sample of native maize from Oaxaca was analyzed, when some stress was induced by the presence of *Aspergillusparasiticus*fungus, both in coleoptile and milk stage maize. For the grain analysis, a sample of *vandeño*breed maize was grown in land and it was inoculate in the pollination stage with an*Aspergillus* stump. Furthermore, the coleoptiles obtained in petri dish were inoculated three days after it germination, and they were analyzed five days later. The protein extraction was produced by using anextraction buffer (NaCl, tris-HCl, β-mercaptoethanol and protease inhibitor) pH 8.In a second stage, a protein analysis of a maize sample was made for identifyingproteins froma one-dimensional electrophoresis (SDS-PAGE) inpoliacrylamide gel at 10%. The protein profiles showed differences in both stages, and a higher expression of protein was observed in the infected maize coleoptile.

Keywords: maize coleoptile, milk stage,*Aspergillusparasiticus*

Capsaicinoid accumulation in *in vitro*cultured *Capsicum chinense* placental tissue depends on *in situ* synthesis of valine and phenylalanine

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In order to evaluate the contribution of *in situ* synthesis of valine and phenylalanine towards capsaicinoid accumulation in placentas from habanero peppers, this tissue was exposed to specific inhibitors of key biosynthetic enzymes of both amino acidic precursors. In the case of valine, chlorsulfuron was used to inhibit acetolactate synthase (ALS), whereas for phenylalanine, an analog of this amino acid (p-fluorophenylalanine) was employedto hinder arogenatedehydratase (ADT) activity. Maximal ALS inhibition (78%) was obtained applying 90 nMchlorsulfuron for 12 hours to the placentas, which resulted in a 50% decrease in capsaicinoid accumulation. On the other hand, p-fluorophenylalanine induced 42% and 85% inhibition, when placentas were treated with a 500 mMdoses for 24 and 72 hours, respectively. It was observed a concomitant lowering in capsaicinoid content to 22% and 38% for the treatments stated above.
Though inhibition of the key biosynthetic enzymes resulted in important decreases of capsaicinoidcontents, the effects over valine synthesis were faster and more pronounced. Under the evaluated conditions, it can be suggested that capsaicinoid accumulation in *in vitro* cultured placentas depend on the *in situ* synthesis of valine and phenylalanine.

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Effect of the R3W, C27G, F211I, and Q279*somaticmutationspresent in humancancercellson Gpn3function.

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Gpn3 is a member of a smallgroup of GTPases, the GPN family, whichincludesothertwomembers: Gpn1 and Gpn2.Thenameisderivedfrom a tripeptideloopconstitutedbythe amino acidsglycine-proline-asparagine, whichisfound ineverymember of the GPN family. AllthreeGTPases share somelevel of homology in their aminoacidsequence, henceit has beenproposedthatthey share a commonancestor. Althoughtheirspecificfunctionisstillunknown, thethreemembers of thisfamily are essentialproteins. Gpn3 has beenreportedtophysicallyinteractwithboth Gpn1 and RNA polymerase II (RNAPII), theenzymecomplexin charge of thesynthesis of everymRNA in thecell. Both, Gpn1 and Gpn3 arenecessaryforthe nuclear localization of RNAPII. Recentgenomicstudieshaveshownthat in a smallpercentage of cancercells Gpn3 ismutated. In thepresentworkwehypothesizedthatthesemutationshave a funtionaleffecton Gpn3. Weemployedthe nuclear localization of RNAPIIto test thisproposalandusedbasic molecular and celular biologytechniquestogenerate molecular constructstoexpressshRNA-resistant(Gpn3R) and flag-tagged Gpn3R-Flagproteinswithone of several Gpn3 mutationsdescribed in human cancercells.TheGpn3 mutantversionsweregeneratedbysitedirectedmutagenesis in thepEYFP vector, followedby Gpn3R-Flag subcloningintothe pLNCX_2 retroviral vector. Thedifferentversions of Gpn3R-Flag (Wt, R3W, C27G, F211I, Q279*)werethenexpressedfromthepLNCX_2 vectorstably in MCF-12A cells.TheGpn3 expressionlevelswereverifiedby western blot and thesubcellulardistribution of RNAPII wasexaminedbyfluorescencemicroscopy. Endogenous Gpn3 wassilencedusingtheshRNA g193 ortheshRNA g239 as a control; in thesenewlyderivedcelllinestheexpression and subcellularlocalization of RNAPII was once againevaluated. Theresultsobtainedsuggestthatthe nuclear localization of RNAPII isdisruptedin MCF-12Acellsexpressingthe Gpn3 mutants F211I and Q279*butnotthe R3W or C27G Gpn3 mutants. Theseresultssuggestthatthe nuclear localization of the RNAPII isstronglydeterminedbytheintegrity of the Gpn3R-Flag C-terminus and thatthefunction of Gpn3 isaltered in a subset of human cancercells.

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Establishment of a Silver Staining System on Polyacrylamide Gels for Electrophoretic Analysis of ProteinPatterns from Human Urine

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Introduction: In Mexico, an important increase has been observed in the prevalence and incidence of kidney diseases as the chronic kidney disease, of silent evolution.It is important to know the present proteins in human urine, since they are a non-invasive way to recognize the kidney status[1].**Objective:** To establish silver staining system on polyacrylamide gels for electrophoretic analysis of protein patterns in human urine.**Methodology:**To assess linearity, electrophoresis was performed in triplicate of 1mm polyacrylamide gels at 10%, in the presence of SDS. Bovine albumin samples were prepared in dilutions of increasing concentrations. Gels were stained with silver and Coomassie Blue as control.To determine the detection limit, electrophoresis of series of bovine albumin dilutions were made, whose concentrations were above and below the detection limit reported for polyacrylamide gels stained with silver or Coomassie Blue. Reproducibility of silver staining system is in process of assessment. Gel images were taken with the GelDoc XRBIORAD system. Subsequently the area was quantified in pixels for each band, using the ImageJ program.The figures and statistical analyzes were performed in Microsoft Office Excel 2007.The analysis of human urine samples of 141 patients with different glomerular filtration rates (GFR), by silver staining system is in process.**Results and discussion:** A linear range of Coomassie Blue staining was found, between 600 to 2000ng (R^2 =0.96). Silver staining linear range was 800 to 1600ng (R^2=0.82).For Coomassie Blue staining, it was found a limit of quantification using the ImageJ program, of 50 ng, which resembles the reported detection limit (50 to 100 ng)[2].A sample of human physiological albumin and a band of 10 ng bovine albumin were observed with the naked eye but not detected and quantified by ImageJ. The bands on silver staining were quantifiable up to 50 ng in one of three gels; however, samples of human physiological albumin were detected and quantified using ImageJ. With the naked eye, bands were observed up to 5ng, which is consistent with the reported detection limits of silver staining (1-10ng)[2].**Conclusion:** The silver staining system established,is suitable for the analysis of protein patterns from human urine.**Acknowledgments:**Scholarship granted by the Dirección General de Investigaciones of the Universidad Veracruzana.

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Evaluation of the cytotoxic activity of organic extracts of *Juniperusmonticola* form *monticola*Martínez on breast human cancer cell lines

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Cancer is the second leading cause of death worldwide, representing a major health problem. Surgery, radiotherapy and chemotherapy are the most used therapeutic treatments. Despite the big diversity of drugs used in chemotherapy, cancer mortality is increasing due to limited activity against various types of cancer, chemoresistance, and secondary effects, among others, so it is necessary to continue the searching for new and more specific drugs. Approximately 60% of the drugs used in cancer treatment have been obtained from natural products, where plants are the main source, so it is reasonable to continue investigating for anti-neoplasic substances in plant species. *Juniperusmonticola* f. *monticola* is a species distributed in Mexico that has not yet been studied. The ethanolic extract of some species of the genus have shown cytotoxic activity in Hela, KB and MDA-MB-468 human cancer cell lines. Therefore, the objective of this study was to evaluate the cytotoxic activity of ethanolic extracts of *Juniperusmonticola* f. *monticola* on human cell lines and identify responsible substances.

Terminal branchlets of *J. monticola*f.*monticola*were collected and identified. Dried terminal branchlets (2.5 Kg) were used to prepared ethanolic extract by maceration. The total extract was partitioned with EtOAc to obtain two phases (organic and aqueous). The three extracts were evaluated in order to determine their effect on cell proliferation by sulforhodamine B assay on two breast cancer human cell lines, MDA-MB-231 and T47D after 24 h of exposure. Our results showed that at 20µg/mL the organic phase inhibits cell proliferation on 49.96±4.75% and 72±7.7% for T47D and MBA-MB-231, respectively. On the contrary, at the same concentration, the total extract and the aqueous phase presented lower cytotoxic activity on both cell lines.

The IC_{50} for organic phase was found at 20µg/mL after 24 h of exposure for T47D and at 12.89µg/mL for MDA-MB-231. Meanwhile, the total extract and the aqueous phase had IC_{50} values over 100µg/mL.The organic phase of *J. monticola* f. *monticola*presented the highest inhibitory activity on the two human tumor lines. According to our preliminary phytochemistry results, the substances responsible for the activity must have medium polarity, since the total extract and the aqueous phase did not show significant cytotoxic activity.

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Relation between simvastatin and mitochondrial uncoupling protein 2 in PAE cells.

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Abstract.

Cholesterol is a component of cell membranes, a precursor of steroid hormones and bile acids. However, in excessive amounts cholesterol becomes a risk factor in the developing of cardiovascular diseases. Although the diet cholesterol may contributewith the change in serum cholesterol level, more than two thirds of cholesterol is synthesized in liver. The principal step in cholesterol synthesis corresponds to HMG-CoA reductase. Statins are drugs that interact with this enzyme, inhibiting its activity and blocking the transition state substrate-product. In clinic, statins are used for the prevention of heart disease; however, its benefits go beyond its control over the levels of serum cholesterol. Hasbeen reported effects of statins in the endothelium function, in the atherosclerotic plaques, in the oxidative stress, in the inflammation, in the immune system, and in the central nervous system. These effects are called "pleiotropic". Statinsinhibit theisoprene synthesis, which are used as adhesion molecules during the posttranslational modifying. As happen with the family of small proteins bound to GTP (small GTPases) Rho, Ras and Rac, whose membrane localization and function isdepend of their isoprenylation. This posttranslational regulation may have consequences in the activity of other proteins, such as PPARs. PPARsinteract with the 9-cis retinoic acid receptor (RXR), and together bind withthe PPAR response elements (PPREs) in the DNA. By this way, the regulation of transcription of several genes will happen. In this work, we propose thatnuclear genes related with the mitochondrial, will be regulated by statins, and one of these genes corresponds to the uncoupling proteins UCP (UCPs). The mechanisms of uncoupling of oxidative phosphorylation, made by the UCPs, have several functions. Has not been described a relation between statins and mitochondrial function. We shows an increase in the amount of UCP2 protein in presence of simvastatin, whichis related with an increase of Rho not-isoprenylated and an increase of PPAR gamma protein, and a decrease in the production of free radicals in the endothelial cells.

Thermodynamic characterization of three Triosephosphateisomerases from representative, yet unexplored species from the *Eukarya* domain.

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Triosephosphateisomerase (TIM) is a ubiquitous oligomericenzyme that catalyzes the reversible interconversion between (R)-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. TIM participates in Glycolysis and the Calvin Cycle andits catalytic efficiency is diffusion-limited. TIM was the first example of a very common topology, named the “TIM barrel”; this scaffoldhas been found in 10% of the enzyme structures deposited in the PDB. The 3D structure of TIM monomers and the mode of association between them has been found to be conserved, nonetheless, the identity between the sequences of homologous TIMs can be as low as 10%.

All eukaryotic TIMs (eTIM) so far described are dimers and their temperature-induced unfolding is either irreversible or presents hysteresis. In contrast, the GdnHCl and urea-induced unfolding of eTIMs is frequently reversible; diverse folding patterns have been described, ranging from a simple two-state model, as observed in humanTIM,to a complex four-state model with irreversible aggregation steps in the TIM from *Trypanosomabrucei*.TIM is therefore an excellent model to study how sequence modulates folding behavior within a particular structural framework.

There are several phyla within the Eukarya domain where there is no information regarding the folding of TIM. Therefore, in this work we characterizedthe catalytic properties andfolding behavior of the TIM from a plant(*Zea mays*ZmTIM), an insect (*Bombyxmori*BmTIM)and a nematode(*Caenorhabditiselegans*CeTIM).

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The three enzymes were successfully purified after overexpression with IPTG induced plasmids inserted in BL21 strands of *E. coli*, followed by a protocol that involves nickel affinity chromatography, removal of the histidine tagand anionic exchange chromatography. The average protein yield was 50 mg per liter of culture. The three eTIMswere found to be dimeric and present the circular dichroism (CD) spectra characteristic of β/α proteins; in addition, fluorescence emission spectra showed that tryptophan residues are excluded from the solvent, whereascatalytic efficiency was found to be in the diffusion-limit.

The temperature induced unfolding of the three eTIMs was followed by CD at 220 nm. We performed temperature ramps to observe thermal unfolding and refolding of the enzymes. ZmTIMshowed a single transition, whereasboth BmTIM and CeTIMshowed two transitions, which indicates the presence of a third, stable, partially folded state.None of these transitions were reversible.

The urea-induced unfolding of ZmTIM was found to be a reversible process, as deduced from the coincidence of unfolding and refolding curves.The coincidence of fluorescence, CD and activity curvesstrongly suggests that this equilibrium transition is a two-state process.

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Influence of Microbial Inoculation on Phenolic Content and the Therapeutic Properties of Common Bean.

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It has been proposed that common bean consumption is related to the prevention of chronic degenerative diseases such as diabetes and cancer. The content of phenolic compounds in the seed has been proposed as the main responsible for this therapeutic effect. The coat color depends on phenols content, and has been reported that black bean varieties are better than lighter varieties in the prevention of these diseases. However, in the literature a high variability in the therapeutic effects are observed because no importance is given to the way of producing the seeds. Whereas phenols are secondary metabolites and one of its main functions is plant-microbe communication, both beneficial and pathogenic relationships, the objective of this study was to determine the effect of microbial inoculants on the content of phenolic compounds in the bean seed and determine whether this change has effect on the therapeutic properties of the crop. The experimental design for this study was an arrangement of two factors, Factor A: bean genotype (1: Negro 8025 2: Rojo INIFAP) and Factor B: inoculation (1: control chemical fertilization, 2: Rhizobium sp, 3: Bacillus subtilis, 4: Trichoderma atroviride). The total phenolic content was determined by the Folin-Ciocalteau method; Phenolics profile and purification was performed by HPLC. The antioxidant activity of the extracts was assayed with the DPPH technique, and the cytotoxic activity was evaluated against HeLa and Caco-2 cell lines. The results showed that inoculation with Rhizobium and Trichoderma produced the highest plant biomass compared to chemically fertilized control (average increase of 82% in Rojo INIFAP Beans and 47% in Negro 8025). Plants inoculated with Bacillus showed the best performance in seed yield (22% increase in rojo INIFAP Red and 54% in Negro 8025 compared to seed yield of control plants). The chromatographic profiles of beans showed only quantitative changes, being more significant in the seeds from plants inoculated with Bacillus. Significant differences in the cytotoxic and antioxidant capacity according to the origin of the seed was recorded

Evaluation of melatonin as an adjuvant therapy in a model of experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is an autoimmuneinflammatory disease of the CNS that is mediated by Tcells and macrophages and represents the paradigmatic model for multiple sclerosis.Oxidative stress appears to play a role in the onset and progression of EAE[1]. It has been suggested that decreasing oxidative stress might ameliorate symptoms and signs of EAE. Melatonin (N-acetyl-5-methoxytryptamine), a pinealneurohormone, is a potent hydroxyl radical scavenger andantioxidant, and plays an important role in the immunesystem[2].Therefore, we evaluate the effect of melatonin as adjuvant therapy in the model of EAE.

EAE was induced in Sprague Dawley ratsby immunization withcomplete Freund's adjuvant plus spinal and spinal cord pig homogenate. Immunized rats were observed daily for signs of paralysis,which is the clinical manifestation of EAE. Paralysis wasgraded in five stages of severity (grade 0, no signs; grade 1,floppy tail; grade 2, mild paraparesis; grade 3, severeparaparesis; grade 4, tetraparesis or moribund condition).The animals were divided into seven groups: control, EAE, EAE + melatonin, EAE + glatiraramer acetate, EAE + interferon β-1b, EAE + melatonin -glatiraramer acetate, and EAE + melatonin -interferon β-1b. After treatment, brains were homogenized and lipid peroxidation products and nitric oxide catabolitеswere evaluated.

We found that melatonin, glatiramer acetate and interferon β-1b decreased the severity of encephalomyelitis and delayed the presentation of clinical signs. A basal level of nitric oxide catabolites and lipoperoxides (LPO) were detected in midbrain and cerebral cortex of control group. A significant increase in nitric oxide catabolites and lipoperoxideswere found in the analyzed tissues of the EAE group. Administration of melatonin, glatiramer acetate and interferon β-1bdiminishes significantlythe increases of nitric oxide catabolitesand lipoperoxideselicited by immunization. These findings suggest that exogenous melatonin attenuates EAE via a mechanism involving diminution of oxidative stress.

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Increased lung apoptosis in *Autophagin-1* deficient mice after bleomycin-induced injury.

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Autophagy is a critical cellular homeostatic process that controls the turnover of damaged organelles and proteins. Impaired autophagic activity is involved in a number of diseases, including idiopathic pulmonary fibrosis suggesting that altered autophagy may contribute to fibrogenesis. However, the specific role of autophagy in lung fibrosis is still undefined. In this study, we show for the first time, how autophagy disruption contribute to bleomycin-induced lung fibrosis *in vivo* using an *Autophagin-1* (*Atg4b*) deficient mouse as a model. *Atg4b* deficient mice displayed a significantly higher inflammatory response at 7 days after bleomycin treatment associated with increased neutrophilic infiltration and significant alterations in proinflammatory cytokines. Likewise, we found that the number of TUNEL-positive cells was significantly increased in *Atg4b*$^{-/-}$ mice compared with WT mice as early as 7 days after bleomycin exposure. Moreover, widespread active caspase 3 immunostaining was detected mainly in bronchial and alveolar epithelium in *Atg4b*$^{-/-}$ mice at 7 days post-treatment. Western blot analysis of lung homogenates corroborated the marked increase of cleaved caspase 3 levels in *Atg4b*$^{-/-}$ compared with WT mice at 7 days after bleomycin. These data indicate that Atg4B deficiency resulted in increased epithelial cell death after bleomycin challenge. At 28 days post-bleomycin instillation *Atg4b*-deficient mice exhibited more extensive and severe fibrosis with increased collagen accumulation and deregulated extracellular matrix-related gene expression. Together, our findings indicate that Atg4B protease and autophagy play a crucial role protecting epithelial cells against bleomycin-induced stress and apoptosis, and in the regulation of the inflammatory and fibrotic responses.

Metabolic effectofconstant high pH on*S. cerevisiae*.

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Growth of *S. cerevisiae* stopped by maintaining the pH of the medium in a pH-Stat (Titration manager) at pH 8.0 or 9.0, withNaOH or KOH. Incubation of fresh cells at high pH did not decrease fermentation, respiration, acid production, or K^+ transport, which on the contrary, increased as the medium pH increased, reaching a maximum and similar values at pH above 7.0. Similar results were obtained even after incubating the cells for 4 h or 6 h at the high pH values.

ATP levels increased to similar levels, indicating that energy mechanisms were preserved. Respiration was similar and sensitive to the addition of an uncoupler. With glucose, as compared to ethanol, at high pH values, the main component of acidification was the production of CO_2, converted to bicarbonate and accumulated outside the cells.

In summary, growth inhibition at high pH was due neither to a decreased metabolic activity or altered energy metabolism, nor to a significantly lower amino acid transport by the cells or its incorporation into proteins. The cell cycle stopped at pH 9.0, arresting cells in G1, probably due to the adjustments needed by the cells to contend with the changes under these conditions.

More work has to be done to conclude that our results may be relevant to industrial fermentations, like bioethanol production.

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Oligomerization of nitrilases in catalytic filaments. Alejandro Evaristo Cáliz Rodríguez, Georgina Garza-Ramos Martínez, Lab. Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Torre de Investigación, Circuito Interior sin número, Ciudad Universitaria, delegación Coyoacán, México, D.F., C.P. 04510, tel. 56232275, ggarza@bq.unam.mx

Nitrilases catalyze the direct conversion of organic cyanides, commonly named nitriles to the corresponding carboxylic acids and ammonia, and found widely in nature. These enzymes are members of the carbon-nitrogen hydrolase superfamily that is characterized by having a homodimeric block with a αββα sandwich and a conserved, catalytic triad of Glu-Lys-Cys (Gong, Jin-Song, *et al.*, 2012). Microbial nitrilases are active only as oligomers that rearranged themselves into long regular helices of variable length by a reversible pH-dependent switching or after removing a segment of C-terminal tail. In this study, we explore the dependence of the activity and stability of the nitrilase from *Rhodococcus pyridinivorans* on quaternary structure by modification of the residues at the C-terminal interfaces leading to the extended fibers formation (Thuku, R., *et al.*, 2007). Monomers of WT nitrilase self-associate to form active dodecamers of 460 kDa, and a phenomenon of activation following assembly showed to be dependent on enzyme concentration. Modified construct bearing truncation in the C-terminal tail of 39 amino acids did show a significant improvement of catalytic activity and thermostability. Nitrilase from *R. pyridinivorans* hydrolyze both aromatic and aliphatic substrates with rather high efficiencies. NitT328 mutant had 6-fold higher activity relative to WT enzyme, without changes in the substrate specificity. The enzyme display high stability in a wide range of temperatures; at 55 ^{o}C had a half-live of 22 h. Dynamic light scattering and electron microscopy study of NitT328 mutant showed homogeneous population of spiral particles with a regular helical array of variable length, 50 to 400 nm and 10 nm diameter. The long helices provide a catalytic ensemble with the optimal properties to explore their biotechnological potential.

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ProtectionofhumanALDH2of the inactivationby lipid peroxidation products by site directedmutagenesis.

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Aldehyde dehydrogenases (ALDHs) catalyze the oxidation of aldehydes to their corresponding acids using $NAD(P)^+$ as coenzyme. It has been determined that ALDHs are responsible for the detoxification of lipid peroxidation products, which are important in the etiology and pathogenesis of different diseases that involve an increase in oxidative stress.AlthoughALDHsdetoxifythelipid peroxidationproducts,it has been reportedthat ALDHsare targets of the inactivation bythesetoxic aldehydes.ALDH2is the most sensitive isoformto inactivation byacroleinand4-HNEcomparedwith ALDH1A1andALDH3A1.Recent data from our group showedthat the presenceofCys residuesother than thereactiveCysin the aldehydebinding site,are responsibleforthe inactivation ofALDHsbylipid peroxidationproducts. Therefore,itis proposedthatchangingthe Cys residuesadjacenttothereactiveCys inALDH2, will produce an enzymeresistant toinactivation bythesetoxic aldehydes.

Themutant ALDH2-Cys301Thr-Cys303Val(H2CTCV), was generated by mutagenesis, purified and characterized. The data indicated that it was resistant tothe inactivation byacroleinand4-HNE, resistingconcentrations 1000-fold higher than those required toinactivateALDH2 (the wild type enzyme).However,the mutant presented acatalytic efficiency3-fold lowerwithpropionaldehydeandacrolein, compared withthe wild type enzyme, butshoweda catalytic efficiency140and15timeshigherforacroleinand4-HNE,respectively, compared with ALDH3A1, whichis thenaturallyoccurring enzymeresistant to inactivationbylipidaldehydes. Finally, the stabilityof the H2CTCVwas evaluatedand comparedwith that of thewildtype enzyme,the results showedno significant difference,suggestingthat the stability ofthe protein wasnot compromisedby the changesin thebinding siteof the aldehyde.These data revealed thatthe Cysresidues near to reactive Cys in ALDH are important in the inactivation process induced by lipid aldehydes.

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Matrix metalloproteinase (MMP)-19 deficient fibroblasts display a profibrotic phenotype

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Idiopathic pulmonary fibrosis (IPF) is a progressive and usually lethal interstitial lung disease of unknown etiology characterized by aberrant activation of epithelial cells that induce the migration, proliferation and activation of fibroblasts. The resulting distinctive fibroblastic/myofibroblastic foci are responsible for the excessive extracellular matrix production, and abnormal lung remodeling. We have recently found that Mmp19-/- mice develop an exaggerated bleomycin-induced lung fibrosis but the mechanisms are unclear. In this study we explored the effect of MMP19 deficiency on fibroblast gene expression and cell behavior. Microarray analysis of Mmp19-/- lung fibroblasts revealed the dysregulation of several profibrotic pathways including extracellular matrix formation, migration, proliferation and autophagy. Functional studies confirmed these findings. Compared with wild type mice, Mmp19-/- lung fibroblasts showed increased alpha 1 (I) collagen gene and collagen protein production at baseline and after TGF-β treatment, and increased smooth muscle alpha actin expression ($p < 0.05$). Likewise, Mmp19-deficient lung fibroblasts showed a significant increase in growth rate ($p < 0.01$), and in transmigration over Boyden chambers coated with type I collagen or with Matrigel ($p < 0.05$). These findings suggest that in lung fibroblasts, MMP-19 has strong regulatory effects on the synthesis of key ECM components, on fibroblast to myofibroblast differentiation and in migration and proliferation.

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“Functional analysis of the NADP-Dependent glutamate dehydrogenase (NADP-*Kl*Gdh1) of *Kluyveromyces lactis*and(NADP-*Kl*Gdh1) of *Lacchanceakluyvery”*

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Generedundancyis a common featureof living beings, which may occur for a single gene, chromosomic segmentor whole genome.Gene duplicationmay be asource for genetic materialusefulto developnew orspecializedfunctions.The genome of the yeast *Saccharomyces cerevisiae (S. cerevisiae),* arose from a whole genome duplication event (WGD), after which,the loss of 90% of the duplicated genes and the subsequent selective retention of a selected group of genes, shaped S. cerevisiae genome, allowing the development of a facultative metabolism. The paralogousgenes *GDH1* and*GHD3* codify for glutamate dehydrogenases; these enzymes are organized inheterohexamers and are implicated glutamate biosynthesis. These two paralogousenzymes, show 87% identity in amino acid sequence. Previous studies from our laboratory have shown that Gdh1 and Gdh3 display different kinetic properties. Gdh1 has a higher affinity for alpha-ketoglutarate than Gdh3. *GDH1* is expressed in glucose as the unique carbon source while *GDH3* is repressed under this condition and its expression is deepressed when glucose is exhausted or when the yeast is grown on ethanol as the sole carbon source. The glutamate synthase pathway for glutamate biosynthesis (Glt1) is present in *S. cerevisiae*, *Kluyveromyces lactis* and *Lacchanceakluyveri*.*Kluyveromyces lactis* (*K. lactis*) and *Lacchanceakluyvery* (*L. kluyvery*), diverged from the *Saccharomyces* lineage before the WGD event and have a unique orthologous NADP-dependent glutamate dehydrogenase (NADP-*Kl*Gdh1 or*Lk*Gdh1).Respectively, Gdh1-*Kl*Gdh1 and Gdh1-*Lk*Gdh1 /and Gdh3-*Kl*Gdh1 and *Lk*Gdh1 have either 80 or 74 % identity in amino acid sequence. The aim of this project is to characterize the *Kl*Gdh1 enzymeand *Lk*Gdh1 enzyme following three different approaches i) Phenotypic analysis of the single *Klgdh1, Klglt1, Lkgdh1, Lkglt1* and double *Klgdh1-Klglt1* and *Lkgdh1-Lkglt1* mutantsgrown under different physiological conditions ii) kinetic characterization of the enzymes and, iii) analysis of the expression profile.So far, we have shown that the *Kl*gdh1 mutant and *Lk*gdh1 mutant are a glutamate braditrophslacking NADP-Gdh activity, and double mutants *Kl*gdh1-*Kl*glt1, *Lk*gdh1-*Lk*glt1 are glutamate auxotroph. The kinetic characterization of NADP-Gdh´sis being analyzed.

Structural Determinants of Amyloid Fibril Formation in Triosephosphate Isomerase.

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The amyloid fibril is one of the most biologically important protein structures due to its implication in numerous degenerative diseases. Although all proteins have the potential to form these aggregates, because their formation relies on main chain interactions, not all of them can form fibrils under physiological conditions. The structural determinants that promote or inhibit the formation of amyloid fibril remain unknown. This study aims to find these determinants in the human enzyme triosephosphateisomerase, whose ability to form fibrils according to recent reports may be associated with Alzheimer's disease. Aggregation kinetics under destabilizing conditions followed by thioflavin T show that triosephosphateisomerase form cross-beta structures reaching saturation within 72 hrs of incubation. The presence of a cross-beta core in non-fibrillar morphology was also confirmed with the antibody WO1 in dot-blot assays and fluorescence microscopy. The cross-beta region was found by a prediction analysis using several predictors. The three regions with the highest score were synthesized and tested under physiological conditions. The region 221-249 was able to form fibrils confirmed with thioflavin T binding, WO1 dot-blot assay and transmission electron microscopy. Despite the presence of a cross-beta region within the structure of triosephosphateisomerase the enzyme formed non-fibrillar aggregates, this may be due to protection by the proline 239 which make a turn in the last β-strand to stabilize a helix α, all in the region cross-beta. This factor could prevent the elongation of the fibril stopping the aggregation in a non-fibrillar state.

Expression and activity of the alternative oxidase in *Ustilago maydis* under different carbon and nitrogen sources.

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The *Ustilago maydis* has an economic impact on the agriculture sector because it is a pathogen that infects corn. Other ustilaginales are also important because they infect several important plants like rice, sugar cane or sorghum. Due to these characteristics, *U. maydis* is considered as a model for various types of research, among which the host – parasite interactions, dimorphism, and gene regulation are important. The pathogenic activity in these fungi depends on the fusion of two compatible haploid strains, which generate a dikaryotic hypha that invades the host plant. The yeast form (haploid) is unable to cause disease and can be propagated on artificial media. *U. maydis* is used to study various biological processes present in animal cells, because there are several similarities in both cell types. It was shown that this organism is more related to humans than *S. cerevisiae*.
A phenotype shared between *U. maydis* and other organisms i.e. plants, is the presence of an alternative oxidase (AOX); the AOX is a monotopic mitochondrial protein located in the mitochondrial inner membrane that allows a cyanide resistant respiration. The AOX is important in energetic metabolism. The protein has two hydrophobic regions and two or three iron binding motives, which participate in electron transfer from ubiquinol to the eventual reduction of oxygen to water. AOX is a protein that does not generate a proton motive force and therefore does not contribute to energy conservation.
Our group found that depending on the growth phase and the culture conditions used to grow *U. maydis* (YPD; minimal medium-glucose, mm-lactate, mm- ethanol; mm-glycerol), there are changes in the activity of the AOX in this yeast; however, it is not known whether this behavior is due to alterations in the expression of the AOX or the allosteric regulation of its activity.

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Structural studies of glutamyl tRNA reductase

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In most bacteria, glutamyl tRNA reductase is involved in the biosynthesis of heme, building the precursor glutamate-1-semialdehyde in a unique reaction that requires charged tRNAGlu in an NADPH-dependent reaction.

We are interested in studyng the role of 1) glutamyl-tRNA in the catalysis of GluTR 2) the heme group on the regulation of the enzyme and 3) the different domains of the enzyme. By a series of biophysical and biochemical studies that include mutagenesis studies, activity assays, thermofluor experiments, biolayer interferometry and light scattering we have explored these topics, and we will present our most recent results.

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Localization of enzymes involved in nitrogen metabolism in *Capsicum chinense* Jacq. using*in situ* hybridization techniques

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Capsaicinoids are secondary metabolites that are exclusive to the pods of the *Capsicum* genus, which have an extensive use due to their flavor and pungency in the pharmaceutical, paint and food industries, among others. From the horticultural point of view, chilis are the most important crop given its extended cultivation areas. *Capsicum chinense* produces high capsaicinoids contents making it the most pungent pods, which are synthesized and accumulated in the placental tissues.

Since several enzymes involved in nitrogen metabolism, namely nitrate and nitrite reductases, glutamate dehydrogenase, glutamine synthetase and glutamate synthase, have been found to be functional in the placental tissue of Habanero peppers, it was important to determine their cellular localization. *In situ* RT-PCR hybridization allowed the amplification of transcripts, using nucleotides labeled with digoxigenin, which later would interact with antidigoxigenin antibodies coupled with alkaline phosphatase. The posterior addition of NBT (nitro blue tetrazolium) and BCIP (5-bromo-4-chloro-3'-indolyl phosphate) generated a colored signal whose intensity was proportional to the transcripts' contents of the studied enzymes. The cell-specific location patterns support the idea of a direct relationship between the *in situ* synthesis of precursors and capsaicinoids accumulation in the pods.

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Study of the interactions of atypical subunit ASA1 in the ATP synthase of *Polytomella* sp.

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The mitochondrial ATP synthase of chlorophycean algae has a structure different from that of other organisms. All the subunits that typically make up the the peripheral arm and those that are involved in the dimerization of the enzyme are missing. In compensation, it has acquired nine subunits of unknown evolutionary origin that have been named ASA1 to ASA9. These ASA subunits are only present in chlorophycean algae and are not found in others closely related algal lineages, such as ulvophycean, prasynophycean and trebuxophycean green algae. Heat dissociation experiments, cross linking studies and electronic microscopy studies have allowed the proposal of a structural model of the ATP synthase of chlorophycean algae in which ASA subunits make up the peripheral arm and participate in the dimerization of the enzyme; however, the localization of ASA1 subunit remains unclear. The objective of this work is to clone and purify the ASA1 subunit to perform interaction studies in order to know which are its neighboring subunits and to propose its topological disposition in the peripheral arm of the ATP synthase of *Polytomella* sp. The experimental strategy is based on the cloning of the corresponding gene, the overexpression of the protein in *Escherichia coli* and the purification of the recombinant protein in order to perform interaction assays. In this work, we report an ASA1-OSCP interaction, which could link a classical protein of the enzyme, such as OSCP, with an atypical subunit unique to the chlorophycean algal lineage.

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The Role of the Erythrocytes in the Serum Enzyme Levels that Regulates Nitrogen Metabolism in Humans: the OTC.

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Introduction

In previous studies we reported that the human erythrocyte is capable of metabolizing arginine into various products such as nitrites, citrulline, ornithine, and urea. This finding was particularly evident when we used human erythrocytes from patients that suffered diabetes mellitus type 2. Therefore, the erythrocyte capacity to catabolize arginine into the products mentioned above could be related to the active presence of oxide nitric sintase (NOS) and arginase type 2. The stoichiometry of the production of ornithine and urea is correlated with the arginase activity. However, the relation between nitrites and citrulline was not maintained because there may be additional production of this last amino acid**.** Results suggest that the human erythrocyte has another enzymatic activity responsible for the extra citrulline synthesis. The ornithine-transcarbamilase (OTC) is the most adequate candidate for the extra production, but an active OTC has not been described until now.

Objective

The objective of this research is to determine whether the human erythrocyte has an active OTC like the one mainly found in the hepatic mitochondria.

Results

We obtained erythrocytes from centrifugation of the blood samples of 50 healthy volunteers. The differential centrifugation of erythrocytes allowed us to separate the cytosol from the plasmatic membranes. We were able to identify the OTC activity in cytosolic fractions of human erythrocytes. We performed enzymatic kinetic studies for these preparations and compared them to the kinetic determination of the same enzyme obtained from human and rat liver to establish if it was the same enzyme. Finally, we carried out immuno-detection studies of the protein at different fractions of human blood.

Conclusions

With the evidence obtained from these studies we can confirm the presence of the OTC in human erythrocytes. According to the kinetic characterization it is an isoform of the hepatic enzyme, which we are naming as OTC type 2-CH.

cDNA cloning of fructose bisphosphatase and tissue expression in the shrimp *Litopenaeusvannamei*

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Glucose is produced *de novo* from non-carbohydrate precursorssuch as lactate, pyruvate, glycerol, and amino acids in gluconeogenesis. Fructose 1-6, bisphosphatase (FBP) is a key enzyme for gluconeogenesis and catalyzes the production of fructose 6-phosphate from fructose 1-6-bisphosphate. In crustaceans, the presence of gluconeogenic enzymes has been reported, however, there are still few studies about cDNA characterization and expression. Here, we report the complete cDNA sequence for the white shrimp FBP that is composed of 1197 bp with start and stop condonsat positions 63 and 1065, respectively. The 5′-untranslated region (UTR) is 62bp and the 3′-UTR is 129 bp long, excluding the poly-A tail. The predicted protein contains 334 residues and has a calculated molecular weight of 36.2 kDa and pI of 6.0, similar to other FBP proteins from invertebrates. Sequence analyses of the deduced FBP showed that this protein shares high homology with the FBP counterparts from *Marsopenaeusjaponicus* (95 %)and with other related taxa FBP. An alignment of the amino acid sequences of different FBP showed that the residues that are in contact with the substrate fructose-1,6-bisphosphate are highly conserved, as well as those in contact with fructose-2,6-bisphosphate and magnesium. Finally, we evaluated the expression of FBP by RT-qPCRfinding that it is present in all tissues analyzed (hepatopancreas, gills, muscle, hemocytes, pleopods and intestine);this suggest that FBP is generating intermediates compounds for either gluconeogenesis or glucose 6-phosphate pathways in these tissues.

Curtobacterium sp. strain MR2 exhibits two lead-resistance mechanisms dependent of cell density

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Introduction

Heavy metals are located in a natural way in the earth crust. These may be converted in contaminants, whether its distribution in the environment is altered due to anthropogenic activities. Lead is a persistent environmental contaminant which is accumulated in the tissues of organisms, generating biomagnification along the food chain. To help with this problematic, characterization of microorganisms able to tolerate heavy metals, that may be useful biological-tools in bioremediation of contaminated sites are needed (Naik & Dubey, 2013).

The goal of this research was the identification of microorganisms associated with the rhizosphere of plants that grow in mine wastes found in the Biosphere Reserve "Sierra de Huautla" and which could have a future application in the remediation of soils contaminated with heavy metals.

Methods

The growth and isolation of bacteria were made in mineral medium with low phosphorus and nitrogen,supplementedwith 200 ppm $Pb^{+2}$by microbiological standard methods. The resilience assays were made followingthemethoddescribed byJin ZM, S. W. (Jin ZM, 2013). PCR amplification of 16S rDNA was done using standard primer set 8F and 1492R and sequencing was done at IBT-UNAM, Morelos, Mexico. DNA sequence was compared with GenBank reference database using NCBI-BLAST search tools (Altchul *et al.*1997).

Results and discussions

Several aerobic rizospherical lead-resistant bacteria were isolated from mining wastes soil, associated with several vegetal species, located on the Biosphere Reserve “Sierra de Huautla”, Morelos, Mexico. These mining wastes contain an average of 1600 mg/Kg of Pb+2. The capacity of resistance of isolates were tested through resilience assays with concentrations from 0.6 to 3.6 mM of $Pb(NO_3)_2$, through these assays were isolated eight strains that tolerate above 2.4 mM of Pb^{2+}. One of the isolates, which can grow in presence of 3.6 mM of Pb^{2+}, in minimal salt medium, was identified as *Curtobacterium* sp. and designated as strain MR2. This strain was associated with *Dalea leporina* roots. Interestingly, this plant is one of those that present a lower concentration of lead in its tissues, only 87.4 mg/Kg. *Curtobacterium* sp. MR2 exhibits two lead-resistance mechanisms in solid mineral medium, dependent of cell density. In lower dilutions $1x10^{-1}$ to $1x10^{-4}$ (high cell density), colonies of this strain, produces an exopolysaccharide for extracellular sequestration of lead. In contrast, in higher dilutions $1x10^{-5}$ to $1x10^{-8}$ (low cell density), colonies take on a brown coloration, possibly by oxidation or precipitation of lead. Because to these phenotypes, this strain could play, an important role in bioremediation of lead contaminated soil.

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Physicochemical characterization of the folding and binding mechanisms of ArtJ, a substrate-binding protein from *Geobacillusstearothermophilus*.

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The folding, structure and stability of proteins have been exquisitely tuned by evolution in order to allow life adaptation to an enormous diversity of environments. Sequence variation allows proteins with the same structure to carry out different functions and display distinctive physicochemical properties. Substrate binding proteins (SBP) are such an example. SBPs share a common bilobular topology composed of Rossman fold motifs. SBPs are in charge of the transport of a variety of ligands inside cells. The folding and ligand binding properties of the lysine-, arginine-, ornithine- periplasmic binding protein from *Salmonella typhimurium* (LAO) has been extensively studied in our lab. At equilibrium, the unfolding transitions induced by urea or temperature are two-state, however, unfolding and refolding kinetics show an intermediate state. Binding is strongly enthalpy-driven, and the heat capacity change is dominated by the conformational change that take place upon ligand binding. In order to determine how these properties are modulated in a thermophilic environment, we undertook the study of ArtJ, the SBP homologous to LAO from *Geobacillusstearothermophilus.* We have successfully expressed and purified recombinantArtJ in *E.coli*. Results on the folding and binding properties of this protein will be presented.

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Rewriting theterminar marking mechanism by deamidation in human triosephosphateisomerase

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Deamidation, the spontaneous conversion of asparagine and glutamine to aspartic and glutamic acid is one of the most commonly occurring posttranslational modifications altering the structure or function of proteins. As deamidation reaction rates are encoded in the protein structure, it was proposed that deamidation can serve as molecular clocks for the timing of biological processes such as protein turnover, development and aging. In human triosephosphateisomerase (HsTIM for example, it has been proposed that deamidation of N15 and N71 signals the terminal marking of the protein by introductionsof adjacent negative charges at the protein interface. In this work, we introduceddeamidations on HsTIM by sited directed mutagenesis and studied the contribution of each deamidation over the functional and structural properties of the enzyme; to this end, three HsTIM mutants (N15D, N71D and N15D/N71D) were constructed and characterized. The results show that the N71D mutant resembles structurally and functionally the wild type enzyme;in contrast, the N15D mutant display all the disruptive effects related to deamidation. The N15D/N71D mutant shows only minor additional effects to the N15D mutation, supporting that deamidation of N71D has negligible effects on HsTIM. The Crystal structure of the N15D mutant shows that deamidation induces conformational changesthat modifies the catalytic site and alters the structure of M14 and loop 3, both critical elements of the HsTIM interface.

Kinetic and metabolic analysis of tumor Krebs cycle

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The Krebs cycle (CK) is an important pathway in the cell due to produces the reducing power to generate the proton gradient necessary for ATP synthesis. In isolated mitochondria from tumor cells the activity of Krebs cycle enzymes and flux increase significantly respect to rat liver mitochondria (RLM) [1]. This change was attributed to increase in the activity of enzymes that exert control in non-tumor cells (isocitrate dehydrogenase, ICDH; 2-oxoglutarate dehydrogenase, 2OGDH) and it was suggested that the control distribution in tumor cells may be different compared with non-tumor cells.

To establish qualitatively a possible difference in the distribution of control in the CK of tumor cells, the kinetic characterization of enzymes of the pathway and other enzymes that are not strictly part of the CK but feed it with intermediaries such as aspartate amino transferase, glutamate dehydrogenase and pyruvate dehydrogenase (PDH) was carried out in isolated mitochondria of AS-30D tumor cells and rat liver. In general, the V_m values were significantly higher in tumor mitochondria (1.8-20 times) compared to RLM, although the affinities (K_m) for each substrate were similar in both types of mitochondria. It was found that enzymes with low catalytic efficiency were the ICDH, 2-OGDH and malate dehydrogenase in both types of mitochondria. These results suggest that these enzymes may be main controlling steps in both types of mitochondria. To validate this hypothesis is necessary determined the physiological concentrations of substrates, products, inhibitors and activators which may modify the activity of enzymes, especially of ICDH and 2-OGDH, whereby the flux distribution may be different between tumor and normal cells.

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Molecular characterization and tissue expression of betaine aldehyde dehydrogenase from white shrimp *Litopenaeus vannamei*

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The enzyme Betaine Aldehyde Dehydrogenase (EC 1.2.1.8; BADH) catalyzes the oxidation of betaine aldehyde oxidation to glycine betaine. The glycine betaine contribution as methyl donor for methionine synthesis and cellular osmoregulation has been described on marine species, but information about BADH gene expression is not well investigated in marine crustaceans. We report the molecular characterization and tissue expression of BADH from white shrimp *Litopenaeus vannamei* using quantitative PCR, PCR amplification, cloning and sequencing. The partial cDNA sequence of BADH was obtained of gills from white shrimp, and is 907 bases pare and code for 150 amino acids. The BADH partial sequence corresponding to ~80% of the full sequence compares to marine invertebrate homologs as the pacific oyster *Crassostrea gigas*. BADH sequence is similar to some vertebrates homolog's and contains the important domains for their function and regulation. Moreover, BADH nucleotide sequence is 97% and 99% identical to two expressed sequence tags of eyestalk cDNA library from *L. vannmei.* BADH expression was detected in gills, hepatopancreas, muscle and heart tissues with the higher expression in gills compare to the other tissues. The present study is the first step for know and understand the molecular mechanism involved in the response of marine crustacean to cellular osmoregulation by BADH.

Key words: Betaine aldehyde dehydrogenase, gene expression, *Litopenaeus vannamei*, molecular characterization

Evaluation of Plant Lectins Interaction with *Aspergillus parasiticus*.Edmar de Jesús Díaz García[1], Marco Antonio Sánchez Medina[1], María del Socorro Pina Canseco[2], Alma Dolores Pérez-Santiago[1]. [1]Instituto Tecnológico de Oaxaca, Av. Víctor Bravo Ahuja No. 125 Esq. Calz. Tecnológico, Oaxaca, Oax. C.P. 68030. [2]Universidad Autónoma Benito Juárez de Oaxaca, Carretera Antigua a San Felipe del Agua S/N. Oaxaca. C.P. 6820. edg.89@live.com.mx

Aspergillus flavus and *Aspergillus parasiticus* are the cause of damage of many foodstuffs; the damages caused in grains during it storage are an important case of it, because they also produce aflatoxins, which are highly carcinogenic compounds. Someproteins (proteases and trypsin inhibitors) can inhibit the development of *Aspergillus* and the production of aflatoxins. Also, the interaction between lectins and microorganisms could modify the normal development of the microorganism. The object of this research was to evaluate the different effects on the interaction between the plant lectins with *A. parasiticus*. In this work, the interaction between *A. parasiticus* spores and mycelium, and lectinswas evaluatedby the agglutination technique reported by Baker in 2009. These lectins were: the Concanavalin A specific to manose and glucose, *Dolichusbiflorus* specific to galactose, and N-acetylgalactosamineand *Ulexeuropeaus*agglutinin I specific to L-fucose, marked with FITC. In fluorescence microscopy, three lectins showed a more intense marking in septas and mycelliuostips; moreover,lectinsonlymarked some zones of *A. parasiticus* spores, and in both cases the cell agglutination was observed. Therefore, we can say that the zone where a great fluorescenceis shown is where specific sacaridstructuresfor lectins exist.

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Identification of the amino acids responsible for the differences in susceptibility to inactivation by methylmethanethiosulfonate of the triosephosphateisomerasesfrom*Trypanosomabrucei* and *T. cruzi*.

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Triosephosphateisomerase (TIM) is a glycolytic enzyme that catalyzes the conversion between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Its amino acid sequence is quite conserved for the corresponding proteins of the human parasites *Trypanosomabrucei* (Tb) and *T. cruzi* (Tc), since an alignment of TbTIM and TcTIM has an identity of 73%. Also, a superposition of the three dimensional structures of these two enzymes has a Root Mean Square Deviation of0.96 Å. Albeit being so similar, or even partially identical, they react quite differently when exposed to certain reagents. A very clear example is the inactivation of these two enzymes with the derivatizing reagent specific for cysteines methyl methane thiosulfonate (MMTS). TcTIM is 70 times more susceptible to inactivation than TbTIM, when incubated with MMTS. To explore which of the 65 different amino acids in both TIMs are responsible for this different behavior, we divided the sequence into 8 regions, taking advantage of the $(\beta/\alpha)_8$TIM barrel structure of the enzyme. We produced several chimeric TcTIMs that had an increasing number of regions from TbTIM. After many experiments we determined that both regions 1 and 4 together of TbTIM are sufficient and necessary for a TcTIM to loose its enhanced susceptibility and to inactivate like TbTIM in the presence of MMTS (García-Torres, I. et al., PLoS ONE 2011). These two regions are separate in the three dimensional structure of both TIMs and have no close range interactions between them.

In this work we set out to identify and determine which amino acids of regions 1 and 4 are responsible for the change in susceptibility of TcTIM and also the minimal number of mutations required for this change. Using a chimera having regions 1 and 4 from TbTIM and regions 2,3,5,6,7and 8 from TcTIM as a template, or a chimera with region 4 of TbTIM and all other regions of TcTIM we have analyzed over 20proteins with different point mutations. Our current results indicate that five amino acids in region 1 and in two amino acids in region 4 are responsible for the resistance to inactivation by MMTS in wild type TbTIM.

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ANALYSIS OF GLYCEROL DEHYDROGENASES IN *Mucor circinelloides* YR-1

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Summary

Key words: alcohol dehydrogenase, glycerol dehydrogenase, hydrocarbons, metabolism.

Alcohol dehydrogenase (ADH) is an enzyme that catalyzes the conversion of alcohols to aldehydes and ketones. The reaction is reversible, reducing carboniles compounds to alcohols using NAD or NADP as coenzyme. Some of the substrates that this enzyme may use are aromatic and aliphatic alcohols with lineal or branched chain (Reid and Fewson, 1994).

Glycerol is a polyalcohol of 6 carbons and it is one of the most abundant biochemical compounds in the biosphere. A number of functions have been addressed to mannitol in filamentous fungal; storage of carbohydrates, stress tolerance, dispersion and/or release of spores. The development of techniques of genetic manipulation in filamentous fungi has accelerated the understanding of its functions and mannitol metabolism

In *Mucor circinelloides* YR-1, there are two inducible $NADP^+$-dependent glycerol dehydrogenase activities, one of them iGlcDH2, was specifically induced *n*-decanol when it was used as sole carbon source in the medium. The other iGlcDH1 was induced by alcohols and aliphatic or aromatic hydrocarbons it uses exclusively glycerol as substrate meanwhile, iGlcDH2 has a much broader substrate specificity having a low activity as an ethanol dehydrogenase with NAD^+ or $NADP^+$ as cofactor. Both isozymes showed an optimum pH for activity of 9.0 in Tris-HCl buffer and are subject to carbon catabolite repression. In contrast the constitutive $NADP^+$–dependent glycerol dehydrogenases (GlcDHI, II and III) were only present in cell extracts when the fungus was grown in glycolytic carbon sources or glycerol under oxygenation and its optimum pH was 7.0 in phosphate buffer. In addition to the five $NADP^+$-dependent glycerol dehydrogenases, a NAD^+–dependent alcohol dehydrogenase is also present in glycerol or *n*-decanol medium and has weak activity as glycerol dehydrogenase.

In the strain YR-1 of M. circinelloides, native of contaminated sites with hydrocarbons, the studies have focused in researching the influence of different kinds of compounds (alcohols and aromatic and aliphatic hydrocarbons), on the production of the ADH enzyme(s) that use short and long chain alcohols and dihydrodiols as substrate and that are involved in the pathways of degradation of aliphatic and aromatic hydrocarbon, respectively (Zazueta et col. 2008). Previous studies have demonstrated that strain YR-1 of M. circinelloides has a great variety of ADHs, which perhaps, are involved in the metabolic machinery able to adjust to degradation and use of aromatic and aliphatic hydrocarbons, and alcohols too.

Amino acids as modulators of root development in habanero pepper (*Capsicum chinense*Jacq.)

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Introduction

L-amino acids represent a major fraction of the lowmolecularweight organic N that is dissolved in the soil. In general, these compounds are found at low concentrations in the soil, approximately 0.01 to 10 μM, but their levels reach millimolarlevels in patches withdecomposingorganic matter. In these patches, glutamic acid, serine, glycine, alanine, and aspartic acid are the most abundant amino acids. In addition to their importance as a N source for some plants in low-N systems, it has been recently suggested that amino acidsmay be sensed in the root tip by specific receptorsand act as signaling molecules that indicate the presence of nutrient-rich patches in the soil. D-aminoacids also occur naturally in several organisms,although the physiological relevance of these substances in higher plants remains unknown.To characterize the effects of amino acids on root growth, we used seedlings of habanero pepper (*Capsicum chinense*Jacq.), one of the most widely cultivated annual and spice crops in the world. The response of this specie to the presence of amino acids was exposition time-, amino acid type-, dosis-, and stereoisomer- specific.

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Structural studies of the human triosephosphate isomerase. Artificial deamidation in the amino acid residues 15 and 71 and his effect over structure.

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The human triosephosphate isomerase (HsTIM) is an enzyme that deamidate spontaneously in the amino acid residues 15 and 71, this deamidation is the conversion of asparagine (N) to aspartic acid (D), it was proposed that deamidations can serve as molecular clocks for the timing of biological processes such as protein turnover. In HsTIM, it has been proposed that deamidation of N15 and N71 signals the terminal marking of the protein by introduction of adjacent negative charges at the protein interface. In this work, we simulated deamidations (artificially) by site directed mutagenesis and studied the contribution of each deamidation over the crystallographic structures. Three HsTIM mutants (N15D, N71D and N15D/N71D) were constructed, crystallized and solved the crystallographic structures. The results show that the N71D mutant resembles structurally the wild type enzyme. In contrast, the crystal structure of the N15D mutant shows that the mutation induces conformational changes that modifies the catalytic site and alters the structure of M14 and loop 3, both critical elements of the HsTIM interface. Unfortunately the N15D/N71D mutant did not render interpretable diffraction patterns and were discarded for analysis. These results indicate that the deamidation in HsTIM is an important process that promotes conformational changes affecting structurally this enzyme and potentially drive it to their degradation.

Changes in affinity and molecular basis of multiple specificity of a periplasmic binding protein for basic amino acids; The LAO-protein. Andrés Escandón Flores, Nancy O. Pulido Mayoral, Alejandro Fernández-Velasco, and Alejandro Sosa Peinado, Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Depto. Bioquímica, Facultad de Medicina (UNAM). Email: buzo_2000@hotmail.com.

The periplasmic binding proteins (PBP´s) in bacteria and archae are the first component during the transport of diverse metabolites from the periplasm to the interior of the cell. The function of PBP´s is to trap the ligand and transfer to the permeases associated to the membrane. These proteins are characterized for a biolobulated structure that binds the ligand at the interface between the lobules. The protein present two structures because this protein change conformation upon ligand binding: the open form without ligand and the closed form in the presence of ligand,

We are interested in the molecular basis of specific of this protein to their ligand for future “protein redesign”; our model of study is the LAO-protein that binds *L*-lisina, *L*-arginina y *L*-ornitina from *Salmonella typhimurium*. This protein has multiple specificity for basic amino acids in the range of nanomolar, except for histidine that binds in the micromolar.

Nature has been achieved a different solution for the affinity for basic amino acids, the homologous protein HisJ, that binds histidine with high affinity, and low affinity for *L*-lisina, *L*-arginina, contrary to LAO-protein. Between the two proteins the percent of similarity is 85 %, thus comparing both structures, the only four residues different at the binding site were mutated in LAO-protein by the sequence present in HisJ protein (Phe-52-Leu, Ser-120-Thr, Tyr-190-Leu, Asp-193-Val). The aim of this study is to understand the molecular basis of the affinity of LAO-protein to basic amino acids, and the changes in molecular specificity.

In this work we present the changes in affinity observed by intrinsic fluorescence for the individual mutants and for the four mutations at once. The data are analyzed in term of structural energetic by the enthalpic and entropic changes observed from the Isothermal Calorimetry titration and molecular modeling of the mutated proteins in sílico.

Characterization of Dystroglycan on Kasumi 1 cell line

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Leukemia are a group of diseases with a significant importance in the clinical field and as many types of cancer, its incidence has increased both among children and adult.Leukemia are diseases of the hematopoietic system produced by a failure in differentiation process as well as in their overproduction; theyoriginate from a hematopoietic stem or from a progenitorcell. Recently proteins of the cell membrane such as dystroglycanhave been implicated to cancer development in solid tumors, where proteolysis and altered glycosylation, causes loss of adhesion and migration of cancer cells. On the other hand, deficiencies in glycosylation of dystroglycan due to a low expression of glycosyltransferasesthat affects the processing of the molecule and subsequent adhesion to the matrix. In this study we characterized dystroglycan molecule in a cell line of Acute MyeloidLeukemia, named Kasumi 1, were performed biochemical assays to determinate the pattern expression of the protein, and through Fluorescence-activatedcellsortinganalysis we describedthe cellular distribution of the protein. To identify the level of dystroglycan glycosylation, cells were processed for flow cytometer. Kasumi 1 cells were also differentiated to macrophage lineage compared to mononuclear cells obtained from healthy individuals. Our results showed the expression of the protein in all cells studied; with adistribution mainly located at the periphery of the cell membrane, dystroglycan phosphorylated beta subunit was found in the nucleus as clusters formed which was diminished in the Kasumi cells differentiated to macrophages. The presence of this protein was not able detected by immunofluorescence technique. Theflow cytometer analysis demonstrated a significant decrease in the glycosylation of dystroglycan in differentiated Kasumi cells compared to cells from the non-differentiated cell line. All together these results will allow us to correlate the decrease of the protein with a reduction of adhesion to the hematopoietic niche and therefore an earlyrelease to bloodstream that is a characteristic feature of leukimia.

Assembly and distribution of the OXPHOS complexes and supercomplexes reflect energy metabolism in two human glioblastoma cell lines

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Tumor cells metabolic profile and those which they originate is not the same, as it the case in tumor cell glycolysis where it provides the energy and substrates required for both the biosynthesis and cell proliferation. Whereas in non-cancer cells it is carried out by oxidative phosphorylation (OXPHOS). In order to explain this abnormal bioenergetic phenotype, investigators have conducted studies to determine the metabolic and molecular alterations that have place in tumor cell mitochondria, where there are some known deficiencies in enzymes of OXPHOS and even changes into protein expression, which have been proposed as biomarkers of disease. Such is the case of β-ATP synthase as well as GAPDH which have been considerate to have relationship as an index or prognostic marker for various cancers. OXPHOS complexes are associated together to form respirosomes or super complexes whose deficiency is associated to different pathologies, so that mitochondrial alterations that occur in cancer may also be reflected in both the organization and rearrangement of mitochondrial respiratory complexes.
In the present work it was used two human glioblastoma cell lines: U87MG and T98G which have glycolytic and oxidative metabolism, respectively, in order to obtain energy. The bioenergetics index and the measurement of oxygen consumption and lactate production, confirm metabolic profile on both cell lines. As for the five respiratory complexes, it was noted by western blot assay that the protein expression, was higher in isolated mitochondria of T98G line, in this line, the organization of complexes is more efficient since ATPase is able to form dimers and tetramers, and in addition, complex III assembles into super complex. Conversely the loss of supercomplex assembly in the U87MG line seems to be accompanied by a reduction/loss of the complex I activity. Proteomic approach by 2D electrophoresis on mitochondria showed some differences on protein expression pattern. It is important continue with this analysis, since protein identification could highlight other mitochondrial metabolic pathways involved in carcinogenesis. .
These results suggest that supercomplexes formation is essential for a good OXPHOS function. Lack of these supercomplexes could be related to defects in mitochondrial respiration and enhanced glycolysis in certain cancers, where there are a decrease in mitochondrial respiratory enzymes expression and a disorganized respiratory chain. An understanding of how the main metabolic pathways are modified by cancer cells, may lead to new strategies for cancer therapy.

Structural and functional characterization of Whiteleg shrimp thioredoxin (*Litopenaeus vannamei*).

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Thioredoxins are proteins of 12 kDa that we can find in every living organism, from bacteria to humans. These proteins tend to have a conserved sequence WCGPC within their active-center which can experiment two different states: reduced or oxidized. Thioredoxins act as an apoptosis inhibitor and cell growth factor, but they also perform many other functions when activating transcriptions factors or repairing proteins. In the case of thioredoxin of Whiteleg shrimp *Litopenaeus vannamei* is one of the most important enzymatic systems for cellular redox control and helps eliminate damage occasioned by oxidative stress.

Structural studies carried out so far, for most thioredoxins suggest that the protein appears to be in a monomeric form. Nevertheless, in the case of human thioredoxin and white shrimp *Litopenaeus vannamei*, crystallographic studies suggest that the protein is a homodimer which consists in a disulfide bridge between cysteine 73 for each monomer. This project is intended to study interactions of the wild type protein and a mutant C73S and their substrates by Nuclear Magnetic Resonances (NMR). We are also studying the oligomeric state of the protein in solution and making comparative studies for the dynamics between thioredoxins from different species. Our research and studies in this field will strongly contribute to the structural and functional characterization of both wild type and the mutant protein.

Effect of chronic exposition to extreme low frequency electromagnetic fields on fatty acid contents and lipoperoxidationin different regions of rat's brain.

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Diary exposition to electromagnetic fields generated by various electric and electronics devices as mobile phones, computer displays and home electrical installation, can origin a chronic exposition to extreme low frequency electromagnetic fields (ELF-EMF). The interaction between this type of radiation and the biological systems still unclear but there are some theories that try to explain this interaction.One of them is the half life extension of free radicals that can produce damage to any kind of tissue.For example the brain is particularly vulnerable to damage bythese free radicals due to its high metabolic rate and its low antioxidant enzymes levels.Lipidsplays an important role in nervous system, involving multiple signaling pathways and synaptic. It is known that physical stress has an effect on oxidative stress leading alipoperoxidation.

The aim of the present study was to evaluate the effects of chronic exposure (2 h/21 days) to ELF-EMF (60 Hz, 2.4 mT), restraint stress (RS) as positive control, and ELF-EMF+RS, in the different brain structure of adult Wistar rats(cerebral cortex, cerebellum and sub cortical structures). The cerebral structures were processed to obtain total lipids and to determine: TBARS (Tiobarbituric acid reactant substances), Non esterified fatty acids (enzymatic kit), and Fatty acid methyl ester (Gas chromatography coupled to mass spectrometry). Also, a plasmatic corticosterone level was determined to corroborate the physiologic stress induced by RS and EMF.

Preliminary result showed a significant increase in corticosterone in all treatments groups, these concentrations have a positive correlation with TBAR's in cerebral cortex. In other hand NEFAS demonstrate an increase in EMF-ELF treatment on subcortical structures. Analysis of FAME's showed a significant decrease in relative content of polyunsaturated fatty acids (arachidonic acid, eicosenoic acid, docosahexenoic acid and docosatretraenoic acid) in cortex and cerebellum in RS and ELF-EMF treatments. All statistical analysis was carried out with one way ANOVA and Tukey test post hoc($p<0.05$).

NMR assignment and dynamics of LAO protein.

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Many proteins exhibit a large-scale movement of rigid globular domains. Among these, bacterial periplasmic binding proteins (PBP) involved in substrate transport, can be used as prototypes for understanding the mechanism of the motions and the associated functions, such as substrate binding, catalysis, and recognition by other biomolecules. After binding their respective substrates, the PBP interact with the membrane-bound complex and translocation of the substrates from the binding proteins to cytoplasm takes place. The LAO (lysine/arginine/ornithine-binding) protein is one of this PBP, its three-dimensional structure have been determined with and without substrate by x-ray crystallograpy. The crystal structure shows two lobes connected by a short linker, with the ligand-binding site located in the inter-lobe cleft. This protein has 238 residues an a molecular weight of 26 kDa. Its presumed “opening-closing” movement of the two domains has been described as a “pac-man” motion. However, despite the three-dimensional structures, relatively few connections between function and dynamics have been established. We are using NMR spectroscopy to study the dynamics and interactions of the LAO protein from E.coli.

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Study of the Pet309 function by site directed mutagenesis in *Saccharomyces cerevisiae* mitochondria.

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Pet309 is a member of the **p**entatrico**p**eptide **r**epeat family of proteins (PPR). It is necessary for translational activation of the *COX1* mRNA present inside mitochondria, which codes for the cytochrome *c* oxidase subunit 1. Pet309 is imported to the mitochondria, where it interacts with the *COX1* mRNA 5'-UTR to activate translation. PPR proteins have 2 to 26 degenerate motifs of 35 amino acids arranged as tandem repeats. Each motif forms a pair of anti-parallel α-helixes and the consecutive helical hairpins result in a super helical structure similar to a solenoid (1). These proteins are mainly located in mitochondria and chloroplasts, they are involved in RNA metabolism, and each member of this family has one or two target RNAs. Single stranded RNA is bound to the inner groove formed by the solenoid and it was proposed that these proteins could form a dimmer in the presence of RNA (2). Recent work with plant PPR proteins has proposed that positions 2, 5 and 35 of each PPR motif are important for RNA binding and recognition (3). However it is not known whether this code is important for yeast PPR proteins. The goal of this work is to try to identify residues that could be facing the surface that interacts with RNA and that could be important for Pet309 function. Our previous work demonstrated that two basic amino acids facing the internal groove of Pet309 are important for protein function (4). In the present work we generated structural models of the predicted 13 central PPR motifs of Pet309 using the I-TASSER and Robetta servers. We identified some basic, acid and aromatic residues that are predicted to face the internal grove of the solenoid, and that could be important for electrostatic or stacking interaction with the *COX1* mRNA. Next we generated point mutants in some of these residues to analyze their phenotype and the effect on cytochrome *c* oxidase function. In addition we analyzed if *in vivo* this protein forms a dimmer. These data contribute to understand the mechanism of RNA binding of Pet309.

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Identification of a lectin from blue mussel (*Mytilusedulis)* belonging to an unclassified family of lectins

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Marine invertebrates lack adaptive defense system, so that the immune response is mainly based on cell components such as the hemocytes and a large number of proteins that are part of a humoral component. Lectins play an important role in the defense of these organisms as they have the ability to recognize surface antigens that are expressed in bacteria such as LPS (lipo-polysaccharides) and carry out agglutination mechanisms and hemolysis. Very little is known about the defense mechanisms from these organisms which are important due to commercial interests.

In this work we report the isolation and properties of a new lectin from edible blue mussel (*Mytilusedulis)*that shows specificity for D-galactose and α-oligosaccharides such as raffinose and α-lactose. The lectin was purified by affinity chromatography using α-lactose-agarose-bound column,its electrophoretic profile showed a single band corresponding to a molecular weight of 17,000 Daltons in SDS-PAGE. Hemagglutinating activity of thelectin was not dependent on divalent cations and the specific activity was 5.3 x 10^6 U/mg. Amino acid sequence was obtained after in-gel trypsin digestion and LC/MS analysis of the peptide fragments. The resulting sequence consisted on 149 amino acid residues and three tandem repeats with high sequence similarity to each other (up to 65%) and does not belong to any known lectin family. This lectin has up to 90% sequence similarity with *Crenomytilusgrayanus*lectin (CGL) which is a GalNAc/Gal-specific member of an unclassified family of lectins. On the base of GalNAc/Gal specificity and other properties, CGL was assumed to be a member of galectins, awidespread animal lectins found in both invertebrates and vertebrates. Amino acid sequence analyis of four CGL peptides with total molecular weight of 6.14 kDa (one-third of CGL molecular weight) showed no similarity to galectins nor other animal lectins reported earlier. According to circular dichroism analysis, we found a lack of secondary structure of *Mytilusedulis*lectin, but when it binds to the ligand, a β-structure folding is acquired.The amino acid sequence of *Mytilusedulis*lectin was used to predict its folding by protein structure homology with help of modeling servers. Prediction of *Mytilusedulis*lectin fold by I-Tasser revealed that this lectin shared a common ß-trefoil fold with ricin B-like lectins. According to FUGUE server, CGL and ricin B-like lectinsCel-III from *Cucumariaechinata* (PDB ID: 1VCL), EW29 from *Lumbricusterrestris* (GenBank ID:BAA36393) and MOA from *Marasmiusoreades* (PDB ID: 2iho) possessed a similar fold.Proteins with the ß-trefoil fold consist of six two-stranded ß-hairpins, three of them form a barrel structure while the other three are in a triangular array that caps the barrel. Proteins from these families possesses distinct functions, significant differences in their sequences, ligands, and their manner of ligand binding; therefore, they are assigned to different protein families.

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New target of proton pump inhibitors: inactivation of triosephosphateisomerase from*Giardia lamblia* by Omeprazole derivatives.

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Proton pump inhibitors (PPIs) are a family of compoundswhose main action is reduction of gastric acid production. These drugs are useful in the treatment of many conditions, such as dyspepsia, peptic ulcer disease, gastro esophageal reflux disease among others. Some clinically used proton pump inhibitors are omperazole, pantoprazole, rabeprazole, lansoprazole and esomeprazole. PPIs act by irreversible blocking the H^+/K^+ ATPase of the gastric parietal cells, mainly by derivatization of Cys residues. Recently, it has been proposed that omeprazole inactivates triosephosphateisomerase from *G. lamblia*(GlTIM). This protein is ahomodimeric enzyme that catalyzes the fifth reaction of glycolysis. GlTIM has five cysteine residues per monomer. Studies that combine site directed mutagenesis approaches and inactivation studies of recombinant GlTIM with sulfhydryl reagents, have been demonstrated that derivatization of Cys 222 is the main event that promotes the loss of enzyme activity. In contrast, recombinant human TIM (HuTIM) is highly resistant to sulfhydryl compounds. In order to evaluate the effect of omeprazole derivatives in GlTIM activity, we studied the kinetic, structural and chemical modifications caused by some PPIs. Using kinetic andspectroscopic techniques, we have found that these compounds inactivate GlTIM through derivatization of Cys 222, without affect HuTIM activityalthough this enzyme is chemically modified. Taking together, we proposeomeprazole and its derivatives as potential antigiardiasicleader molecules for further drug design studies.

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Is the loss of protein interaction in Ribosome Biogenesis the cause of Shwachman-Bodian-Diamond Syndrome?

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Ribosomes are essential molecular machines responsible of decoding the mRNA into proteins. These macromolecules are made of one large subunit and one small subunit, named in eukaryotes 60S and 40S, respectively. The pathway to create ribosomes is the Ribosome Biogenesis and it starts in the nucleolus, going through the nucleus and finishing in the cytoplasm. This pathway involves the synthesis, modification, assembly and transport of fourrRNAsand 80structural proteins. To make this possible, the cell needs the intervention of more than 200 proteins with different functions. Two of these accessory proteins are EFL1 and SBDS that together releasethe anti- association factor, eIF6, from the pre-60S subunit.

Ribosome dysfunction has been implicated in several syndromes. Shwachman-Diamond Syndrome (SDS) is an autosomal recessive disorder characterized by skeletal abnormalities, haematological dysfunction, pancreatic exocrine insufficiency, and predisposition of developsleukemia. In

90% of the patients, the SBDS gene is mutated, which makes us believe that the cause of this syndrome is the loss of protein function. SBDS interacts with EFL1 acting as a guanine exchange factor. In this work, we incorporated site- specific an unnatural amino acidthat forms covalent bonds upon UV irradiation. Using this system wefound that residues in SBDS capable of directly interacting with EFL1 corresponded to residues mutated in the disease. Finally, using fluorescence anisotropy we measured the interaction of these two proteins. SDS mutations weaken the interaction with EFL1.These results suggest that mutations in SDS abrogate EFL1 regulation due to the loss of interaction between the two proteins.

Structural and functional characterization of four pathologic variants causing Glucose-6-phosphate dehydrogenase deficiency in humans.

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most frequent enzymopathy in humans with a global prevalence of 4.9%. The disease is heterogeneous at genetic level with around 160 mutations described worldwide. In Mexico, 18 mutations have been described with a prevalenceof 0.75%. G6PD deficiency is characterized by chronic or acute hemolysis in response to oxidative stress, which is related to the low cellular activity of G6PD in erythrocytes. In this work, we studied the structural and functional consequences triggered by three mutations causing G6PD deficiency in our population. With this aim, we cloned and expressed the human *g6pd*gene in a bacterial recombinant system and constructed the pathologic variants Yucatan (K429E), Nashville (R393H), Valladolid (R136C), and Mexico City (R227G) by site directed mutagenesis. Proteins were purified by two chromatographic steps (affinity and ion-exchange) with yields of 2-6 mg per liter of culture and purity higher than 95%. The kinetic parameters determined for the G6PD WT corresponds with the previously reported[1,2]; however the catalytic efficiency was decreased in greater proportion for the Nashville (R393H), Yucatan (K429E), Valladolid (R136C), and Mexico City (R227G), respectively. Thermal stability parameters indicate that the mutants are more susceptible to denaturation by urea and heat relative to the G6PD WT. Furthermore, circular dichoism and fluorescence spectra of the mutants show significant changes in their secondary and tertiary structure respect at the G6PD WT. We concluded that the stability of the variants decrease in concordance with the severity of the clinical characteristics, indicating that instability is a main factor related to the physiopathology of these mutations.

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Reduction of nitrate and ammonium assimilation during induced capsaicinoid accumulation in *in vitro* cultured placentas of habanero pepper (*Capsicum chinense*Jacq.).

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It has recently been demonstrated that *in vitro* cultured placentas of habanero pepper have the ability to synthesize capsaicinoids. This alkaloid accumulation can be stimulated by addition of inducing agents, such as salicylic acid (AS) or by decreasing the total content of nitrogen in the culture medium. On the other hand, it has also beenshown that for such accumulation to occur, it requires the activation of one of the ammonium assimilation pathways, the GS-GOGAT cycle.

In order to study the regulation of N metabolism in placental tissues, we have analyzed nitrate reduction (NR and NiR) and the ammonium assimilation pathways (GS-GOGAT and GDH) during induction of capsaicinoid accumulationusing AS in an *in vitro* culture of placentas, under nitrogen limiting conditions (10 mM).

Preliminary results suggest that even when a combined treatment (1/6 total N +500 µMAS) increased capsaicinoid contents in placentas, it does not require a concomitant increase in the activity of NR and NiRin order to accumulate these alkaloids *in vitro.*

Computational Models of Pores Made of Light Chain Variable Domains: Another Cytotoxic Species?

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Amyloidosiscomprises a group of heterogeneousdiseasescharacterizedbytheabnormaldeposition of proteins in regular aggregateswith beta conformation, calledamyloidfibers. Despitetheubiquity of fibers in thesepathologies, thenature of thetoxicspeciesassociatedtocelldeathisstillunder debate. Thereisincreasingevidencethattheseverity of thesedisordersisbettercorrelatedwith soluble oligomers, smalltointermediatesize, thantotheamount of fibers (1). Althoughdisruptingmembranefunctionappearsto be thehallmarkforthetoxicityinducedbyoligomers, thereis no consensusaboutthemechanismassociatedtoit. One of theproposedexplanationsistheassembly of oligomericstructures similar toPoreFormingToxins, withtheabilitytoinsert in themembrane and induce ion leakage (2). Thishypothesisissupportedbymeasurements of conductivity in solutionscomposed of amyloidprecursors (3), and thevisualizationby AFM of annularoligomersformedbyamyloid-relatedproteins (4). Light ChainAmyloidosis (AL), a systemicdiseasecausedbytheuncheckedexpression of antibody light chains and theiraccumulation in multipleorganslikeheart and kidneys, is no exceptionregardingthisdiscussion: Despitethepresence of fibers, there are severalreportsshowingthecapability of soluble light chainsto induce celldeath (5).

In thiswork, wedesignedbycomputationalmeans a series of oligomericstructureswith beta barrelarchitecture and suggestthem as possibletoxicspecies in AL. Guidedbytheirgeometry and size, weselected as templatescrystallographicstructures of α-Hemolysin and the Human voltage-dependentanionchannel (VDAC) fromthe PDB (ID's 7AHL and 2JK4, respectively),and used a partiallyunfoldedlgdomainfromthe lambda 6a class as themonomer (PDB ID: 2W0K). Afterassemblingthem, eachmodelwasimmersed in a POPC bilayer and atomistic molecular dynamicssimulationswereperformedwiththe CHARMM 36 Force Field in NAMD. Theelectrostaticpotential of themodelswasestimatedwith APBS, and thispropertysuggeststhecationselectivity of poremodels. Theanalysis of thesimulations shows thatsmallermodels are inherently more disordered and unstablethanthosewith more monomers. Thedistribution of chargeswithintheprotein in thecontext of theporeappearsto be thekeyfeatureassociatedtothestability of thestructure.

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TRACHEAL CRYOPRESERVATION: CASPASE-3 EXPRESSION IN TRACHEAL EPITHELIUM AND IN MIXED GLANDS

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ABSTRACT

Background: Cryopreservation has an immunomodulating effect on tracheal tissue as a result of class II antigen depletion due to epithelium exfoliation. However, not all the epithelium is detached. We evaluated the role of apoptosis in the remaining epithelium of cryopreserved tracheal grafts. **Methods:** Caspase-3 expression of tracheal epithelium was studied on canine tracheal segments cryopreserved with F12K medium, with or without subsequent storage in liquid nitrogen. **Results:** Loss of structural integrity of tracheal mixed glands was observed in all cryopreserved tracheal segments. Caspase-3 expression in tracheal mucosa and in mixed glands significantly decreased, in contrast with control group and among cryopreserved tracheal segments which remained high, due to storage effect in liquid nitrogen (ANOVA+TUKEY, $p<0.05$). **Conclusions:** We concluded that the apoptosis in epithelial cells can be triggered during tracheal grafts harvesting even prior to cryopreservation, and although the epithelial caspase-3 expression is reduced, in the tracheal cryopreservation, this could be explained by increased cell death. Apoptosis can not be stopped during tracheal cryopreservation.

Is glicerol employed asosmolytein the symbiotic dinoflagellate *Symbiodinium?*

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Glycerol is a small polyalcohol employed by many microorganisms as anosmolyte when challenged by high osmotic conditions. The dinoflagellate genus *Symbiodinium* Freudenthal is a common symbiont of invertebrate hosts. Cultured symbiotic cells exposed to high concentrations of solutes as well asfreshly isolated symbiotic cells exposed to host homogenatelead to an increase in glycerol. Several investigations have indicated that glycerol may be a mobile photosynthate in the *Symbiodinium*-cnidarian endosymbiosis.However, very little is known about the osmoregulatory abilities of dinoflagellates. We have studied the response to high osmotic shocks in two species of *Symbiodinium* examining glycerol production and growth, specific activity of the enzyme glycerol 3-phosphate dehydrogenase and the expression of the coding gene. Results show that when *Symbiodinium* cells are exposed to high osmolarity conditionsglycerol synthesis is induced,but it is not effectively retained inside cells. While other osmolytes may compensate the loss in osmotic pressure, the glycerol response is not turned-off, resulting in a reduced growth rate. Even though glycerol is a metabolite induced by osmotic stress, our results do not support a role as osmolyte. However its liberation from cells when in symbiosis could have found a divergent yet important role, being co-opted by the cnidarian host that benefits from it.

Unexpected redox homeostasis in the free-living platyhelminth *Dugesia* sp.

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In parasite flatworms such as tapeworms (Class Cestoda) and flukes (Class Trematoda), the redox homeostasis is dependent on a single multifunctional disulfide oxidoreductase. This enzyme, which has been named thioredoxin-glutathione reductase (TGR), is able to reduce both GSSG and thioredoxin in a NADPH-dependent mode. In these organisms the typical glutathione reductase (GR) and thioredoxin reductase (TR), which are present in a majority of the living world, are absent. However, in the genome of a representative (*Schmidtea mediterranea*) of the free-living flatworms (Class Turbellaria) the genes coding for the two latter enzymes were recently found, suggesting the redox homeostasis between parasite and free-living flatworms could differ. In order to test the possibility that in a free-living flatworm the three above noted enzymes could be expressed, in the present work its purification from the free-living flatworm *Dugesia* sp was attempted. Using either DTNB or GSSG as substrates to monitor TR or GR activity, respectively, the crude extract was processed through a three-step purification protocol involving ion-exchange chromatography, hydroxyapatite and affinity chromatography. The two disulfide reductase activities were co-purified through the process. By using denaturing PAGE, the resultant final preparation showed two protein bands: a major one (over 84 % of total protein) corresponding to a protein with a molecular mass of 65 kDa, and a minor one (<16 % of total protein) of about 55 kDa. By mass-spectrometry analysis, peptide fragments with a high identity to both GR and TR were identified in the minor band, demonstrating in *Dugesia* both enzymes are expressed. However, the main disulfide oxidoreductase did correspond to TGR, located at the major protein band. Through the use of the gold-compound auranofin (AF) as inhibitor of TGR, it was possible to identify the fraction of glutathione reductase activity corresponding to GR. Under these conditions, about 20 % of residual glutathione reductase activity was observed. The kinetic parameters K_m and V_m for GSSG reduction in the AF-treated preparation were determined. The resultant K_m (135 ± 8.4 µM) was in the range expected for a GR. The results obtained in the present work revealed all the three enzymes (i.e. TGR, GR, and TR) are expressed in *Dugesia* sp, in sharp contrast with the situation existent in its parasite counterparts. However, TGR represents the main disulfide oxidoreductase, as in parasite flatworms.

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Intracellular localization of the six *Arabidopsis thaliana* isoforms of soluble inorganic pyrophosphatase.

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In plants, the inorganic pyrophosphate (PPi) accumulates in the cytosol and its concentration does not appear to suffer large variations (1). PPi is considered a byproduct of the biosynthesis of carbohydrates, nucleic acids, proteins and some lipids (2). The soluble inorganic pyrophosphatases (PPa) hydrolyze PPi into orthophosphate molecules. Several Mg^{+2}-dependent pyrophosphatases isoforms are present in plants. In *Arabidopsis thaliana* T-DNA insertion mutants lacking isoforms AtPPa2, AtPPa4 and AtPPa5 showed changes in phenotype and each one had a different tolerance to specific types of abiotic stress.

In this work, the aim was to study the intracellular localization of the six isoforms of PPa in *Arabidopsis* (AtPPa1-AtPPa6). Transgenic homozygous plants of *A. thaliana* plants expressing the isoforms AtPPa1- AtPPa5 fusion to fluorescent proteins was used. Confocal microscopy data revealed a differential cytosolic distribution pattern for each isoform. AtPPa6-YFP fusion has a clear chloroplastic distribution, consistent with an intrachloroplastic localization *in vivo*. The chromatographic profile of the PPa activity in extracts form these transgenic plants was compared to the wild type, and each transgenic showed a distinct pattern. Immunoprecipitation (IP) of the protein extracts from the trangenic plants, using anti-GFP antibodies, pulled down some protein bands observed in SDS-PAGE gels of control IP, in addition to the expected fusion protein. The analysis of these IP by mass spectrometry identified some interesting proteins with possible relation to the PPa proteins. The data taken together suggest a possible interaction of PPa isoforms with other proteins likely to be related to their regulation.

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Purificación y caracterización parcial de una enzima tipo quimotripsina de *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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Prostephanus truncatus, es un coleóptero responsable de grandes pérdidas en cultivos de maíz en América Central, América del Sur y África. Este insecto posee serín peptidasas como parte principal de su maquinaria proteolítica intestinal. Se ha planteado la posibilidad de que los inhibidores de proteasa representen una alternativa para el combate de esta plaga. El objetivo de este trabajo fue aislar, purificar y caracterizar una de las enzimas tipo Quimotripsina de *Prostephanus truncatus*, la cual muestró características poco comunes de proteasas de insectos. Se llevó a cabo la purificación por diferentes métodos cromatográficos hasta la obtención de una proteína pura. La enzima aislada de tipo quimotripsina, reconoce únicamente al sustrato Glt-F-*p*NA (sustrato corto), lo cual resulta poco usual, ya que no reconoce al sustrato convencional Suc-AAPF-*p*NA que es reconocido por la mayoría de las enzimas de este tipo en insectos. Presenta una masa molecular aparente de 110 KDa. Además de su especificidad diferente, esta enzima no presentó un pH óptimo alcalino, como la mayoría de proteasas de este tipo, sino un pH acido-neutro (pH 6). Su actividad máxima fue a los 50°C, perdiendo actividad a los 55°C. Un aspecto muy importante que puede estar relacionado con la resistencia a Inhibidores de plantas, fue que resultó insensible a inhibidores de serín peptidasas presentes en granos, que sí inhiben a las demás quimotripsinas encontradas en este insecto y solo esta enzima es inhibida por una fracción de inhibidores de bajo peso molecular presentes en la semilla de chan *Hyptis suaveolens* (HCPI).Esta enzima podría jugar un papel importante en la adaptación de *P. truncatus* a los efectos negativos encontrados en pruebas *in vivo,* realizadas con el inhibidor de Chan. Esta enzima pudiera representar una alternativa para el desarrollo de estrategias de el control biológico de este insecto plaga.

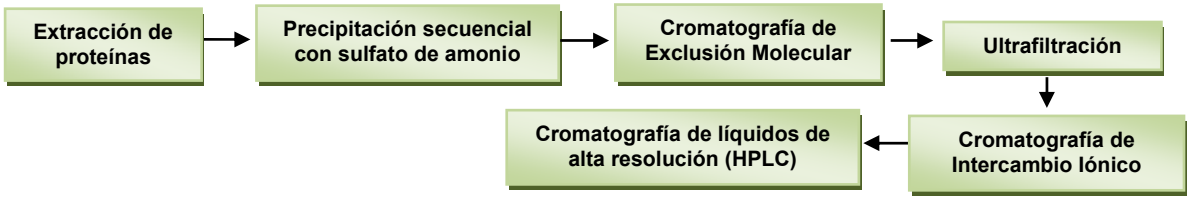

Figura. Esquema general de purificación de la enzima tipo quimotripsina.

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Synthesis of omeprazole analogs with potentialcapacity to inactivate the triosephosphateisomerase of Giardia lamblia(GlTIM)

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Introduction. Omeprazole is an inactivatorof the gastric proton pump (PPIs) H^+K^+ ATPase,acting through thechemical modificationof itsamino acid residue Cys813. Becauseof themechanism of action previously described, we tested its potentialutility as an inactivator of GlTIMand then, evaluatedto it as potential new antigiardiasic drug. Upon performedincubationassaysinGlTIMand human TIM, it wasdetermined that omeprazole inactivate specifically to the former enzyme.Besides, it was found that the chemical modification of the Cys222 by omeprazole is responsible ofthe GlTIMinactivation.

Objective. Synthesize and purify omeprazole analogscompounds and study theireffectsinthe enzyme activity of GlTIM.

Material and methods. The analogs wereprepared by condensation method of 5-methyl-2-mercaptobenzimidazole with 2-chloromethylpyridine,properly substituted by 1,2-dimethoxyethane in a basic medium. The sulfoxidesforms were then obtained by oxidation of sulphidesusing 3-chloroperoxybenzoic acid. The purity of these compounds was evaluated by thin layer chromatography on silica gel plates. The structures were confirmed by spectral data analysis (^{1}H NMR, ^{13}C NMR). The effect of the omeprazole analogs on the enzyme activity of GlTIMwas evaluated by following the decay of the NADH absorbance at 340 nm, using a coupled enzymatic assay at 25°C.

Results. TheanalogsBHO-1, BHO-2 and BHO-3 showing the following structural characteristics:allof them possess a methyl group in the 5 position of the benzimidazole ring.Respect to the pyridine ring, BHO-1 does not have substituentsgroupswhile BHO-2 has two methyl groups and one methoxy group; BHO-3 containstwo methyl and one trifluoroetoxy group. We evaluated theability of omeprazole and the three analoguesto inactivate GlTIM. An IC_{50} of 225 μM was obtained for omeprazole while for BHO-2 and BHO-3, theIC_{50}were 67 and 100 μM, respectively. BHO-1 only inactivated15% at concentration of 500 M.

Conclusions. BHO-2 and BHO-3 exhibited IC_{50} less than theomeprazole, showing that the substituents on the template of omeprazole have important effects on their ability to inactivateGlTIM. The BHO-3 showed 2.2 times greater potency than omeprazole; however, BHO-2 showed the best result being 3.5 times more potent than omeprazole.

Analysis of theO-GlcNAcylationstate of the vitamin D receptor (VDR) in human monocytes cultured under hyperglycemic conditions.

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Vitamin D plays an important role inglucose homeostasis and insulin secretion through transcriptional mechanisms mediated by its receptor (VDR). Interestingly, the O-GlcNAcylation(enzymatic addition of β-D-N-acetyl glucosamine residuesto proteins under chronic hyperglycemia), has been implicated in the glucose toxicity and in the promotion of insulin resistancein micemodels of diabetes. However it is not known ifthe VDR is target of this posttranslational modification,which affects the activity, electrophoretic mobility and subcellular location of multiple transcription factors. **OBJECTIVE**: In this work we assessed the VDR O-GlcNAcylation in the pro-monocytic cell line THP1 and in human monocytes from healthy donors. **METHODS**:THP1 cells and human monocytes were cultured in the presence of high glucose (30mM), or PUGNAc (a pharmacological inhibitor of the O-β-N-acetylglucosaminidase). Then the subcellular location and the electrophoretic mobility of VDR were analyzed by Western blot. The glycosylation of VDR was detected by antibodies that recognize O-GlcNAc groups attached to Serine or Threonine residues.**RESULTS**: Neither the total amount of VDR nor its nuclear import, were affected by high glucose or PUGNActreatments in THP1 cells;but in monocytes, the higher electrophoretic mobility isoforms of VDR increased in the nuclear compartmentwithPUGNAc. The OGlcNAcylation of VDR was observed in untreated THP1 cells and monocytes, but increased under hyperglycemia.**CONCLUSION**: High glucose inducesthe O-GlcNAcylation of VDR in human monocytes.

Comparative study of *Synechococcus* PCC 7335 and CCC9, two different strategies for cyanobacterial nitrogen fixation and the harvesting of light

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The phycobilisome (PBS) is asupercomplex composed of chromophorebinding proteins (phycobiliproteins) and linker proteins. PBS collects light energy,funnels it to the core and then transfers it to photosystems.The typical PBS consists of several peripheral rods that project radially from the core subcomplex.The core consists of a bundle of two (bicylindrical), three (tricylindrical), or five (pentacylindrical) core cylinders, each of which is composed of allophycocyanin (AP). Tricylindrical six peripheral rods PBS are bound to the core, while pentacylindricalPBS have eight rods. Rods are composed of phycocyanine (PC) and phycoerythrine (PE). Linker proteins are essential for PBS assembly, those that assemble the AP core with the thylakoid membrane (L_{CM}),
those assembling rods of PC (L_R^{PC}), or of PE (L_R^{PE}) and the linkersthat bind the rods with the core (L_{RC}).

Cyanobacteria have many capacities of adapting, some may produce secondary metabolites of biological interest, few acclimate to changes in light colorby complementary chromatic adaptation (PBS changes the ratio of phycocyanine to phycoerythrine in rods)[1] or someothersfix nitrogen. The central enzyme for N_2 fixation,nitrogenase, is sensitive to O_2. In order to avoid oxidative damage of this enzyme, diazotrophic cyanobacteria have developed strategies such as different time (*Synechococcus*PCC 7335 fixes N_2 during the night) or spatial separation of N_2 fixation (heterocysts) from the photosynthesis (vegetative cells) as in CCC9.

In Mexico, there are areas with a large variety of cyanobacteria as in Cuatro Cienegas, Coahuila; Guerrero Negro, Baja California Sur; Puerto Peñasco, Sonora; Alchichica, Puebla and Bacalar, Quintana Roo. In CuatroCienegas, filamentous cyanobacterium CCC9 (CuatroCienegasCultive) was isolated. A study of CCC9 using 16S rRNA gene sequence analysis and 16S-23S internal transcribed spacer (ITS) found a 98%and 88% identity of *Nodulariaspumigena*CCY9414 respectively. The conclusion is that CCC9 is a closely related species to *N. spumigena*though it is not identical. Genome of *N. spumigena*is completelysequenced[2]but it has not beenannotated, therefore *in silico*analysis was made to find phycobilisome and nitrogen fixation genes and compare them to the
experiments in CCC9.

On the other hand, in Puerto Peñasco, J. B. Waterbury found Sonora *Synechococcus* PCC 7335[3] as a unicellular cyanobacteriumfrom a snail shell. Its genome has already been sequenced and is in process of being annotated. This cyanobacterium is capable of N_2 fixation, presents CCA and produce extracellular polymeric substance (EPS). The cells were grown in red and white light and the harvested cells broken by French press. The whole cell extract after triton-solubilization was applied to a sucrose gradient to isolate the PBSs. In previous studies a problem arises in this process due to the capture of water by EPS, it causes a dilution of the strength of the buffer needed not allowing the levitation of the membrane components and disassembling the PBSs. New results will be shown with these changes in the methodology for isolating assembled PBS.

In both strains Nase activity was measured at different stages of the dark/light cycle by acetylene reduction. We also present new strategies to identify the components of nitrogen fixation system.

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Effect of Mixed Inoculation on the Yield and Content of Phenols in the Common Bean Seed (Phaseolus Vulgaris L.)

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Common bean is now considered a nutraceutical food, since several studies have shown a correlation between the consumption of this crop with the prevention of chronic degenerative diseases such as diabetes and cancer (1). Studies with laboratory animals indicate that darker the bean's seed, greater protective effect against cancer (2-3), which supports the hypothesis of the dominant role of phenolic compounds present in the seed in the therapeutic effects. It has also been reported a high variability in these results, the protective effect was even questioning. However, the main problem is that these pharmacological analyzes were performed with seed purchased in local markets, not knowing the origin of the crop and agronomic conditions. Phenols are considered secondary metabolites and one of the characteristics of such compounds is that their biosynthesis and accumulation depends on various biotic and abiotic factors that influence the growth of plants. Previously in our group, we found that bean plants inoculated with Bacillus subtilis and Trichoderma atroviride generate seeds with higher content of phenols. The aim of this study was to determine the effect of mixed inoculants on the content of phenolic compounds in beans seeds and crop yield. The experimental design was an arrangement of two factors; Factor A: bean genotype with 2 levels (1: Negro 8025 2: Rojo INIFAP) and Factor B: inoculation with 5 levels (1: control chemical fertilization, 2: Rhizobium + Bacillus, 3: Rhizobium + Trichiderma, 4: Bacillus + Trichoderma, 5: Bacillus + Rhizobium + Trichoderma). Plants were grown under greenhouse conditions, where agronomic crop yield parameters were taken. The total phenolic content was determined by the Folin-Ciocalteau method. The results showed that in general terms the Negro 8025 variety had a better performance the Rojo INIFAP. In the triple inoculation a greater number of nodules per plant was observed. Significant differences in phenolic content between treatments were observed

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Rol of autophagy in altered hydrotropic response (*ahr1*) mutant of *Arabidopsis thaliana*

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Plants have developed and evolved mechanisms that are called tropisms, that involves the bending of the plant organs and even the regulation of the growth toward or away from the perceived stimulus. Tropisms determine the root growth direction and orientation in nature, also help in the development of root system and are responsible of anchoring the root to the ground; they are also considered as fundamental to avoid drought. Hydrotropism is the tropism that involves the perception of water and consequently the change of the root's growth direction to the water source. This process is considered an adaptive strategy to resist the drought. The plants' roots have used hydrotropism to avoid areas with little water and grow towards wetter areas. This process is crucial for the establishment of the root structure and the survival of the plant under water limiting conditions.

The *ahr1* mutant (altered hydrotropic response) develops a system of long roots in the presence of a water potential gradient; this results in an altered hydrotropic response compared with the wild type (*wt*), Col-0. Also, the *ahr1* mutant, in contrast to *wt*, has the ability to overcome the growth arrest of the root, maintain the integrity of the root tip and the ability to respond to water potential.

Several factors have been described that are involved or regulate the hydrotropic response in *Arabidopsis thaliana*. One of them induce autophagy under hydrotropic response. Autophagy is a catabolic process that degrades and recycles cytoplasmic content, is induced in response to various kinds of stress and protects the cell until it is controlled. It is known that autophagy degrades amyloplasts of the root tip during the hydrotropic response, but its role in the hydrotropic mutants is not know, so we propose that the perception of water stress on *ahr1* mutant's root induces autophagy as a protective mechanism that allows the cells to survive until the situation becomes more favorable.

In this work we measured the participation of autophagy in the hydrotropic response using fusions between GFP and Atg8 proteins.

Carbonic anhydrases and their role in mammalian sperm physiology

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymespresent in prokaryotes and eukaryotes that are encoded by four evolutionarily unrelated gene families (i. e., α, β, γ, δ and ζ). In mammals, 16α-CA isozymes(CA1-CA16) or CA-related proteins have been described, with different calatytic activity, subcellular localization and tissue distribution. CAs catalyze the reversible reaction $CO_2 + H_2O \blacktriangledown HCO_3^- + H^+$, which is involved in many physiological and pathological processes, including respiration and tumorigenicity.

In mammalian sperm the modulation of intracellular pH is fundamental to regulate basic processes such as motility, capacitation and acrosome reaction. So far the available information regarding the presence and function of CAs in mammalian sperm is still scarce; it is known that certain CA isoforms are present in mouse and human sperm, and at least in mouse sperm CA4 seems to be involved in the regulation of motility. Unfortunately some evidences in the literature are contradictory. For all these reasons we decided to explore indetail the presence and function of CAs in both species.

Our results by immunoblot confirmed the presence of CA2 and CA13 in total protein extracts of mouse and human sperm. Besides, CA4 is also present in mouse sperm. The immunocytochemistry assays demonstrated for the first time the subcellular localization of CAs in sperm from both species. Specific antibodies against CAs 1, 2 and 13 were tested; the three isoforms are present throughout the human spermflagellum. On the other hand, CA2 is present in the midpiece and acrosome of mouse sperm and no signal was detected for the other two CAs.

The direct activity of CAs was measured using a tetrapole mass spectrometer,in non-capacitated and capacitated sperm. Our results demonstrate an activity of similar magnitude in both species andphysiological conditions. The CAs activity decreases in the presence of either general or highly specific permeable inhibitors against different CA isoforms.

Finally, to determine the contribution of CAs in the regulation of intracellular pH in mammalian sperm, the ratiometric fluorescent dye BCECF-AM was used to measure pH in the absence orpresence of CA inhibitors.

Structural studies of Mu-class glutathione S-transferase from shrimp *Litopenaeus vannamei*

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Glutathione S-transferase is ubiquitously distributed in nature, it is found in diverse organisms from microbes, insects, plants, fish, birds to mammals. Currently, four types of GST enzymes: called Pi, Mu, Alpha and Theta have been characterized. These enzymes can catalyze the conjugation of reduced glutathione (GSH) with compounds that present an electrophilic center (like pesticides or xenobiotics) through the formation of a thioether bond between the sulfur atom of GSH and its substrate. Experimental data suggest that sub lethal concentration of certain metals may affect the capacity of the organism to detoxification, against some pesticides or xenobiotics. Many of them are presents in the marine sediments. Our work is focused on the structural description of a Mu class GST of *Litopenaeus vannamei,* leg white shrimp (LvGST). Mu-GST present a glutathione (GSH) binding site (G site) and hydrophobic substrate binding site (H site). We have purified this protein and performed experiments in order to obtain crystals at different conditions. The crystals of LvGST were diffracted at the APS (Advanced Photon Source) and at the NSLS (National Synchrotron Light Source I). Currently we are refining the native structure. The structural analysis of the crystallographic structure will allow us to test the binding capability of GST protein with heavy metals, and then evaluate the possible effects over its structure and function.

Flavonoids acacetin, chrysin and 4´,7-dimethyl narangenin as photosynthesis and seedling growth inhibitors

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As part of an intensive study of the bioactive secondary metabolites that inhibit photosynthesis, that could lead in the development of "green herbicides", in previous works has been found that flavones as tephroleocarpin, glabraninand methylglabranin isolated from the *Tephrosia*spp.inhibited different photosynthetic activities.[1]Pachypodola flavonoid isolated from the plant *Croton ciliatoglanduliferus* behaves as Hill reaction inhibitor on photosynthesis *in vitro*, tested on isolated chloroplasts from spinach leaves[2]. In this work we investigated the activity of the three flavonoids isolated from Mexican propolis: acacetin (**1**), chrysin (**2**) and 4´,7-dimethyl narangenin (**3**) on the electron transport chain and H^+-ATPase on thylakoids, and their effects on germination and seedling growth of *Physlisixocarpa*, *Loliumperenne*and *Echinochloacrusgalli.*

The results showedthatflavonoids **1**-**3** inhibited the photophosphorylation in fresh lysed spinach chloroplasts, being **2** more potent as inhibitor; **2** is hydrophilic, this property of **2** may allow to reaches its target.**1** behaved as uncoupler and **2** at low concentrations also acted as uncoupler, however at higher concentrations (up to 100 µM)it behaved as a Hill reaction inhibitor. Compound **3**acted as Hill reaction inhibitor. Chl*a* fluorescence results suggesting that **2** interacts with the PQ-pool and **3** affects the acceptor side of PS II in a similar way as DCMU.

*In vivo*assays showed that **1**, **2** and **3** did not affected the chlorophyll *a* fluorescence neither the dry biomass of plants treated, indicating that they did not penetrate the leaves of the plants.

Flavonoids**1**-**3** did not affect the germination of *P. ixocarpa,* however, the roots length was increased, and the shoot lengths were lightly inhibited by them. On the monocot seeds of *L. perenne*and *E.crusgalli*the three flavonoids inhibited partially the germination as well roots and shoots length at concentration dependent manner, being

1 the most active. The results show that these flavones interfere with the photosynthetic electron flow and also with the monocot plants growth process.

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Importance of the GTPase Gpn3 in the nuclear accumulation of RNA polymerase II in breast cells with increasingdegrees of malignancy.

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Gpn3is a GTPase that, along with Gpn1 and Gpn2, belongs to the GPN protein family. These three proteins are essential for life,their sequence is highly conserved in all eukaryotes, and theyall contain a conserved sequence of glycine (G)-proline (P)-asparagine (N) that originates their name.Gpn3 interacts withRNA polymerase II (RNAPII), the enzyme that transcribes all protein coding genes, as well as many other small nuclear RNA genes.In our laboratory we demonstrated previously that Gpn3 is necessary for the nuclear localization of RNAPII in the non-tumorogenic MCF-12A breast cells. Interestingly, in four different breast tumorogenic cell lines Gpn3 is not longer necessary for Rpb1 nuclear accumulation. This may imply that the tumorogenic cells have developed a molecular mechanism independent of Gpn3 for the RNAPII nuclear accumulation.In this work, we determined if there is a relationship between the degree of cell transformation and the importance of Gpn3 for RNAPII nuclear targeting.To this end, we employed progressively more malignant derivatives of the originally non-tumorigenic MCF10 breast cells. These derivatives include the MCF10AneoT, MCF10AT1, MCF10ATK1.cl2, MCF10CA1d.cl1 and MCF10CA1a.cl1 cells. We suppressed Gpn3 expression employing a specific shRNA, and evaluated the subcellular localization of Rpb1, the largest subunit of RNAPII. Gpn3 silencing was verified by Western blot, and the subcellular localization of Rpb1 was analyzed by immunofluorescence.We will present and discuss the results of our investigation at the meeting.

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Effect of methanol extracts of *Lentinula edodes* on the gelatinases A and B (MMP2 and MMP9) and urokinase (PLAU) in breast cancer cells MCF7.

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Breast cancer is formed in the ducts and lobules of this organ. The ability to migrate, invade and metastasize is a characteristic of malignant tumors. The extracellular matrix (ECM) is the first barrier that cancer cells must cross in order to generate metastasis. Matrix metalloproteinases (MMP) are a family of proteinases that degrade all the components of the ECM, in this group we can find gelatinase A (MMP2) and gelatinase B (MMP9) which are involved in the invasion and metastasis of breast cancer cells and its overexpression correlates with aggressive tumors. The plasminogen/plasmin system has been considered as one of the initial steps in the activation of the proteolytic cascade in cell migration. There are two types of plasminogen activators: urokinase type (PLAU) and tissue-type (PLAT). These activators are involved in the formation of plasmin from plasminogen. Some alterations in the regulation of this system are associated with disease such as cancer. A promising group of cancer preventive agents are those derived from natural products. The use of products derivate of fungi has increased the interest of the scientific and clinical community because of its effectiveness against diseases and metabolic disorders such as cancer. *Lentinula edodes*, known as " shiitake " is a mushroom which has been attributed antitumor properties, so the objective of this study was to evaluate the effect of methanol extracts of fruiting body and mycelium of *L. edodes* at concentrations of 25, 50, 100 and 200 µg/mL on the content of MMP2, MMP9 and urokinase (PLAU), in the breast cancer cell line MCF7. To meet this objective, a MTT assay was performed to determine the effect of these extracts on the growth of MCF7 cell population, zymographies in SDS-polyacrylamide gels copolymerized with gelatin were made to measure the content of gelatinases MMP2 and MMP9 and an amidolytic assay was used to evaluate the activity of PLAU. It was found that when extracts of mycelia and fruiting bodies of *L. edodes* were applied an inhibition of 60% in the growth of the cell population was observed, also it decreased the concentration of MMP2 and MMP9 secreted in the conditioned medium and reduced the ability of transform plasminogen into plasmin by decrease PLAU activity. These results indicate that the methanol extracts of shiitake can be, potentially useful as adjuvant therapy or tumor chemo-preventive agents. Supported by PAPIIT, DGAPA, UNAM, project IN 223014-3.

Structure and function characterization of SdrP protein from *Thermus thermophilus*.

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Transcription is a highly regulated process that takes place in all living organisms; this process can be activated or repressed by proteins called transcription factors (TF). In bacteria there is a TF family called CRP/FNR, members of this family are dimers of identical subunits. The Catabolite Gene Activator Protein (CAP) is the best-characterized member of the CRP family. CAP functions by binding, in the presence of the allosteric effector cAMP, to specific DNA sites in or near target promoters. Four members of the CRP/FNR family have been described in the extremophile bacteria *Thermus thermophilus*, one of them is the stationary phase-dependent regulatory protein (SdrP) which is a dimer of identical subunits of 202 amino acids. SdrP positively regulates the expression of genes related to nutrient and energy supply, redox control and nucleic acid metabolism. Interestingly, it has been demonstrated that transcription activation by SdrP does not require any allosteric effector, thus SdrP is a member of the CRP/FNR family that is always in the "on" conformation. In this work we have cloned the SdrP DNA sequence in the pET28 expression vector (NdeI-BamHI sites). The purification protocol of SdrP comprises IMAC, ion exchange and size exclusion chromatography. In order to determine whether the recombinant SdrP protein was functional, a mobility shift assay using size exclusion chromatography was performed with a consensus sequence of DNA (38 bp) and SdrP protein. Thermal stability experiments following intrinsic fluorescent were performed in the presence and in the absence of trehalose. Single crystals of the SdrP-DNA complex were obtained in crystallization experiments. The final goal of this project is to determine the structure of the SdrP-DNA complex by protein X-ray crystallography.

A derivative-based steroidal androstane skeleton compound (OM4) inhibits FcεRI-dependent degranulation after IgE/Antigen stimulation in mast cells

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Introduction

Crosslinking of the high affinity IgE receptor trough IgE/Antigen in mast cells (MC) lead to release of preformed mediators, process known as anaphylactic degranulation. Inhibition of degranulation has been proposed as an important therapeutic target for allergic reactions. Anti-inflammatory actions of steroids have been exploted to control allergies with important results in the late phase of the inflammatory response but with limited effects on the acute, early anaphylactic degranulation of MC. Since anti-inflammatory actions of glucocorticoids have been reported and those compounds can be artificially synthesized we decided to analyze the effects of a group of 12 new androstane derivatives on the early IgE/Ag-induced degranulation of murine bone marrow-derived mast cells (BMMCs).

Methods

BMMCs were sensitized overnight with a monoclonal anti DNP-IgE. The day after, cells were pre-treated 15 minutes with different concentrations of the compounds and then were stimulated with the specific antigen (DNP-HSA). Degranulation was determined measuring the activity of □-hexosaminidase on supernatants of stimulated cells.

Results and conclusions.

From the 12 compounds used, only one of those (OM4) inhibited FcεRI-induced degranulation in a concentration-dependent manner. This suggests that androstane derivatives could serve as anti-inflammatory agents for the treatment of the acute (early) phase of allergic reactions.

Expression of MUC1 glycoprotein in HEK293 cells for studying the role of MAL and GGA2 proteins on its cellular trafficking.

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MUC1 is a hetero-dimeric trans-membrane glycoprotein, expressed on the apical surface of mucosal epithelial cells. Typically it is endocytosed to recycle through the Golgi complex to the plasma membrane (PM). MUC1 is over-expressed in several cancers and it alters many cellular properties including intercellular adhesion and immune recognition, which favor neoplasm progression and metastases.Our group is interested in characterize themolecular regulation of MUC1 cell trafficking, particularly by determining the role of myelin and lymphocyte protein (MAL), and Golgi-associated γ-ear-containing , ADP-ribosylation-factor-binding protein-2 (GGA2) on this process. HEK-293 cells are not expressing endogenously MUC1, thus we take advantage of this fact and we generate different stable cell lines by transfection and antibiotic selection. HEK293-cell lines expressing human MUC1, MAL-GFP, and wild-type and truncated GGA2-GFP were generated.The expression of transgenic proteins was confirmed by western blot, and fluorescence microscopy. Sub cellular distribution of transgenic proteins was the expected. Currently we are analyzing the phenotypes of transfectectedthrough changes in distribution of MUC1 by western blot of different cellular fractions, (i.e. PM, nucleus, and cytoplasm), and by immunofluorescence. So far we found that expression of MUC1 in PM of HEK293-MAL-GFP cells was not detected when compared to PM of HEK293-MUC1 cells, by western blot. We hypothesize that in cells over-expressing MAL (MAL-GFP),MUC1 could be somehow unstable, probably due to accelerated degradation. Currently we are working to test this assumption, as well as in elucidating the phenotypes showed by the other cell lines.

Quality Control Test for Sequence-Phenotype Assignments

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Relating a gene mutation to a phenotype is a common task in different disciplines such as protein biochemistry. In this endeavour, it is common to find false relationships arising from adaptive mutations that may be depurated using a phenotypic assay; yet, such phenotypic assays may introduce additional false relationships arising from experimental errors. Here we introduce the use of high-throughput DNA sequencers and statistical analysis aimed to identify incorrect DNA sequence-phenotype assignments and observed that 10-20% of these false assignments are expected in large screenings aimed to identify critical residues for protein function. We further show that this level of incorrect DNA sequence-phenotype assignments may significantly alter our understanding about the structure-function relationship of proteins.

Functional Divergence of Genes Implicated in the Leucine Biosynthesys in *Sacharomycescerevisiae*and the Ancestor Type Yeast *Kluyveromyceslactis.*

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Gene duplication is an important force of evolutionary innovation, allowing the acquisition of a new function or the improvment of ancient ones. The paralogous pair of genes *LEU4* and *LEU9*present in *Saccharomyces cerevisiae* (*S. cerevisiae*) encode two alpha isopropyl malate synthases which catalyze the formation of α-isopropilmalate from α-ketoisovalerate which is the first step in leucine biosynthesis, both genes were originated from the event of whole genome duplication (WGD) of the ancestor of *S. cerevisiae* this event had place approximately 100 million years ago. *K. lactis* is a yeast related with S. cerevisiae which derived his lineage before the event of WGD, nevertheless *K. lactis* also has two genes *KlLEU4* and *KlLEU4bis* which codify two α-isopropyl malate synthaseisozymes. Synteny analysis of *K. lactis* and *S. cerevisiae* genome suggests that the duplication of the ancestor type *KlLEU4bis* originated *LEU4* and *LEU9* in *S. cerevisiae*.

The presence of the gene *KlLEU4* on *K. lactis* that doesn´t have such a direct relationship with the genes in *S. cerevisiae*offers a new perspective to analyze the physiological role of paralogous pairs in *S.cerevisiae* and in the ancestral type yeast *K. lactis*, which has not been previously analyzed.

This work has the objective of determinate the reciprocal complementation between the genes of *K. lactis* and *S. cerevisiae*, this study will provide a new point of view of the fate of paralogous genes. The analysis of single mutants *Klleu4*Δ and *Klleu4bis*Δ shows a decrease of the α-isopropyl malate activity in crude extracts,this phenotype is similar to that found for both *leu4*Δ and *leu9*Δ. The mutant Leu4Δ shows a minor growth rate, nevertheless the mutant Leu9Δ doesn't show a decrease in grow rate.The mutants *Klleu4*Δ and *Klleu4bis*Δhave similar growth rate than wild type stain on glucose or ethanol. The double mutant *S. cerevisiaeLeu4*Δ*Leu*Δand the double mutant *K. lactis Klleu4Δ Klleu4bisΔ* are leucine auxotroph.

Inhibition analysis of the proteins by the final pathway product (leucine) shows a remarkable difference, *Leu4* is highlyinhibited by leucine otherwise *Leu9* is poorly inhibited. *KlLeu4* and *KlLeu4BIS*shows anintermediateleucine inhibition. We believed that the difference showed in *S. cerevisiae*proteins is necessary for homeostasis in ethanol as carbon surce.

INFLUENCE OF MIF ABSENCE ON HEPATIC MITOCHONDRIAL FUNCTION USING A MURINE MODEL OF DIABETES MELLITUS TYPE TWO.

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Introduction: MIF (macrophage migration inhibitory factor) is a proinflamatorycytokine expressed on different tissues; it also works in immunologic and inflammatoryresponse. Diabetic patients present high plasmatic levels of MIF in comparison to non-diabetic patients. It has been observed that deletion of MIF reduces cytokines production and the diabetes development is less, which indicates that inhibition of MIF production represents an alternative approach for diabetes treatment. The hepatic failure is another alteration on diabetic patients. The liver regulates glucose homeostasis due to a great amount of mitochondria (500-4000 per hepatocyte) to cover the object energetic demand. The principal function of mitochondria is ATP synthesis from oxidative phosphorylation, in which reactive oxygen species (ROS) normal production are generated. A high sucrose diet increases the
ROS levels and the permeabilityby Ca^{2+}. The enzymatic alterations of hepatic mitochondrion, by hyperglycemic conditions, can be estimated by oxygen consumption.

Objective: The aim of this work is to evaluate the alterations of the hepatic mitochondrial respiratory chain activity on a murine model of diabetes mellitus type two (DMT2) in the absence of MIF.

Methodology: The diabetes mellitus experimental model was established on male BALB/c mice (wild type; MIF +/+) using a 130 mg/kg dose ofstreptozotocin (STZ) and in knockout mice ofMIF gene (MIF -/-). The physiologic parameters of blood glucose level and corporal weight were recorded each week during two months; a Glucose tolerance curve test and the quantification of water and food consumption using metabolic cages on the 7^{th} week were also recorded. The livers were extracted from each group and the mitochondria were isolated. Oxygen consumption was measured with a Clark-type electrode and respiratory control (RC)enzymatic activity of mitochondrial complexes were measured, as well as mitochondrial swelling and hydrogen peroxide production (H_2O_2) were evaluated.

Results: The physiologic parameters showed an experimental simulation of DMT2.Blood glucose levels were maintained over 200 mg/dL in the diabetic group (MIF+/+ STZ) while in the other groups the blood glucose levels oscillated between 100-120mg/dL, the same response was observed on the glucose tolerance test.The body weight of diabetic mice during the study decreased while an increase in weight was observed in others till the period of observation was over.Therespiratory control of isolated mitochondriawas measured using glutamate/malate as substrates to initiate the oxygen consumption of complex I. Comparatively, MIF+/+ STZ hepatic mitochondrion had a less RC than the non diabetic mitochondrion. The MIF-/- mitochondrion had a RC lower than the rest of the mitochondrion using glutamate/malate for complex I. This can be explained by the presence of high amounts of lipids during mitochondrial isolation. By using succinate as a substrate, the RC decreased only in the groups with STZ, however the differences were not significant.MIF-/- mitochondrion had amajor response to 60 µM of extracellular calcium likewise to H_2O_2production,while those mitochondrion treated with STZ showed a similar response to calcium and H_2O_2 production.

Conclusion: Absence of MIF does not increase bloodglucose levels therefore diabetes mellitus type 2 does not develop or is milder in the KO. A diminution of RC on MIF +/+ STZ and MIF -/- STZ was observed.

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Polyphosphate increases cadmium resistance in Methanosarcina acetivorans.

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We previously reported that Methanosarcina acetivorans cultured in presence of cadmium (Cd^{2+}) is able to show different resistance mechanisms such as intracellular sequestration and biofilm formation[1]. However, when cells were cultured in acetate plus 54 µM $CdCl_2$ for first time (CdP1) or pre-adapted by growing them permanently with 54 µM $CdCl_2$ for one year at least (CdPA), the increase in the intracellular content of molecules with thiol groups as cysteine, coenzyme M and sulfide (0.4-0.8 µmol of total thiols) were not enough to complete binding of Cd^{2+}, because the –SH groups/Cd^{2+} ratio was < 2. Nevertheless, methane production did not change compared with control cells, suggesting that there must be alternative mechanisms that protect the cells from the Cd^{2+} toxicity. Hence, other known mechanisms were evaluated as: i) content of organic acids (malate and citrate) and ii) polyphosphate content (linear chains of orthophosphate linked covalently; polyP), which have been reported to bind heavy metals (Cu^{2+}) with high affinity.

Malate and citrate increased in both, CdPA and Cd-1P cells, although this increase was negligible in comparison with the content of the thiol groups, suggesting that organic acids are not a mechanism to contend against Cd^{2+} in methanogens.

In cultures with acetate, the intracellular content of polyP determined in control, CdPA and CdP1 cells were: 0.4±0.3, 11± 6 and 4±1 µmol $Pi_{released}$/mg protein, respectively, suggesting that polyP is a complementary mechanism to the thiol molecules synthesis in response to the Cd^{2+} exposure. Because the increase in thiol groups was <1 µmole in CdPA and CdP1 respect to control, data suggested that the increased content of polyP is the primary mechanism to bind and neutralize Cd^{2+}, as described for archaea[2], where it has been reported that synthesis and degradation of polyP are important in heavy metals resistance[3]. On this regard, M. acetivorans showed an exopolyphosphatase activity (PPX) of 6 nmol $Pi_{released}$/min*mg protein. In turn, transcripts of PPX decreased 0.2-0.6 times in CdPA and CdP1 cells respect to control cells whereas transcripts of PPK increased 2.4 times. This data suggested that the metabolism of polyP plays a key role in archaea when are exposed to different stressors.

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"Expression, purification and characterization of CGI58/ABHD5 enzyme"

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CGI58/ABHD5 is an enzyme that participates in the lipid metabolism as an activator of adipose triglyceride lipase(ATGL). Recently it has been reported that CGI58 has a lysophosphatidylacyltransferase activity, so it has been associated with the "signaling processes" in the production of eicosanoid lipids. Although CGI58 has been the subject of several studies, the three-dimensional structure has not yet been determined. The main goal of this work was to generate a construct that expresses CGI58 in a soluble form and at suitable concentration to initiate crystallization experiments; this will allow us to solve its structure by X-ray crystallography in the near future. Here, we report a homology model of human CGI58 performed with I-TASSER, which was used to design a truncated protein of 50 residues at the N-terminus. Two constructs of mouse CGI58 were generated, comprising the wild type and the truncated enzymes. Two optimized DNA sequences for the human CGI58 were designed, for each one we have the corresponding construct that express the wild type and the truncated enzyme. So, six protein constructs were generated in this work. Four of the six constructs have been expressed as recombinant proteins in *E. coli*; all of them have been purified by nickel affinity chromatography. We found that the detergent octylglucoside increases the solubilityof CGI58 protein in solution about ten fold. Finally, we observed that the use of 10% glycerol, 2 mM magnesium and pH 7.5 had a positive impact on CGI58 thermal stability. However, the evaluation of these additives in the purification step has not been tested yet.

C**haracterizationof cyclin proteins implied in cell cycle regulation of *Trichomonas vaginalis***

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Trichomonas vaginalis is a microaerophilic protozoan of early evolutionary divergence that causes trichomoniasis, a genitourinary sexually transmitted infectionin humans. The trophozoite, that is the infectant form, has four anterior flagella and one recurrent that forms the undulating membrane, which together confers spasmodic motility.

T. vaginalis reproduction is asexual and divide by binary fission. At the present time, the studies on the cell cycle in trichomonads are poor. However, the steps comprising mitosis have been structurally characterized by electron microscopy and it has been found that during this stagethe nuclear envelope remains intact.

Most of cells perform an orderly sequence of coordinated events to reproduce and divide in two genetically identical cells, process known as cell cycle. In order to occur the different events which constitute the cell cycle, it is necessary the participation of several molecules that are responsible of regulating and control the division. The components of the cell cycle control system are members of a family of kinases proteins known as cyclin-dependent kinases (Cdks) and in turn, the cyclic changes in the activity of Cdks are controlled by a family of proteins known as cyclins.

The cyclin proteins are characterized by the oscillation of their concentration levels that are dependent on synthesis and degradation during the cell cycle. Based on their behavior during the cell cycle, cyclins can be classified into four classes as cyclins D, E, A and B. In general, the cyclins are very different from each other in their amino acid sequence, however, the homology between them is limited to a conserved domain of approximately 100 residues known as the cyclin box. This domain is the binding site to the Cdk, necessary for the first activation of the complex.

At present, there are not studies about these important cell cycle regulators in*T. vaginalis*. Using BLAST analysis there were identified at least 26 putative cyclins in the *T. vaginalis* genome. All of the found sequences contain the cyclin box and some other contain motifs characteristic of cyclins. For a preliminary study, four sequences which are most likely to be active according to cyclin proteins compared with other organisms were selected.

Using a yeast deficient in D-type cyclins, we have made complementation assays with each one of the putative cyclins in study and the results show that at least three of four proteins rescues the function of yeast D-type cyclins, suggesting that these proteins act as cyclin proteins.

Additionally, using the yeast two-hybrid system we determined that only the putative cyclin that has not rescued the mutant mentioned above, shows interaction with some Cdk proteins of *Trichomonas vaginalis*, which also suggests a possible role as a cyclin protein.

In the near future, we would like to determine in which stage of *Trichomonas vaginalis* cell cycle are expressing the putative cyclins that we are using in this work, through synchronization of *Trichomonas vaginalis* cultures.

Role of S-adenosylmethionine and Methionine Adenosyltransferases in a sequential model of cirrhosis-hepatocellular cancer induced by DEN treatment.

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The methylation cycle involves the conversion of methionine, vía S-adenosylmethionine (SAM) and S-adenosyl homocysteine (SAH), into homocysteine followed by reconversion of homocysteine into methionine. This cycle provides SAM for the methylation of numerous essential cell constituents and its synthesis and degradation occurred mainly in the liver. Its synthesis is catalyzed by methionine adenosyltransferase (MAT), MAT1A is expressed only in the liver, and it is the product of gene expression of the *Mat1α*, while isoform MAT2A is widely distributed and it is result of gene expression *Mat2α*.

Various chronic liver disorders, as a cirrhosis and cancer, markedly decrease the hepatic activities of MAT1A and increase activities of MAT2A. In addition to this, AMPK is the essential regulator which is activated by cancer and it is an important regulator of MAT2A protein expression. We showed that an adenosine derivative (IFC305) (UNAM Patent US8,507,459 B2 and MX 316096) has an anticarcinogenic effect and we studied the effect of this compound in S-adenosylmethionine (SAM) synthesis.

We investigated the effects of IFC305 in a sequential model*in vivo* of cirrhosis-hepatocellular cancer (HCC) described by Schiffer (2005). Male wistar rats were treated with DEN for cirrhosis induction for 12 weeks or for cancer induction for 16 weeks, and two different groups were simultaneously treated with IFC305. The liver samples were used for measured of MAT1A, MAT2A, and AMPK protein expression by Western blot; gene expression of these isoforms of MATs by qPCR and cellular SAM levels were measured by HPLC at the cirrhosis and cancer stage. Liver samples of the HCC groups were divided into no-tumoral and tumoral site.

The results of this assays showed in cirrhotic and cancer samples induced by DEN treatment, than the cellular SAM levels were low. Added to this levels expression of the MAT1A protein were low, according to what was reported by several authors, and it is caused by of low gene expression of *Mat1α*. However, the treatment of DEN causes high expression of the protein MAT2A, apparently without affecting levels of the *Mat2α* gene transcript. In addition, this treatment causes activation of AMPK which also favors the expression of protein MAT2A.

In contrast, these effects were reversed with the administration of this adenosine derivative. Levels of S-adenosylmethionine in cirrhotic and cancerous samples were elevated. This is correlated with low cellular levels of S-adenosylhomocysteine, main inhibitor of methylation reactions, when this compound was administered. In addition, the IFC305 was able to increases the levels of MAT1A protein and reduces of MAT2A protein, which could be related to the AMPKp inhibition in the different groups treated with IFC305.

The resultsobtained in cirrhosis and cancer are similar to those reported by other groups, so that the effect of IFC305 could be explained at different levels, for example: gene regulation, post-transcriptional and post-translate regulation or epigenetic regulation of the enzymes involved in SAM synthesis. Experiments are in progress to determine the IFC305 molecular mechanism.

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Triosephosphate isomerases of Arabidopsis thaliana

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Triosephosphate isomerase (TIM) is involved in glycolysis, gluconeogenesis and Calvin cycle. To date, more than 25 crystal structures of TIMs have been solved and studied biochemically, but none of these TIMs is an enzyme of the Calvin cycle. Herein, we present the crystal structures of the cytoplasmic and chloroplast TIM from the model plant *Arabidopsis thaliana*, their regulation by oxidants and a study involving specific amino acids in this regulation.

Crystals of Cytosolic TIM (citoTIM) and Chloroplastic TIM (CloroTIM) diffracted to 1.7 Å and 2.4 respectively, both proteins exhibit a TIM barrel fold composed of a staggered arrangement of eight alpha helices and eight beta strands. Each protein contains four cysteines in their amino acid sequence. Inhibition study indicates that both cloroTIM and citoTIM are inhibited by H2O2 and both proteins react with the tripeptide glutathione *in vitro*. However, they show differential inhibition by methylmethane thiosulfonate ((MMTS) citoTIM requires 5 microMolar of MMTS to present 50% of inhibition, wheres cloroTIM requires 50 micromolar, this data confirming that the inhibition of plant TIMs occurs by a cysteine residue. We postulate that the reaction with glutathione in plant TIMs can occur through cysteine 13 or 15 that is located at the dimer interface. Currently we are identified cysteines capable of reacting with glutathione in both enzymes by mass spectrometry and site-directed mutagenesis. We postulated that the way that glutathione regulates the activity of Arabidopsis' triosephosphate isomerase is through alterations in monomer-dimer equilibrium of these enzymes.

Membrane supercomplexes and complexes of oxygenic photosynthesis in the marine cyanobacterium*Prochlorococcus* MIT 9313.

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Cyanobacteria are the only prokaryotes that perform oxygenic photosynthesis. In this process, oxygen is released and an electron flow begins from the cleavage of water(transmembrane charge separation).They use two photosystems whose reaction centers are widely conserved.Cyanobacteria have developed distal antennas, as phycobilisomes, to improve the capture and energy transfer to reaction centers. Genus *Prochlorococcus* do nothavephycobilisomes,instead they useintrinsic membrane proteins with six transmembrane α-helices (Pcb) that possess special types of chlorophyll: divinyl chlorophyll *a* and *b*.Pcb-photosystemssupercomplexeshas been described by electronic microscopy techniques. To describe these associations from a biochemical approach, thylakoid membranes were isolated from the whole cell extract, solubilized with dodecyl maltoside and analyzed by either clear native electrophoresis (CN-PAGE) or sucrose gradient centrifugation.The CN-PAGE was followed by a second dimension SDS-PAGE and identification of proteins by mass spectrometry.By mass spectrometry identification, western blot and diaphorase activity, we found that Ferredoxin:$NADP^+$ reductase (FNR)of *Prochlorococcus* is an integral protein in the thylakoid membranes andcomigrating with the cytochromeb_6f complex fraction, suggesting a supercomplex whose function could be related to the cyclic electron flow.We did do not obtain Pcb-photosystem supercomplexes and of F_1F_0 ATP synthase.This could be due to the excessive strength applied with the French press to disrupt the cells.Therefore we implemented a softer method of cell disruption.Preliminary experiments with of *A. maxima*and Cyanobacterium CCC9membranes were obtained by mildly grinding the cells with sand, in which F_1F_0 ATP synthase is abundant, gave support to the above conclusion. Other results about this approach will be presented.

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Energetic basis of molecular recognition of guanine nucleotides by the ribosomal GTPase Efl1.

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In yeast, Efl1 GTPase involved in the final stages of maturation ribosomal and acts together with the SDO1 protein to promote the release of anti-factor Tif6 association pre-60S subunit. Very little is known about the biochemical and biophysical properties of Efl1. In our research group we are interested in studying the molecular recognition of nulceotidos guanine at energetic level by GTPase Efl1 in order to understand the underlying basis of the functioning of this protein. In order to thermodynamically characterize the binding of guanine nucleotides to Efl1 with and without Mg(II) and Sdo1 protein, in this work the overexpression and purification of recombinant Efl1 in *Saccharomyces cerevisiae* was performed. The content of secondary structure was estimated by circular dichroism and Efl1 stability by differential scanning calorimetry (DSC). Efl1 interaction with guanine nulceotidos GDP, GTP and GMPPNP was characterized using isothermal titration calorimetry (ITC) at different temperatures in the presence or absence of Mg(II) and the protein Sdo1. The results are shown and discussed in detail in the presentation of the poster. Acknowledgment to financial support of projects CONACYT 129239 and PAPIIT IN205712. A doctoral scholarship number 269390 to A.L. and financial support PAEP-UNAM.

Aminopeptidases as potential markers of celular aging in *Schizosaccharomyces pombe*

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Aging is defined as a collection of damage accumulated in the molecular and cellular structure that increase metabolism sprawl, leads to pathology and death. Know the molecular mechanisms related to ageing and identify markers of the process could offer therapeutic targets to improve the quality of life of humans. An aspect little studied of cellular aging is the change in the activity of proteases. In the search for markers of aging this worked focused on the identification of aminopeptidases whose activity is modified in aged cells of the yeast *Schizosaccharomyces pombe* (*S. pombe*). To achieve this objective are cultivated strain 972 h^- *S. pombe* between 1 and 30 days in EMM medium. Subsequently, measured 5 aminopeptidases activity using substrates coupled p-nitroanilide. The measurement of the activity was performed both in young cells (1, 2, 3, 4 days) as in cells on chronological Aging (5, 10, 15 and 3 days of culture). The two enzymes with increased enzyme activity in young as in aged cells were leucine aminopeptidase (LAPyspII) and dipeptidyl aminopeptidase (dpa) being LAPyspII which had increased activity was significantly increased at day 5 (day that is considered to start of aging in yeast) and then decreases to the 10 day to disappears to the 30 day. The activity of the dpa was detected with a good activity in young cells, but its peak was detected to day 5, decreased to 10 day but remained at the same level until the last day of cell culture and was the same between day 1 and 30. About the phenylalanine, valine and glycine aminopeptidases, the activity registered was very small compared with the above-mentioned enzymes. The higher activity of these was detected between 1 and 5 days. The activity was not detected after 5 day of culture, it is likely that these enzymes have a higher activity to the first hours of cell culture. With the obtained results it is possible to propose that the dipeptidyl aminopeptidase is a crucial protease for the maintenance of life in yeast. While the leucine aminopeptidase enzyme could be a marker of cellular aging due to the decrease in enzymatic activity in aged cells. Although the exact role and the mechanism by which is related to cellular aging will have to be analyzed in subsequent studies.

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Preliminary localization by confocal microscopy of a protein DING in seeds of habanero chili (*Capsicum chinense*Jacq.).

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The family of DING proteins has been described in different species of prokaryotic and eukaryotic organisms (Bernier, 2013). Their name is associated with its N-terminal highly conserved amino acid sequence, aspartic acid (D), isoleucine (I), asparagine (N) and glycine (G) (Bernier, 2013). They have a molecular weight of ~40 kDaalthough some potential precursors having higher molecular weights have been described(Perera et al, 2008). The phosphatase enzymatic activity is the characteristic that unifies all them due to their ability to bind phosphate.

Several reports have associated to the DING proteins with the inhibition of diseases such as cancer (Darbinian, et al., 2009), inhibition of the formation of kidney stones (Kumar, et al 2004) and the inhibition of the replication of HIV-1 virus (Darbinian-Sarkissian, 2006).There are controversies regarding to the cellular localization of this proteins family, Kumar et al. (2004) described that the DING protein called CAI and involved in the inhibition of adhesion of calcium oxalate crystals to kidney epithelium, is localized in cytosol and nucleus. In contrast, Shah et al. (2013) described that the DING PA14 protein from*Pseudomonas aeruginosa* was secreted and was localized in external appendages together with the PstS protein, a subunit of an ABC transporter.

In the working group, Brito-Argáez et al. (2009), waspurified a peptide of fraction called G10P1.7.57 from seeds of habanero chili. The fraction G10P1.7.57 showed antimicrobial and phosphatase activities. The amino acid sequencing of the polypeptides contained therein, showed the presence of a DING protein. The comparison and alignment of the amino acid sequences of the DING protein from habanero chili with the amino acid sequences from other DING proteins allowed select the internal peptide "ITYMSPDYAAPTLAGLDDATKVAKVGKDV" highly conserved in the family of DING proteins. This peptide was synthesized and the antibody-COM2 was generated andimmunopurified against the peptide. Western blot experiments with this antibody showed that in seed extracts, the COM2 antibody recognizes a polypeptide of ~40 kDa. Because the group is interested in establish which is the cellular localization of the DING protein from habanero chili, then in the present work, the antibody-COM2 was conjugated to dabsyl chloride and by confocal microscopy experiments it has been established that the antibody reacts with intracellular structures of the embryo. In this work, we describe and discuss such results.

The structure, stability and fibrillogenesis of a model amyloidogenic protein as a function of pH.

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6aJL2 is a modelprotein that contains the variable domain of the light chain encoded by the 6a family gene. This subgroup is strongly associated with AL amyloidosis, a disease characterized by the deposition of fibrillar aggregates of a monoclonal light chain. In AL patients 6a-derived deposits are found in the kidney. Being this organ partially responsible for pH homeostasis, in this work we followed the conformational properties of 6aJL2as well its fibril formation kinetics in the pH range from 2.0 to 8.0. The spectroscopic, calorimetric and hydrodynamic properties of the protein show minor changes in the 3.5-8.0 pH range. Urea-induced unfolding experiments indicate that maximal stability was observed near pH 5.0. In more acidic conditions, gross structural changes were detected by all the spectroscopic probes employed; notably, a drastic reduction of the hydrodynamic radius. The protein is severely destabilized as determined by the reduction in Tm and ΔG. Acid conditions alsodecreased thelagtimeand increased the rate of fibril formation. Molecular dynamics simulations and NMR data were then used to obtain a molecular description of the process and to correlate the conformational changes observed in the soluble protein with the increased aggregation propensity for fibril formation.

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Structural and functional characterization of two chitinase-like proteins from *Hevea brasiliensis* belonging to the glycosil-hydrolase family (GH19)

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The rubber tree *Hevea brasiliensis* is constantly subjected to stress due to latex extraction. A plethora of defense-related proteins are expressed throughout this process. Chitinases are among the most important defense-related proteins in plants. They hydrolyze chitin cell walls of various pathogens including insect exoskeletons. Recently, plant chitinase-like proteins (CLPs) have been described, which lack enzymatic activity, due to mutations in one or more of their amino acids required for catalysis. Nonetheless, only the CLPs belonging to the glycosyl hydrolase (GH) family 18 have been structurally and functionally characterized. In this study, two CLPs of the GH19 family, HbCLP1 and HbCLP2, were cloned and expressed. The former has 100% identity with the allergen Hev b 11.0101, which has previously been described by others, while HbCLP2 is an isoform that has not yet been described. It consists of an unusual chitin-binding domain (CBD) that contains a hevein-like domain, a linker, a half hevein-like domain and another linker. Sequence alignments showed that in both proteins, the catalytic residues Glu117 and Glu147 in HbCLP1 and HbCLP2, respectively, were mutated to Ala, accounting for the lack of activity. Nonetheless, both CLPs bound chitin and chitotriose $(GlcNAc)_3$ with high affinities, as evaluated with chitin-affinity chromatography and tryptophan fluorescence experiments. The crystal structures of the HbCLP1 individual recombinant domains (CBD and CatD) were determined and possess almost exactly the same folding as the active class I chitinases. The results from binding studies and in silico docking experiments suggest that conserved residues in the CatD are involved in chitin binding. Endochitinase activity was restored in both proteins by mutating residues A117E (HbCLP1) and A147E (HbCLP2). HbCLP1 and HbCLP2 were highly thermostable and exhibited antifungal activity against *Alternaria alternate*, implying its participation in plant defense mechanisms.

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The glucose transporter 1 -Glut1- plays an important role in the white shrimp *Litopenaeusvannamei*in the response to hypoxia

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The Pacific white shrimp suffers fluctuations of oxygen levels in the natural environments as well as in shrimp farms.In response to hypoxia (low concentration of dissolved oxygen -DO-), the shrimp utilizes anaerobic glycolysis to obtain energy by increasing activity of the glycolytic enzymes hexokinase and Lactate dehydrogenase accelerating the anaerobic pathway. We hypothesize that during hypoxia in the white shrimp, the precise supply of glucose is facilitated by GLUT1, and the aim of this work was to measure the effect of hypoxia on GLUT1 mRNA expression in gills and hepatopancreas of the white shrimp, as well as to determine the importance of GLUT1 by silencing its expressionusing RNA interference during hypoxia. A bioassay was performed with healthy shrimp under controlled conditions and four treatments: a) normoxia (5.3 ± 0.3 mg DO L^{-1}), b) hypoxia (1.45 ± 0.2 mg DO L^{-1}), c) normoxia (5.2 ± 0.3 mg DO L^{-1}) injected with GLUT1-dsRNA and d) hypoxia (1.49 ± 0.5 mg DO L^{-1}) injected with GLUT1-dsRNA. Gills and hepatopancreas were dissected from five shrimps of each treatment to quantify expression of GLUT1 by RT-qPCR, and the metabolites lactate and glucosewith commercial kits respectively. The results show that hypoxia induces ($p<0.05$) an increase (3.7 fold-change) of expression levels of GLUT1 in gills after 3 h, however at 24 and 48 h the expression return to normallevels. In hepatopancreas,the expression of GLUT1 increases ($p<0.05$) after 3 h of hypoxia (3.7 fold-change) and remains at 24 and 48 h at the same concentration of DO. The GLUT1-dsRNA treatment decreased($p<0.05$)the GLUT1 transcripts in gills in both normoxic(-11.3fold-change)and hypoxic (-22.3 fold-change)groups compared to the controls, whereas in hepatopancreas the fold change in the hypoxic group was -3.11 and no effect was found in the normoxic group. Lactate as a final product of anaerobic glycolysis increased in the organisms subjected to hypoxia compared to normoxia and glucose remains constant in both treatments. In conclusion, the glucose transporter 1 plays an important role in the response to hypoxia in the white shrimp supplying sufficient substrate to obtain energy.

"Posttranslational Regulation of maize Spermine Synthase 1: A Key Enzyme In Polyamine Metabolism"

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Polyamines are low molecular weight aliphatic compounds involved in various biochemical, cellular and physiological processes in all organisms. In plants, genes involved in polyamine biosynthesis and catabolism are regulated at transcriptional, translational, and posttranslational level. Previously, we characterized a PEST sequence (rich in proline, glutamic acid, serine, and threonine) of the maize spermine synthase 1 (ZmSPMS1) by translational fusion between 123 bp encoding 40 amino acids of the C-terminal region of the ZmSPMS1 enzyme containing the PEST sequence and GUS reporter gene, it fusion was unstable in *Arabidopsis thaliana* transgenic lines and onion monolayers transient expression system. Now, we are studying the molecular mechanism involved in ZmSPMS1 stability. We found that ZmSPMS1 forms homodimers complexes in yeast two-hybrid "split ubiquitin" system; also, we are interested in the ZmSPMS1 protein dimerization regions. These data could provide some evidences about the molecular mechanism involved in ZmSPMS1 enzyme.

Identification of beta-glucosidaseisoenzymes (*TdGlu1 y TdGlu2*) from teosinte*Zeadiploperennis*

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Seeds of teosinte*Zeadiploperennis* were germined in plastic trays with damp paper and incubated at room temperature.To the fifth day of growth, total RNA was extracted using TRIzolreagent (Invitrogen). The cDNA synthesis was performed using the One Step RT-PCR kit (Qiagen) and specific primers to amplify the mRNA of β-glucosidase1 and β-glucosidase2 called *TdGlu1* and *TdGlu2,* respectively, were used. The actin gene *Mac1* was considered as control. The PCR products were analyzed on 2% agarose gels with electrophoresis conditions of 80V for 90 min.Approximately 1800 and 1850 bp were the amplicon sizes of *TdGlu1*and *TdGlu2,*respectively. The partial sequences of *TdGlu1*and *TdGlu2* amplicons showed 98% identity with the mRNA of β-glucosidase1 (*ZmGlu1*) maize (NCBI, access no. NM_001111984) and β-glucosidase2 (*ZmGlu2*) maize (NCBI, access no. NM_001111422)that allowed us to identify some changes that affect the amino acid sequences comparative with *Zea mays*. At the present we are working to confirm the results and determine if these changes alter the three dimensional structure proteins, becauseit is thought these isoenzymes are involved similarly in a defense mechanism that has been preserved for more than ten thousand years in maize.

Keywords: β-Glucosidases, teosinte, *Zeadiploperennis*, maize.

Identification of an allosteric GlcNAc6P-binding site with a novel structure

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The GlcN6P deaminase (EC 3.5.99.6) catalyzes the isomerization- deamination of glucosamine 6-phosphate (GlcN6P) releasing fructose 6- phosphate (Fru6P) and ammonium ion (NH_4^+). This enzyme plays a key role in amino sugar metabolism and, in some species, it catalyzes an important metabolic regulatory step, since it is allosterically activated by *N*- acetylglucosamine 6-phosphate(GlcNAc6P).

Most GlcN6P deaminases studied belong to a structural group that shares sameRossmann-like fold. Recently was found that the GlcN6P deaminase from *Shewanellaoneidensis* (SonNagBII), a non-homologous isofunctionaldeaminasehas an entirely different fold, characteristic of many sugar isomerases and known as SIS-fold. This enzyme shows allosteric activation by GlcNAc6P; this aspect of SonNagBII has been detected but not studied before us.

SonNagBII is filogenetically related to the synthase domain (GlmS) of glutamine: fructose-6-phosphate amidotranferase (GFAT). The sequence conservation analysis suggests that SonNagBII emerged early on evolution from aGFAT-like ancestor. The residues of the active site are conserved and their arrangement indicated that the role of the catalytic-site residue His242 couldbe the proton donation or proton abstraction at the sugar ring-opening step. This step was predicted from our kinetic results. To verify the proposed role of this histidineresidue we constructed the mutant His242-Gln. It is worth to mention that GFAT, and NagBIIdeaminases are similarly inhibited by the same substrates or transtition state analogs. This is consistent with the conserved structural and functional properties of their active sites.

In contrast with the active site, the structure of the allosteric site is not conserved, and has a novel local geometry. The responsible residue of the binding of GlcNAc6P phospho group is Lys218, this was verified by site- directed mutagenesis.The sequence alignment shows that Lys218 and other key residues at the allosteric site appear only in some *Shewanella* species. The presence of these residues strongly suggests that these deaminases exhibit allosteric activation. This GlcNAc6P-binding site has not been described before.

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MDM2 E3 ubiquitin ligase activity is regulated by inter and intra molecular interactions

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MDM2 has more than 100 interacting partners and the number increase continuously. 21 of those that have been characterised bind in its C-terminal RING finger domain, and among those, we can found proteins and RNA molecules. Some of these biomolecules act as substrates or modulators of its E3 ligase activity, some others show different functions that are important in different moment of the cell life to regulate certain important signal pathways. The questions that arise then are how the same protein can be involved with so many different interactions? What is the key issue that regulates its substrate specificity? Our results show that after DNA damage conditions, a post- translational modification is indeed able to change the conformation of MDM2 and this will controls the oligomerisation status of the protein, the hetero- oligomerisation with MDMX, the specificity towards their interacting partner and finally will control the function of MDM2.

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Mmp8-Mmp13 double deficient mouse is more sensitive to experimental pulmonary fibrosis.

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Collagenolysis is critical in lung development and homeostasis and its disturbance contributes to numerous pathologies such as pulmonary fibrosis. Collagenolysisis mediated mainly by matrix metalloproteases (MMPs), such as MMP-1, MMP-13 and MMP-8. We have generated a mutant mouse containing loss-of-function deletions in both Mmp8 and Mmp13 genes as a deficient degradative system model to evaluate the role of these collagenases in extracellular matrix remodeling after lung injury. In order to explore the role of collagenases in lung fibrosis development and resolution*, Mmp8-Mmp13* double knockout mice were treated with bleomycin and the inflammatory and fibrotic response were analyzed at 7 and 21 days. Collagen lung content was assessed measuring hydroxyproline. Stained lung sections were scored blindly for severity and extent of the lesions and percentage of fibrosis. MMP-2 and MMP-9 levels were examined in lungs and BAL by gelatin zymography. *WT and Mmp8-Mmp13* double knockout (dKO) mice experienced progressive weight loss during the first week after bleomycin instillation. However, *Mmp8-Mmp13* dKO mice showed a higher weight loss and never recovered the initial weight. Histopathological analysis revealed increased inflammatory cell infiltration in *Mmp8-Mmp13 dKO* mice, characterized by enhanced neutrophilicinflitration at 7 days after bleomycin. Likewise,*Mmp8-Mmp13 dKO* mice exhibited more extensive, dense and severe fibrosis characterized by increased lung collagen deposition than the WT mice. Additionally, protein level of α-SMA was markedly higher in *Mmp8-Mmp13 dKO* mice compared with WT littermates, as well as higher numbers of αSMA-positive stained cells were observed in their lung. Recent data suggest that MMP2 and MMP9 may also express type I collagenolytic activity under physiologic conditions. In order to determine whether MMP2 or MMP9 could at least partially compensate for the absence of MMP8 and MMP13, we next evaluate MMP2 and MMP9 activity by zimography in BAL and lung tissue extract form WT and double deficient mice. We found that the levels of both MMP2 and MMP9 were significantly higher in both BAL and lung from *Mmp8-Mmp13* dKO mice compared with WT bleomycin-treated mice after 7 and 21 days of treatment. Our findings indicate that the absence of both MMP8 and MMP13 increases the severity of bleomycin-induced pulmonary fibrosis, suggesting a protective role for these proteases. We propose that *Mmp8-Mmp13 double knockout* mice represent a novel *in vivo* model to elucidate the functional relevance of both collagenases in fibrotic lung disorders and fibrosis resolution.

A histological and immunohistochemical study of beta cells in streptozotocin diabetic rats treated with *Carica papaya* leaf extractchloroform

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Medicinal plants have been used in Mexico since prehispanic times to improve people's health. Besides undergoing a treatment with hypoglycemic drugs, many patients use medicinal plants as tea, infusions or extracts. Moreover, *Carica papaya* is a plant grown for its edible fruit, and it is used as a therapeutic remedy (immune- stimulants, antioxidants, and oral hypoglicemiant). In this study the anti-diabetic effects of the chloroformic extract of the leaf of *the C. papaya* was evaluated in rats with diabetes. Diabetes was induced by the intraperitoneal administration of 60mg/kg of streptozotocin (STZ). The chloroformic extract of the leaf of the *C. papaya* was being provided orally for 20 days with doses of 31-125 mg/kg to diabetic and non-diabetic rats (62mg/kg).At the end of the treatment, the rats were sacrificed by decapitation. A sample of blood, liver, and pancreas were extracted from each animal to determine biochemical parameters, insulin in serum, and in the lipids liver. Furthermore, the presence of β type cells was identified in the pancreas by using immunohistochemistry. The collected data shows that ECCP (31mg and 62mg/kg) normalizes the weight in diabetic rats, decreases the levels of glucose, cholesterol and triglycerides in serum. It also diminishes the hepatic lipids. No hepatotoxic effect was shown, and it is suggests that the β pancreatic cells are protected. This data indicates that ECCP has an anti-diabetic effect in rats with induced experimental diabetes by STZ.

A new experimental model tostudy primary and secondary metabolism in*Capsicum chinense*Jacq.:cell suspension from placentas

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Habanero peppers *(Capsicumchinense*Jacq.)accumulate capsaicinoids inthe placental tissue in blister-like structures. These compounds are of greatcommercial interest given their many applicationsin different industries.Since the location of the enzymes that synthesize capsaicinoids coincides with those involved in the production of their precursors, placentas are considered an excellent model to study the regulation of both primary and secondary metabolisms. A single cell suspension has been obtained from placentas of green immature habanero pods in MS mediumwithoutgrowth regulators. Thiscell suspensionhas been propagatedfor over a period ofsix months. The characterization of the cell suspension comprised growth parameters, such asfresh weight, dry weight andnumber of cells per fixed volume, and viability. Growth data indicated that the cell culture accumulated a maximumbiomass of1.34g after 36days. Its viability is above 80% and the microscopic observation of a sample showedisolated single cells, with numerous chloroplasts, even after this six-month period.

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ANTIPROLIFERATIVE ACTIVITY OF *Tournefortiamutabilis*VENT. IN CELL LINE MCF-7 OF BREAST CANCER

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ABSTRACT

Cancer is the second cause of mortality in developed countries to increasing incidence, there is a major medical and social interest in this disease1. In Mexico in 2010, the main malignant tumors in the adult female population were breast cancer (24.3%) and cervical (9.7%) [2]. Plants have played an important role as a source of antitumor agents; over 67% are derived from plants, marine organisms and microorganism[3]. The Boraginaceae family has species of economic importance, mostly as medicinales[4] plants, antitumor activity has been observed in genera *Arnebia* (HL-60, HeLa and MCF-7) 5; *Lithospermum* (B16F10) 6; *Onosma* (SBcl2, WM35, WM9, and WM164) 7. So the interest of evaluating the antiproliferative activity of *Tournefortia* (Boraginaceae) known as the "herb of cancer" and used in traditional medicine as a home remedy for various diseases including cancer.

Was performed leaf extracts of the plant in three different fractions, used as a solvent chloroform, methanol and water (50g of tissue: 500ml of solvent) by the technique of maceration for 24 hours, each fraction obtained was dried with nitrogen gas and was kept at 4 ° C for later use. The extracts were tested in the MCF-7 cell line for breast cancer at different concentrations (2.5, 5, 10, 20, 40 and 80 ug/ml) for 48 hours at 37 ° C, 5% CO2, was made assays MTT and crystal violet for inhibition of the metabolic activity and number of dead cells. The MCF-7 cell line showed sensitivity to *Tournefortiamutabilis*Vent extracts at a concentration of 80 ug/ml and fractionated in aqueous extracts showed a greater antiproliferative activity (inhibition of metabolic activity and inhibition of cell number) in the chloroform extract 48.8% and 36.4% respectively.

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Antibacterial activity of*Lophocereusschottiii* (Muso) and*Pachycereuspecten-aboriginum* (Etcho) against*Escherichia coli* (ATCC 23922)

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ABSTRACT

Gastrointestinal infections are a common cause of disease in the world and the indiscriminate use of antibacterial drugs has originated multi-resistant strains. *Escherichia coli*is the main etiologic agent and in recent years the emergence of strains with extended-spectrum beta-lactamases represent a serious public health problem. Research has shown that plants with medicinal properties represent potential sources for the obtaining of new drugs, including antibiotics against resistant strains. This study evaluated the antibacterial activity of *Lophocereusschottiii* (Muso) and *Pachycereuspecten - aboriginum* (Etcho) methanol extracts and four fractions against *E. coli* (ATCC 23922), using the broth microdilution method. Only the extract from *Pachycereuspecten-aboriginum* showed significant inhibition values. A 800 µg/mL showed 50, 84, 95 and 100 of inhibition at 6, 12, 18 and 24 hours of incubation. From the fractions obtained from the methanol extract of *Pachycereuspecten-aboriginum*, the ethanol fraction was found to be the most active, showing a800 µg/mL an inhibition of 41% to 85% during 24 hours of incubation. Results have shown that only *Pachycereuspecten-aboriginum* has antibacterial activity against *E. coli.*

Key words: Antibacterial activity, plant extract, Escherichia coli.

Antifungal activity of *Zizyphusobtusifolia* (Jutuki) against *Aspergillusniger* (NRRL3)

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ABSTRACT

The immunodeficiency and the emergence of resistant strains factors increase the incidence of environmental and nosocomial mycoses by *Aspergillusspp* or *Candida spp*. The new drug development trough study of medicinal plants for the search of bioactive natural compounds is an option to face mycosis infection. The objective of this study was to assess the antifungal activity of the root bark methanolic extract of *Z. obtusifolia* and four fractions against *A. niger*, applying the agar dilution method. The methanol extract presented values of inhibition of 100%, 90%, 78% and 74% at 1mg/mL during 1, 3, 5 and 7 days of incubation, respectively. The residual fraction from the methanolic extract showed inhibition at lower concentrations. At 0.5 mg/mL inhibited 100%, 80%, 73% and 59% of the growth during the 1, 3, 5 and 7 days of incubation. The results revealed that *Z. obtusifolia* presents antifungal activity and the main bioactive responsible compounds were isolated in the residual fraction.

Key words: Antifungal activity, Plant extract, Aspergillusniger

Direct analysis of biomolecules from microorganisms by Low-Temperature Plasma Ionization coupled to Mass Spectrometry Imaging (LTP-MSI)

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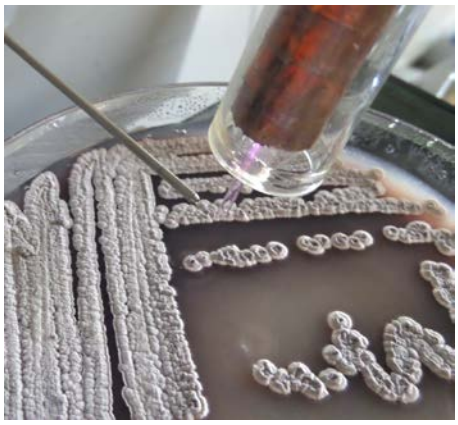

Microorganisms such as fungi and actinomycetes produce a plethora of natural products of ecological and medical importance. Mass spectrometry (MS) is the main tool to analyze them. However, conventional MS methods require extraction and purification prior to measurement. Further, the necessary sample work-up and the physical conditions of ionization hamper the analysis of compounds of high volatility, signaling molecules, etc. Online measurements are therefore difficult.

Ambient ionization is a form of ionization, where the ions are formed under atmospheric pressure and ambient temperature. Current technologies also enable MS *imaging* (MSI), the generation of images which represent the distribution of compounds in a sample [1].

Recently, we developed a system for MSI, based on low-temperature plasma (LTP) [2,3]. *W*n this work we show the direct detection of geosmin, a volatile signaling compound, from cultures of *Streptomyces coelicolor*. Our results demonstrate the potential of our LTP-MSI prototype in the investigation of biosynthethic processes of microorganisms.

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ENERGY DISTURBANCES IN METABOLIC SYNDROME ARE RELATED TO DEVELOPMENT OF NEURODEGENERATIVE DISEASES.

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Metabolic syndrome (MS) is a risk factor for developing and worsening neurodegenerative diseases such as dementia and Alzheimer disease (AD), this last is characterized by cellular lesions called neuritic plaques, formed by beta amyloid depots, and neurofibrillarytangles formed by Tau protein, mainly localized at hippocampusand hypothalamus. Metabolic alterations of MS such as energy deficit linked to insulin and AMP activated protein kinase (AMPK) pathways are thought to predispose appearance of these AD features. In this work we aim to study the relationship between energetic disturbances and degenerative markers in hippocampus (HC) and hypothalamus(HT) tissues in a rat model of MS.

We used adult male Wistar rats (250g) to induce MS by giving them30% sucrose in their drinking water during 16 weeks. One MS group was additionally treated with oral metformin (an AMPK activator) (100mg/kg) for five weeks more. Sex and weight matched rats were used as control and only received tap water.All groups had standard chow diet *ad libitum*. After the treatment the animals were sacrificed and the HC and HT structures were dissected from the brain. AMPK and AKT content and activation were assessed by Western Blot using specific antibodies recognizing total and phosphorylated forms. Expression of amyloid precursor protein (APP) was also measured. Brain creatine kinase (CK) activity was evaluated in terms of determining energy status.

Total content of AMPK and AKT was increased in HC and HT of MS rats, whereas the basal phosphorylated form was reduced, indicating low activation caused by MS. APP was also increased in the brain of MS rats in both HC and HT structures, but this increase was enhanced in HC. CK activity was severely decreased in MS brain (table 1). Metformin treatment failed to recover AMPK or AKT phoshorylation in spite it diminished MS markers such as TG, insulin and visceral fat in the whole animal.

Table 1. Brain CK activity

nmol/min/mg protein	Control	MS	MS + met
HC	8.66±3.44	6.80±0.91*	5.75±0.47*”
HT	10.12±0.71	4.83±0.85*	7.34±1.10*”

*vs control,” vs MS p≤0.05 one way ANOVA.

Conclusions: MS causes energy disturbances that blunt AMPK and AKT pathways in HC and HTwhich could in turn produce neurodegenerative processes and dementia. Alternative treatments are needed for prevention of neurological complications in MS.

Mass Spectrometry Characterization of Trypanothione and Novel Peptides of Medical Importance Isolated from *Acanthamoebapolyphaga*

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Abstract: This paper presents unequivocal results about the presence of trypanothione and its precursor glutathionespermidinefrom the opportunistic human pathogen *Acanthamoebapolyphaga.* They were isolated by RP-HPLC as thiolbimanederivatives and characterized using matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF/TOF).Additionally RP-HPLC demonstrated that thiol-bimane compounds corresponding to cysteine and glutathione were also present in *A. polyphaga*. Besides trypanothione, we want to report four new peptides in trophozoites, a tetrapeptide, ahexapeptide, a heptapeptide and a nonapeptide. Trypanothione and two of the thiol peptides, the hexapeptide and heptapeptide,are oxidized since the reduced forms increase in amount when the normal extract is treated by DTT or by electrolyticreduction that convert the oxidized forms to reduced ones. On the other hand, they disappear when the amoeba extractis treated with NEM or when the amoeba culture is treated with various inhibitors of NADPH-dependent disulfidereducingenzymes. Comparison of the thiol peptides, including trypanothione from *A. polyphaga*with extracts from humanlymphocytes showed that they are not present in the latter. Therefore, some of the peptides here reported could beused as antigens for rapid detection of these parasites. In regard to the presence of the enzymes that synthesize and reducetrypanothione in A. *polyphaga*we suggest that they can be used as drug targets.

Characterization of the forms of U / DAPC complex in vascular smooth muscle cells of human

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Abstract

The Distrophin/Utrophin associated proteins complex (U/DAPC) is conformed by membrane and cytoplasmic proteins organized into three sub complex that links the cytoskeleton and extracellular matrix. DAPC is mainly found in skeletal muscle, other tissues as vascular smooth muscle also presents UAPC. Both complexes keep the stability of the cell membrane during muscular contraction/relax process although some of them can be involved in cell signaling.

The U/DAPC distribution may be different according their functional role and other related proteins (Cav-1, eNOS, aquaporin,etc) These protein interactions could define between mechanical or signaling performance (Rivier) Furthermore endothelial and smooth muscle cells stretching mechanical causes a U/DAPC complex and other signaling proteins over expression, suggesting a regulatory role on vascular tone. However it is unknown if U/DAPC forms different complex or do other protein interactions which define complex functions.

In this work, the main U/DAPC distribution and sub complexes conformation differences between skeletal muscle and smooth muscle cells were analyzed by 2D electrophoresis (BN-PAGE/SDS-PAGE) and WB.

U/DAPC first dimension profiles and WB identification show complexes with different molecular weight and a probable different composition. Skeletal muscle shows at least two Dp-βDg-βSg complexes and also a complex built with Utr and these subunits. Vascular smooth muscle cells show different Utr complexes, one of them interacts with Cav 1. This data were confirmed by 2D electrophoresis, although the complexes differ on molecular weight, its composition or stecheometry remains unsolved.

Evaluation of the possible herbicide activity of an alkaloid isolated of *Ricinuscommunis* L.

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The increase in human population has generated proportional food needs, however the agricultural productivity is constantly affected by different factors, one of them are weeds, which cause a constant reduction in the quantity and quality of important crops, making their elimination a common necessity. The overuse of herbicides to minimize the impact of weeds has created the development of resistant species [1]. Now, the goal is to find natural herbicides with new action modes that will fight this problem. To identify these allelochemicals in the laboratory we use the strategies bioassay-directed search [2].
Ricinuscommunis Linn. (Euphorbiaceae) grows as a weed on the long roadsides in tropical warm regions; it is cultivated for its oilseeds. The oil is used externally for dermatitis and ailments of the eye; the leaves have flavones and tannins, whereas the roots contain alkaloids and the seeds also contain the alkaloids along with flavonoids and tannins [3]. In this bioassay-directed search from the leaves of *R. communis* L. was isolated the alkaloid (ricinine), a majoritary compound in green leaves [4], and it is associated with molluscicidal, insecticidal and larvicidal activity [5,6], However, there are not studies of its herbicide activity.
The isolated compound was evaluated in a germination assays in mono and dicotyledons seeds, different concentrations of ricinice (7.5 a 200 µM), the results showed that dicotyledons seeds germination was inhibited with 100 µM of ricinine (34.48 and 100 % for *Physalisixocarpa* and *Lactuca sativa* respectively), on monocotyledons seeds, ricinine inhibited the *Triticumaestivum* germination 33.33 % and increased the *Loliumperenne* seeds germination (73.30 %). Also, in these assays were determinated the stem and root elongation; the biggest inhibition was observed with 200 µM (stems and roots of *T. aestivum* were deceased 75.87 and 64.75 % respectively, and 100 % for both elongations in *L. sativa*); while the stems and roots elongation of *L. perenne* were not affected by any ricinine concentration, and *P. ixocarpa* stems were increased 78.2 % at 200 µM.
On the other hand, the photosynthetic electron transport was uncoupled by the ricinice at concentrations lower than 50 µM. These results suggested that ricinice can be a good pre-emergent herbicide for dicotyledonous seeds, however more assays need be done to found the site and action mechanisms of ricinine at post-emergent herbicide.

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Effect of protein phosphatases secreted by promastigotes and amastigotes of *Leishmania mexicana* in human macrophages

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INTRODUCTION*:* Parasites of the genus *Leishmania* are the causative agent of leishmaniasis. It is known that the immune response of the host is regulated by several strategies and virulence factors that alter macrophage signaling by promote the survival of the parasites. On the surface of *Leishmania* promastigotes it has been studied different virulence factors such as (lipophosphoglycan) LPG and gp63 wich are the most studied molecules. Although phosphatases have been identified as virulent factors in many pathogenic microorganisms, the role of phosphatases present in *Leishmania* parasites has been poorly studied. Phosphatases are enzymes that remove phosphate groups from amino acid residues of proteins. Also, it is known that some species of *Leishmania* are able to secrete proteins into the culture medium and these molecules could play a role in parasite virulence. Recently, we reported a protein with PTPase activity which was secreted by promastigotes of *Leishmania mexicana*. The aim of this work was to identify phosphatases secreted by promastigotes and amastigotes of *L. mexicana* and analyze these molecules in the production of cytokines by human macrophages. **MATERIALS AND METHODS:** In order to analyze the secreted molecules, promastigotes and amastigotes of *L. mexicana* were incubated for 7 hours and 1 hour, respectively in culture medium. Secreted by these parasites was concentrated 10 times by ultrafiltration. The presence of protein phosphatases was determined by Western blot using two specific antibodies: a monoclonal antibody anti-PTP1B from human placenta and a polyclonal antibody anti-PP2C from *L. mexicana*. Additionaly the concentred medium was used for: 1) phosphatase activity 2) co-incubation with human macrophages to measure cytokine production as TNF-α, IL -12p40, IL-12 p70, IL-1β and IL-10) by ELISA. **RESULTS:** We found that promastigotes and amastigotes secreted to the culture medium proteins with phosphatase activity and this activity was higher in promastigotes than amastigotes. Electrophoretic analysis showed that the proteins secreted by promastigotes, oscillated in a range of molecular weight from 16 to 150 kDa, while the proteins secreted by amastigotes showed a molecular weight that oscillated from 14 to 103 kDa. Finally, the proteins secreted by both stages of the parasite were co-incubate with human macrophages to stimulate cytokine production. The outcome showed a higher production of cytokines with the proteins secreted by amastigotes.

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Putative Transcription Factors In *Trichomonas vaginalis*

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Background

T. vaginalis is a flagellated parasite from theprotozoafamily, which causes trichomoniasis, the most common non-viral sexually transmitted disease. The mechanism by which transcription occurs has been poorly studied in early divergent organisms. In *T. vaginalis*, the promoter region of genes transcribed by RNA polymerase II isbipartite and contains a conserved Inr element surrounding the transcription initiation site. This element isrecognized byIBP39, a transcriptional factor forwhichno homologueshave been identified in other organisms, and it has been proposed that IBP39 may recruit the Pol II transcriptional machinery. Although no canonical TATA boxes have been identified in *T. vaginalis*, in an on going project in our laboratory two proteins with homology to TBP (TATA box binding proteins) have been identified: TvTBP1 and TvTBP2;these proteins do not recognise*cis*elements in the DNA, but may have a role in the assembly of the transcription machinery. The aim of this work is to identify additional transcription factors in this ancient parasite.

Experimental approach

The *T. vaginalis*annotated genomewas*in silico* analysed by BLAST,in order to identifygenes that codify for putative transcriptional factors. From ten genes initially selected, an *in silico*interactome was performed using STRING 9.1 with the protein sequences of TvTBP1 and TvTBP2. This study allowed us to identify two proteins with putative binding to the TvTBPs: TvBRF1 and TvTFIIB. Primers were designed for the PCR amplification of the respective genes in order to clone them andexpress the recombinant proteins. These proteins were used to test protein-proteininteractions with the recombinantTvTBPs and the recombinant IBP39 bythepulldown technique using a GST tag. Additionally, EMSA assays were used to evaluate the ability of these proteins to recognise DNA sequences alone or in combination with other factors.

Results and Perspectives

TvBRF1and TvTFIIB, identified by*in silico*interactome analyses with TvTBPs, contain domains that arecharacteristic for the transcription factor IIBfamily since theycontain a cyclin fold in the carboxyl end and zinc fingers at the amino end. GST pulldown assays show that both TvTFIIB and TvBRF1 are able to interact with TvTBP1 and TvTBP2, additionally, TvTFIIB also interact with IBP39. TvTBPs were not found to interact with a Pol II promoter evaluated by EMSA assays. The recombinant IBP39 was found to interact with a Pol II promoter, as expected, and a supershift of the probe in an EMSA assay was detected in the presence of TvTFIIB. Antibodiesα-TvTBP1 was produced in our lab with the aim of using a co-immunoprecipitation assay to identify additional protein-protein interactions with putative transcriptional factors in a transcriptional context.Antibodies α-IBP39 are being raised for a similar purpose.

Inhibition of amyloid fiber formation for the protein 6aR24G, associated with light chain amyloidosis disease.

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The light chain amyloidosis (AL amyloidosis) is a fatal disease characterized by the deposition of monoclonal immunoglobulin light chains as insoluble amyloid fibrils in organs and tissues of the body causing death within 24 to 48 months. The germline Vλ6a has been closely related to this condition, moreover, the mutation R24G is present in 25 % of the proteins of this germline in patients. In this work, five small molecules were tested as inhibitors for the formation of amyloid fibrils from the 6aR24G protein. We have found by ThT flourecense and transmission electron microscopy that a polyphenol inhibits 6aR24G fibrillogenesis. Using Nuclear Magnetic Resonance we have determined that the inhibition is given by the union to the protein in its native state interacting mainly with aromatic residues.

Digestive physiology and characterization of the enzymatic activity during larval development of spotted rose snapper *Lutjanusguttatus*.

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We studied morphological and physiologicalaspects of the digestive capacity in spotted rose snapper*Lutjanusguttatus*larvae. At hatching, larvae have an undifferentiated digestive tract with mouth and anus closed; however, the activity ofdigestive enzymes like phosphatases, trypsin, chymotrypsin, amylase, lipase andleucineaminopeptidase is present. Afterfirst feedinga regionalization of the digestive tract was observed and an increase in all the enzymatic activities was detected. Lipase showed the highest activity during the larval development and two bands of lipolytic activity were detected. Most enzyme activities tested showed a significant increase around day 20.At day 25 a functional stomach with the presence of gastric glands was observed. Pepsin activity was also recorded from this day, but the zymogram of acid proteaseshowed a band with a molecular weight of 68 kDa from 20 days after hatching. These results suggest that the maturation of the digestive and absorptive functions starts around days 20-25 in the spotted rose snapper larvae.

Bio-guided isolation of antibacterial compounds from*Rhizophora mangle* L. (mangle rojo) against*Staphylococcus aureus* (ATCC 6538P)

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ABSTRACT

Plants with medicinal properties representan important natural source for obtaining new antimicrobial drugs. In the State of Sonora, Mexico, *Rhizophora mangle* L. is a plant used for infectious disease treatments, and recent studies have been shown that bark extract has antibacterial activity against *Staphylococcusaureus*. In this study we isolate compound of methanolic extract from *R. mangle L.* to determinate the antibacterial activity against *S. aureus*. The extract constituents were separated by partition solid-liquid adsorption chromatography and the fractions was analyzed by IR-FT, RMN^{1}H, and MS/MS, while the antibacterial activity was determined by the broth microdilution method.From the obtained fractions, the number two, eluted with hexane:ethylacetate:methanol (8:1:1) from the ethyl acetate fraction, presented the highest values of *S. aureus* growth inhibition. Chromatographic analysis revealedthatthereisa mixture of compoundsandbyspectral analysisthe presenceofdi(ethylhexyl) phthalate (DHEP) was identified. Although natural sourcesof this compound, isunlikely to bea bioactivecompound synthesizedby*R. mangle*L.The antibacterial activity of methanolic extract along with spectrophotometric analysis suggests that the antibacterial activity is due to the action of multiple chemical constituents, and not froma specific compound.

Key Words: Antibacterial activity, extract plant, Staphylococcus aureus

New functions for known proteins involved inmitochondrialproteinimport

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Mitochondria are fundamental organelles for eukaryotic organisms. Originated by an endosymbiotic event, mitochondria participate in several cellular processes like: carbohydrate, protein and lipid metabolism, iron-sulphur cluster biogenesis, energy production and cellular signaling.

For these functions mitochondria use approximately 1000 proteins in *Saccharomyces cerevisiae*, most of them encoded in the nuclear genome and synthesized by citosolic ribosomes. Thereby a system is required to recognize and deliver these proteins to the mitochondrial outer membrane for subsequent intramitochondrial sorting. While there are detailed studies about the machineries that participatein the sorting to all intramitochondrialcompartiments and membranes, there is a lack in the knowledge about the factors that deliver mitochondrial proteins to the TOM complex in the outer mitochondrial membrane (the TOM complex is the main entrance for mitochondrial proteins).

In this work we investigate citosolic proteins and their role in protein import. By genetic and biochemical studies in yeast, our results suggest that these proteins participate in recognition during early stages of protein synthesis.

Protein phosphatase PP2C in *Leishmania mexicana* parasites

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INTRODUCTION*:* Cutaneous leishmaniasis (CL) is a disease caused by protozoan parasites of the genus *Leishmania*, which are transmitted to humans by the bite of sandflies. In Mexico this disease is caused by *Leishmania mexicana*, resulting in either localized cutaneous leishmaniasis (LCL) or diffuse cutaneous leishmaniasis (DCL). *Leishmania* presents two phenotypically distinct stages during the life cycle: promastigote and amastigote. Promastigotes have been observed to interfere with the host signal transduction a way that the effector function the macrophages and dendritic cells is impaired. Signalling pathways inside the cell are tightly regulated by protein phosphorylation, and levels of cellular protein phosphorylation are controlled by the activities of both protein kinases and phosphatases. Phosphatases are enzymes that remove phosphate groups from amino acid residues of proteins and are classified in terms of substrate specificity in: Protein tyrosine phosphatases (PTP) and Serine/Threonine Phosphatases, which can be subdivided in Phosphoprotein Phosphatases (PPP) and Metal-dependent Protein Phosphatases (PPM), which includes PP2C. Some phosphatases of pathogenic microorganisms are associated as a virulence factors. Our laboratory has been interested in the study of the protein phosphatases from parasites and has reported previously the presence of a membrane-bound PTP and PP2C in *Leishmania major* promastigotes. In this work we show the cloning, purification and characterization of a protein phosphatase 2C in *L. mexicana* promastigotes. **MATERIALS AND METHODS:** The gene LmxM250750 was cloned and amplified by PCR from genomic DNA of *L. mexicana* promastigotes using specific oligonucleotides. The recombinant protein was expressed in *Escherichia coli* BL21 (Codon Plus) and purified by metal affinity chromatography. The peak in the elution profile with maximal protein concentration was analyzed by SDS-PAGE and was used to generate monoclonal antibodies. The phosphatase activity was measured in the presence of divalent cations (Ca^{2+}, Mn^{+2} and Mg^{+2}), and differents pH. The effect of specific inhibitors was tested. The antibodies anti- PP2C were used for the immunodetection of the PP2C by Western blot and fluorescence (FL) assays in *L. mexicana* promastigotes. **RESULTS:** The PP2C recombinant protein was able to dephosphorylate a specific threonine substrate. The optimal pH for the activity of PP2C was 8.0 in the presence of divalent cations (Mn^{+2} and Mg^{+2}). The PTPs specific inhibitors as an ammonium molybdate inhibited the enzymatic activity and interestingly, the
activity was inhibited by sanguinarine, which is a specific inhibitor of PP2C. By Western blot the anti-PP2C antibodies recognized a molecule of 44.5 kDa and the immunodetection by FL showed that phosphatase is localized in the *L. Mexicana* promastigotes.

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Binding thermodynamics in periplasmic binding proteins: A comparison between HisJ and LAOBP

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PeriplasmicBinding Proteins (PBPs) are a family of soluble bilobed proteins that bindwith high affinity diversemolecules such as carbohydrates, ions and amino acids. These proteins are crucial components of prokaryotictransport systems and are required for chemotaxis and quorum-sensing. All PBPs display an open/closed “Venus flytrap” conformational change upon ligand binding. We have previously found that Arginine and Histidine binding to LAOBP is enthalpically-driven, with heat capacity changes of -264 and -299 cal $mol^{-1}K^{-1}$ respectively. These heat capacity changes can be accounted for by the changes in surface area that result from the conformational change upon ligand binding. Here we studied HisJ, the other PBP component of the Histidinepermease complex. In the 20 to 35°C range, we found that Histidinebinding is enthalpically favorable and entropically unfavorable On the contrary,the complexes formed by HisJ and Arginine are enthalpically unfavorable. Heat capacity changes upon ligand binding are -838 and -328cal $mol^{-1}K^{-1}$,for Histidine and Arginine binding, respectively. The heat capacity change calculated from the conformational change upon Histidine bindingaccount for only -577 cal $mol^{-1}K^{-1}$, indicating that coupled equilibria should be responsible for a significant fraction of the heat capacity change.

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Effect of β3-adrenergic agonist (carazolol) on calcium regulation of Sarcoplasmic Reticulum from rat slow skeletal muscle.

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Skeletal muscle can be divided into two general types: fast SM (white-glycolytic), or movement muscles and slow SM (red-oxidative), or postural muscles. Fast SM experiences the physiological phenomenon known as fatigue, while slow SM is resistant to fatigue. *Carazolol* is an impermeable molecule in the cell which has a potent blocking effect on β1 and β2 adrenergic receptors and is an agonist to β3 receptors. This drug t has been utilized in the industry of animal production to improve the quality of meat and in the doping of athletic race horses during training to fortify muscular performance. However, experimental evidence of the mechanism of action that this drug has on the SM does not exist. In the present work, we have studied the effect *Carazolol* has on the mechanical properties of both types of muscle: *Carazolo*l reduces the contraction force in a dose-dependent manner in both types of muscle, favoring fatigue and also reducing the fatigue contraction force. However, both types of muscle treated with Carazolol recover and enhance the contraction force after fatigue, though it has a stronger effect on slow muscle. The effect Carazolol has on loss of force and in enhancement after recuperation, suggests an effect on the Sarcoplasmic Reticulum Calcium ATPase (SERCA), which is the protein in charge of calcium capture and muscle relaxation. The differential effect Carazolol has on SERCA1 (found in fast SM) and SERCA2 (found in slow SM) hydrolytic activities in Sarcoplsamic Reticulum isolated from fast and slow muscle, is used as a tool to understand the molecular mechanism of muscle fatigue, a physiological phenomenon which is currently unknown.

Kinetic mechanism of thioredoxin-glutathione reductase from *Taenia crassiceps*.

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Thioredoxin-glutathione reductase (TGR) represents an isoform of the high-molecular weight variant of thioredoxin reductase. Both enzymes are NADPH-dependent flavoproteins and have a catalytically essential selenocysteine residue. However, in TGR a Glutaredoxin-like domain has been appended to the N-terminal end of the subunit, conferring to the enzyme the ability to reduce the disulfide form of glutathione (GSSG) as well as to catalyze thiol/disulfide exchanges. In parasite flatworms, TGR is the only disulfide reductase involved in the regeneration of the reduced forms of both glutathione and thioredoxin. An interesting kinetic property of the enzyme was found when the progress curves were allowed to proceed until NADPH exhaustion in the presence of moderate or high concentrations of GSSG. Under such conditions, a significant lag time was observed. In spite of the potential regulatory role *in vivo* of the phenomenon, no comprehensive model to explain it is yet available. A knowledge of the kinetic mechanism of the enzyme is a prerequiste in order to propose such model. In the present work the kinetic mechanism of TGR from Taenia crassiceps is described.

Initial velocity experiments, with either GSSG or DTNB as the variable substrates, revealed parallel plots families in double reciprocal plots, suggesting a ping-pong bi bi kinetic mechanism. However, the inhibition patterns by the product $NADP^+$ were unexpected. With NADPH or GSSG as the variable substrate, a competitive and an uncompetitive inhibition pattern, respectively, were observed. Such inhibition patterns are consistent with an ordered sequential mechanism. In order to elucidate the apparently contradictory results, an additional experiment in which both NADPH and GSSG were varied at a constant ratio was carried out. The corresponding double-reciprocal plot revealed intersecting straight lines, supporting a ping-pong bi bi kinetic mechanism. The unexpected inhibition patterns by $NADP^+$ are explained by assuming the nucleotide is able to form a dead-end binary complex with the unmodified form of the enzyme while its dissociation from the enzyme during the catalytic cycle is considered as an irreversible event. The second product of the reaction (GSH) was a poor inhibitor. At 8 mM GSH, barely 10 % of inhibition was observed.

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Stability analysis of the perchloric acid-soluble protein (Tv-PSP) from *Trichomonas vaginalis*

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Introduction: Recently, a perchloric acid-soluble protein was identified in *Trichomonas vaginalis* (termed Tv-PSP) a protozoan parasite that infects the urogenital tract in humans. Nowadays, a little is known about the function of this protein in the parasite however, it might be classified into the YER057c/YjgF family, which members are highly conserved within all species, suggesting that its important role was maintained throughout evolution. The majority of the members of this family are homotrimeric proteins, however, its stability dynamics is until unknown. The aim of this work was to determine the stability of the Tv-PSP from *T. vaginalis*. **Methodology:** The purified recombinant Tv-PSP was used to generate polyclonal antibodies, which were used for immunodetection of the native Tv-PSP in total protein extract of *T. vaginalis* by Native-PAGE. The quaternary structure of rTv-PSP was obtained using salt, EDTA and DTT. The interaction type between the rTv-PSP monomers was determined by SDS- and reducing agents-treatments. In order to determinate the protein stability the rTv-PSP was incubated with several urea concentrations (1, 3, and 6 M) and the stability was determinate by densitometric analyses. **Results**: One single band of ~53 kDa was immunorecognized by the polyclonal antibody anti-rTv:PSP suggesting that native Tv-PSP has a quaternary structure in the parasite, which is formed by ~13.4kDa monomers. Interestingly, the rTv-PSP is capable to acquire quaternary structure which is stable at least for 6 months at -20°C. SDS- and reducing agents-treatments destroyed the quaternary structure of rTv-PSP, suggesting that covalent bonds are involved in the interaction between monomers.Surprisingly, the rTv-PSP is stable at high urea concentrations and the quaternary structure of Tv-PSP might be formed by four monomers.
Conclusion: The quaternary structure of Tv-PSP is stable at high urea concentrations and it appears to be formed by four monomers of 13.4kDa each.

Plant proteasomes

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The proteasome is widely recognised as the central enzyme of non lysosomal protein degradation. It is responsible for intracellular protein turnover and it is also critically involved in many regulatory processes and, in higher eukaryotes, in antigen processing. The 26S proteasome is the key enzyme of the ubiquitin/ATP dependent pathway of protein degradation. The catalytic core of this unusually large (2000 kDa, 450Å in length) complex is formed by the 20S proteasome, a barrel shaped structure shown by electron microscopy to comprise of four rings each containing seven subunits. Based on sequence similarity, all fourteen 20S proteasomal subunit sequences may be classified into two groups, alpha and beta, each group having distinct structural and functional roles. The alpha subunits comprise the outer rings and the beta subunits the inner rings of the 20S proteasome. Observations of the eukaryotic proteasome and analysis of subunit sequences indicate that each ring contains seven different subunits (alpha7 beta7 beta7 alpha7) with a member of each sub family represented in each particle. Each subunit is located in a unique position within the alpha or beta rings. 20S Proteasomes degrade only unfolded proteins in an energy independent manner, whereas 26S proteasomes degrade native and ubiquitinylated proteins in an ATP dependent manner. The native protein substrates are recognized by subunits, some with ATP binding sites, of the outer 19S caps of the 26S proteasome.
Blue native PAGE (BN-PAGE) can be used for one-step isolation of protein complexes from total cell and tissue homogenates. It can also be used to determine native protein masses and oligomeric states and to identify physiological protein–protein interactions. Native complexes are recovered from gels by electroelution and could be used for 2D crystallization and electron microscopy or analyzed by in-gel activity assays or by native electroblotting and immunodetection. We use BN-PAGE to characterize *Arabidopsis thaliana* and *Agave tequilana* proteasome complexes. This single step procedure was adapted to purify all existing physiological proteasome complexes associated to their various regulatory complexes and to their interacting partners.
So far, four majoritary (and five not-so-abundant) anti-20S positive bands can be detected from a proteasome enriched fraction obtained by differential ultracentrifugation and analyzed by BN-PAGE and western blot. Electroeluted proteins obtained from each band, showed a distinctive but similar “map of dots” by O’Farrell 2D gels. We suggest that molecular weight proteasome differences observed, are given by 20S catalytic particles interacting with a particular set of proteins. Under stress conditions (paraquat or hydrogen peroxide addition, osmotic stress or an increase in culture temperature) we detect drastic changes in proteasome pools. We pretend to characterize each proteasome assembly by proteomics, biochemical and structural approaches to eventually understand their role in the context of plant physiology.

High-performance planar chromatography (HPTLC) and low temperature plasma ionization mass spectrometry (LTP-MS) for the analysis of bioactive compounds in guava fruit *(Psidium guajava)*

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Guava *(Psidium guajava)* is an appreciated crop around the world because of the sweet flavor of the fruits. The leaves and bark have been used in traditional medicine against different aliments such as cough, fever, diarrhea and malaria.

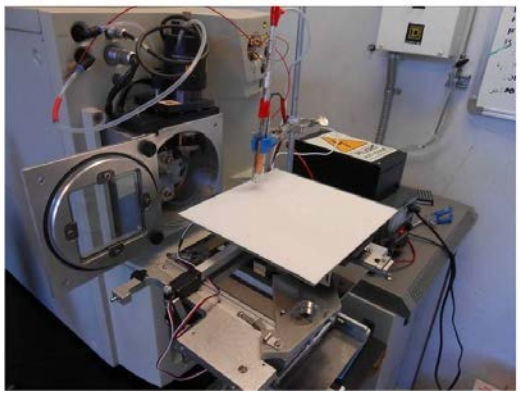
The healing capacity of this plant has been associated to bioactive compounds. These compounds can neutralize free radicals, which are associated to degenerative diseases. Furthermore, some of these molecules can inhibit the proliferation of pathogens.

For this reason, *in vitro* and *in vivo* assays have been developed to understand the healing properties of these bioactive compounds, as well as screening methods to discover them in complex mixtures.

High-performance planar chromatography (HPTLC) is a popular technique in natural products chemistry, because of the availability of many staining protocols and the possible screening of multiple samples in parallel. HPTLC antioxidant fingerprinting can give a pattern of the antioxidant capacity of compounds present in a complex sample.

Mass spectrometry (MS) on the other side is used to identify molecules by their mass charge ratio (m/z). The basic principle of mass spectrometry is to use an ionization source where the molecule is electrically charged and then introduced into a mass analyzer. Various ion sources have been developed with the purpose of ionize different type of molecules. Recently, low temperature plasma (LTP) has emerged as a novel ionization source with the advantage that analyses can be performed in ambient conditions with minimal o no preparation of samples. The direct detection of compound such as naproxen and high volatile compounds as caffeine and vanillin in different excipients has been achieved with this new method.

The aim of this work is to develop a technological platform where bioactive compounds of biological samples separated by HPTLC can be identified directly on the plates with no previous treatment by using LTP-MS. Extracts of guava fruits tissues will be used in bioactive assays.



Direct analysis of HPTLC with LTP ionization using a Waters ZQ micromass simple quadrupole analyzer

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Effect of omega fatty acids (3, 6, or 9) against diabetes.

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Type 2 diabetes mellitus (T2DM) is a progressive chronic disease characterized by hyperglycemia due to insufficient insulin production orinsulin resistance. This disease is associated tohigh morbidity and mortality rates and it is a leading cause for cardiovascular diseases, renal failure, and blindness. The basis for treatment of T2DM is improvement of glycemic control, which is typically achieved by lifestyle changes such as exercise anddiet. There areproposals concerning the effect of diets rich in polyunsaturated fatty acids (PUFAs), such as omega-3, omega-6, and omega-9 fatty acids(the last from avocado oil), that they have beneficial effects against diabetes, through several mechanisms including lipoperoxidation inhibition, transcriptional regulation, and changing membrane physicochemical properties. In this study we analyze the effect of omega fatty acids (3, 6, or 9) on a rat T2DM model. If these omega fatty acids somehow participate on hyperglycemia control, it is expected to be reflected not only on physiological parameters, but also in the membrane fluidity and fatty acid composition of erythrocyte membranes.

The rat T2DM model was developed by intraperitoneal injection of streptozotocin (STZ) in citrate buffer at 125 mg/kg body weight, to 48 hours-old newborn males. Control groups were injected with the citrate buffer only. Nursery of rats lasted 4 weeks. Animals were housed in cages and fed *ad libitum* on standard diet. Additionally, animals were supplemented from monday to friday with anenrich source of omega 3 (flaxseed), 6 (evening primrose) or 9 (avocado oil) fatty acids, at 125 mg/kg.Body weight and blood glucose were determined weekly; cholesterol, triglycerides, and glucose tolerancecurves, monthly.Animals were killed at 1, 3, or 6 months-old and ghost erythrocytes obtained from blood. Erythrocytes fatty acid composition was determined by transesterification of fatty acids to their respective methyl esters and analyzed in a Perkin-Elmer gas chromatograph. Membrane fluidity was measured spectrofluorometrically with the fluorescent probes dipyrenylpropane (DPyP), diphenylhexatriene (DPH), and trimethylamine-diphenylhexatriene (TMA-DPH).

Tolerance curves show that at the doses supplemented, the omega-9 fatty acids have partial control of hypeglycemia at initial stages, but not very lasting. There were not significant differences between groups about cholesterol and triglycerides. Some changes were detected in fatty acid composition and membrane fluidity by the three different fluorescent probes. In conclusion, at long term periods, none of the analyzed omega fatty acids, have long lasting beneficial effect controlling hyperglycemia, at least at the concentrations used.

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Contribution of tryptophan residues on the Human Gamma D-Crystalin stability: Real Time NMR folding study.

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Cataract is the second cause of blindness worldwide, and it is cause by aggregation of proteins inside the eye lens. Age, UV radiation, oxidation, deamidation, and truncations are the causes of covalent protein damage that induce aggregation. Crystallins are the main proteins in the lens; there are three families, alfa, beta and gamma. Alfa crystallin, is member of the small heat shock

protein family with chaperone function. Meanwhile, beta and gamma crystallins are a family of proteins with 173 amino acid, two homologous domains, N-terminal (1-82 amino acid) and C-

terminal (88-173 amino acids). Each domain contains two Greek key motifs forming a beta- sandwich of eight intercalated beta-strands. Human Gamma D crystalline (HgD) is located in the

center of the lens, and the lens nucleus, and it is a highly stable protein. HgD has four buried

tryptophan at positions 42, 68 in the N-terminal domain, and 130 and 156 in the C-terminal domain. It is been proposed that the backbone conformation of these tryptophans in HgD may have evolved to enable the lens to become a very effective UV filter, which may diminished the UV radiation damage, and, consequently, the aggregation of the protein. In this work, in order to study the effect of each tryptophan on the HgD miss folding process, different mutations were prepared, single, double and triple Tryptophan were substituted by phenylalanine, and were studied by real time NMR.

Relevant aminoacids for the differences in reactivation of the triosephosphate isomerases from *Trypanosoma brucei* and *T. cruzi*.

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Triosephosphate isomerase (TIM) is a widely studied glycolytic enzyme. TIM is distributed in all known cells including the parasites *Trypanosoma cruzi* y *T. brucei*. The TIMs from *T.cruzi* (TcTIM) and *T.brucei* (TbTIM) are homodimers, they have similar catalytic properties and three dimensional structures. The enzymes of both organisms are very similar. Their overall sequence identity is 73% and, in the case of the 40 interface residues, 85%. Their three dimensional structures overlap with a Root Mean Square Deviation of 0.96 Å (Hernández Alcántara G. *et al*., Biochemistry 2002). Contrasting with the high structural and sequential similitude between these two enzymes, there are many differences in their functional properties. For example, their different susceptibility to digestion with subtilisin; their different susceptibility to sulfhydryl reagents like methylmethane thiosulfonate; and the different velocity and extent of reactivation from unfolded monomers with guanidine hydrochloride (GdnHCl). The reactivation is faster and more efficient in TcTIM than in TbTIM. These last properties are of particular interest to the present study.

In previous work in our laboratory we gradually converted one enzyme (TbTIM) into the other (TcTIM) exchanging equivalent regions of these enzymes, to determine the relevant segments involved in the refolding of both proteins. To accomplish this progressive grafting we divided the enzyme into 8 regions, with each region consisting of a beta-sheet, an alpha-helix and the corresponding connecting loops. Following this strategy, ten “TcTIM-TbTIM chimeras” were constructed. The profile of reactivation was assayed for each chimera and the results showed that regions 1 and 2 are responsible for the different reactivation behavior.

In the present study we prepared another six chimerical enzymes with all possible combinations between regions 1 and 2 of both enzymes. Our results suggest that region 2 is implicated in the speed of reactivation, but both regions (1 and 2) are involved in the extent of reactivation. Two of these chimeras showed a very low efficiency of reactivation after the treatment with GdnHCl. These chimeras were chosen to prepare enzymes with additive mutations, exchanging the different residues contained in regions 1 and 2 of both proteins. Our results show that the first 5 residues of each region are important for the speed and the extent of reactivation. This has allowed a more detailed study to determine the residues implicated in the different reactivation of TbTIM and TcTIM.

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Expresion de peptido amiloide βen los vasos de las leptomeninges cerebrales en individuos con enfermedad de Alzheimer y su relación con el genotipo ApoE.

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Introducción. Los vasos arteriales y venosos localizados en las leptomeninges, representan la mayor parte de la circulación cerebral externa. Se ha descrito que el péptido ameloide (AB) se acumula formando placas o racimos fibrilares en los vasos. Los cambios adaptativos que sufre el sistema vascular puede afectar su función y su estructura, y relacionarse con la degeneración vascular. Se ha descrito una fuerte asociacion entre el genotipo ε4 de la apolipoproteína E (Apo E), y el riesgo para desarrollar Alzheimer (AD). Se desconoce de que manera, el transportador puede influir en la expresión de proteínas relacionadas conAD, el depósito de AB o en la remodelación vascular.**Objetivo**: Estudiar la expresión de proteínas relacionadas con la remodelación vascular y AD y su relación con el genotipo Apo E. **Método**. Las leptomeninges fueron obtenidas de autopsias de 15 sujetos dentrodel programa de donación de órganos del Instituto Banner en Sun City, Arizona, USA, de los cuales, cinco tenian enfermedad de Alzheimer diagnosticada (AD), con genotipo ApoE 3/3, cinco con AD y ApoE 4/4 y cinco sujetos control sin demencia (NDC) con ApoE 3/3. Se extrajeron las proteínas solubles mediante homogenización con TRIS-HCL y ultracentrifugación, el botón se fraccionó y una porción fue nuevamente homogenizada con buffer de urea para la técnica de DIGE2 o con GDFA (glass destilated flouroacetic acid) para la obtención de proteínas de matriz extracelular. Los extractos proteicos fueron analizados mediante las técnicas de DIGE2 FPLC, HPLC y Western Blot (WB).**Resultados y conclusiones**. Mediante DIGE2, se encontró que las proteínas más expresadas en los extractos de vasos piales fueron ACTA, MYF9, Vimentina, Apo J, ERk2, Colágena IV, Colágena VI, Colágena XXV y VEGF. El análisis por WB mostró marcadas diferencias entre grupos para AB40 y AB42 y de manera menos marcada, para Apo J y Vimentina. El análisis de las fracciones resultado de las columnas de FPLC y HPLC solo mostró diferencia en el patrón de expresión para colágena XXV entre el grupo AD ApoE 4/4 y NDC ApoE 3/3.La expresión de las isoformas AB40 y AB42se relacionaron directamente con el genotipo de ApoE, donde la isoforma ε4 (ApoE 4/4) se asocia icon un aumento en la formación de AB42. Por otro lado, durante el desarrollo de AD, se ha observado que hay modificación estructural de los vasos cerebrales, lo cual puede estar asociado con cambios en los componentes de la matriz extracelular. Aunque en nuestros estudios solo se pudieron apreciar diferencias significativas en la expresión de la colágena XXV y vimentina, es necesario continuar el estudio de estas asociaciones mediante diferentes metodologías para poder cubrir un espectro más amplio.

Increase in pH causes conformational changes in laccase from bacteria *Thermus thermophilus* producing loss in activity.

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The laccase is a multicobre oxidases with a potential biotechnological applications currently (Zheng *et al*., 2012), due to the high resistance to the thermal denaturation, as well as the ability to oxidize various organic substrates possessing. The laccases have been applied in the removal of a large number of pollutants environmental, such as alkenes, chlorophenols, dyes, herbicias, policiclicos and benzopyrene aromatic hydrocarbons (Cuoto and Herrera, 2006). The ability of laccases to oxidize phenolic compounds as well as their ability to reduce molecular oxygen to water has led to intensive studies of these enzymes.

Mainly industrial processes in which include the participation of the laccasesat high temperatures, low-cost, which includes reuse the enzyme and to producethem on a large scale. Several studies have shown a high loss of activity of several free lacasas implying limitingin its operational stability (Zhang *et al.*, 2009;Nicolucci *et al.*, 2011). This project is focused on providing knowledge about the stability of the free laccase, and thus find strategies that allow to improve the use of these enzymes in industry and biotechnology. In the present investigation was considered to analyze the stability laccasefrom extremophilic bacteria *Thermus thermophilus*.

Analysis of the stability of the enzyme is via differential scanning calorimetry (DSC). From the difference in enthalpy (ΔH), and the difference of heat capacity (ΔCp) and the average temperature of denaturation Tm, stability was calculated at different temperaturas and pH. Likewise, the stability of the lacasa research is carried out by limited proteolysis at different pH.This technique describes the general procedure for setting up a limited proteolysis reaction. This experiment like this is useful for determining the sizes, timed order of appearance, and relative amounts of fragments produced from the protein of interest by digestion with protease. Following digestion with a protease, samples are analyzed by SDS-PAGE to identify cleavage products. From the results, we infer the presence of stable subdomains and conformational changes.

Detection of the genes which encode to the ICp55 peptidase, methionine aminopeptidase 1 and methionine aminopeptidase 2 in the yeast *Schizosaccharomyces pombe*

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Schizosacharomyces pombe (*S. pombe*) is a yeast which is an excellent model for the study of aging, due to the similarity in genes and metabolic pathways with eukaryotic superiors as a human. An aspect little studied about the aging is the proteases profile, whose expression is deregulated and that could contribute to advance of process. However, the information about the proteolytic system of *S. pombe* is very limited, therefore in this work were detected to the proteases ICp55, methionine aminopeptidase 1 and methionine aminopeptidase 2, only have been predicted through the sequencing of the genome of the yeast at the Sanger Center and in aged mice such enzymes showed its deregulation. For this purpose the 972 h- strain of *S. pombe* was cultured to the exponential phase in YE medium. Further, the chromosomal DNA was purified, which was used to the amplification of structural region of the genes ICp55, fma1 and fma2 using oligonucleotides specific. PCR products were cloned on pJET-2.1.blunt vector and sequenced with the further in silico analysis with programs in ExPASy. The obtained results do not show changes in the reported sequences in GenBank for any of genes analyzed. Further, was subsequently analyzed t whe sequence primary and predicted secondary and tertiary structure of the three proteins. About the analysis of the ICp55 gene that encode the mitochondrial intermediate cleavage peptidase or SPAC12B10.05, show that is a protein with a character largely hydrophilic which location intracellular is the mitochondrial matrix. ICp55 is a protein of the AMP_N (Aminopeptidase P, N-terminal domain) family and Peptidase_M24 (PFAM and Superfamily). This protein is a manganese-metallopeptidase. The enzyme matures to proteins previously hydrolyzed by the enzyme MPP. For other side, were detected the genes that encoded the methionine aminopeptidase (MAP), which is responsible for the removal of the N-terminal methionine from nascent eukaryotic cytosolic proteins if the penultimate amino acid is small and uncharged. In this work, were detected two enzymes named MetAP_1 and MetAP_2, encoded by the fma1 and fma2 genes, respectively. MetAP1 would reside in cell nucleus and MetAP2 in cytoplasm. Both enzymes are cobalt-metallopeptidases encoded by the fma1 gene, also Known as SPBC3E7.10 and fma2 gene or SPBC14C8.03. Both proteins belong to Clan MG, family M24, subfamily A M24.002. Still is not know the function of any enzymes or their role in the aging in *S. pombe*, which will study in further studies.

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Cytochrome c oxidase Cox2 subunit expression from the nucleus and its import into yeast mitochondria

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Cytochrome*c* oxidase's (COX) subunit II(Cox2) is foundin the mitochondrial DNA in most eukaryotes. Nevertheless, the *cox2* gene is nucleus-localized inapicomplexan parasites, chlorophycean algae and some legumes.Functional transfer ofa mitochondrial gene to the nucleus is known as allotopic expression,which has a potential application in the development of gene therapies for mitochondrial diseases, but has proved to bedifficult to achieve for highly hydrophobic proteins. In the yeast *Saccharomyces cerevisiae*, a Cox2 mutant was designed to be expressed from a plasmid, and its protein product was functionally assembled into COX. This protein mutant was fused to a mitochondrial targeting sequence (MTS) and has one mutation in the first of the two transmembrane segments, a tryptophan for an arginine in position 56 (W56R). This mutation lowers Cox2 hydrophobicity enough to allow its import into the inner mitochondrial membrane.Upon transformation of the Cox2^{W56R}gene into a yeast strain lacking mitochondrial *cox2* (Δ*cox2*), growth in respiratory media was restored, although at lower rates than the wild type.Biochemical analysis of the transformed strain showed a 60% recovery of the abundance of COX and a 75%recovery ofthe oxygen consumption rate of the purified complex, compared tothe wild type strain. Cox2^{W56R} protein, synthesized in the cytosol, clearly follows a different biogenesis route than its mitochondrial counterpart. Our aim is to understand how the W56R mutation affects the biogenesis and activity of COX.The enzyme's biogenesis is highly controlled and involves multiple factors; even so, allotopic Cox2^{W56R} is functionally integrated intoCOX. We want to explore the possibility that mitochondrial proteases are degrading a fraction of the Cox2^{W56R}coming from thecytosol. We will generate double mutants lacking *cox2* and the mitochondrial protease *yme1*. Also, to study weather both allotopic Cox2^{W56R} and native Cox2 compete to assemble into COX, we will simultaneously express both proteins.Additionally, we will generate a strain with the *COX2^{W56R}* gene coded in the mitochondrial genome. The corresponding protein should follow the canonical biogenesis route but will also contain the W56R mutation. Finally, we will mutate position 56 with residues exhibiting similar properties as arginine.In all cases, we will study growth rate in respiratory media, oxygen consumption and COX activity in blue native gels.

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"Expression and isotopic labelling of sea anemone toxin BcsTx1"

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The cnidarians (classes Anthozoa, Scyphozoa, Cubozoa and Hydrozoa) have evolved a large amount of pore-forming toxins, phospholipases A_2, protease inhibitors, neurotoxins and toxic secondary metabolites. The biological and ecological roles of these toxins present in cnidarians venom are 1) immobilization and death of the prey, 2) defense against predators and 3) intra- and inter-specific competition.

Sea anemones (Cnidaria, Anthozoa) produce many polypeptide toxins and proteins that are potent ion channel blockers and cytolysins. Sea anemone venom is an important source of bioactive compounds used as tools to study the pharmacology and structure-function of voltage-gated K^+ channels (K_V). These neurotoxins can be divided into four different types, according to their structure and mode of action.

In Mexico there are three research groups working with the extraction, purification and biological characterization of sea anemone's toxins, however, a disadvantaged of native toxin are small quantities obtained after purification. In this project we are working to produce recombinant toxin of BcsTx1 from sea anemone *Bunodosoma caissarum*. BcsTx1 has 37 residue peptide containing six half-cystines and acts voltage- gated potassium channels.

The amino sequence of BcsTx1 was cloned to GeneScript into expression vector pGS-21a, the plasmid was expressed in Rosetta2 (*E. coli)* cells. Now, we are searching the optimal conditions for express in minimum medium (^{15}N and ^{13}C) and adequate purification, once obtained the best conditions, we intend to elucidate the structure with NMR experiments.

Aluminum induces low phosphate adaptive responses and modulates primary and lateral root growth by differentially affecting auxin signaling in Arabidopsis seedlings

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Abstract

Plant productivity in acid soils is limited mainly by aluminum (Al) toxicity and phosphorus (P) deficiency, both of which are characterized by a rapid inhibition of root growth. In this work we investigated Al and P interactions in roots of *Arabidopsis thaliana* seedlings. Al inhibited primary root growth by affecting cell cycle progression and causing differentiation of cells in the root meristem. These effects were reduced in *low phosphorus insensitivelpi1-3* and *low phosphate resistantlpr1-1 lpr2-1* Arabidopsis mutants. Al also activated the expression of the low phosphate-induced P transporter *AtPT2* in roots indicating that it activates a low P rescue system. The role of auxin as a mediator of morphogenetic changes by Al was evaluated by using the auxin-signaling mutants *tir1*, *tir1 afb2 afb3*, and *arf7 arf19*. Our results show the requirement of auxin receptors for Al-induced LR formation and reveal an important role of ARF7 and ARF19 transcription factors in both primary and lateral root responses to Al. We conclude that lateral root formation in response to Al toxicity and P deficiency may involve common signaling mechanisms, while a pathway involving AR7 and ARF17 is important for primary root growth inhibition by A

Respiratory complexandsupercomplexesarrangementofmitochondriopathy primary skin fibroblastscultures

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Oxidative phosphorylation (OXPHOS) is the process where ATP is formed as a result of the ordered electron transfer through five respiratory complexes (I-V) located at mitochondrial cristae. OXPHOS complexes could be found as individual enzymes or assembled into supercomplexes which confer advantages to the OXPHOS metabolism. Molecular damage on at least one OXPHOS complex raises amitochondriopathy, multisystemic diseases consequence ofmitochondrial or nuclear pathologyDNA mutations, affecting protein function. Although some mutations are well characterized some other not, suggesting defects on complex/supercomplex assembly. Blue Native- PAGE is a useful tool to analyze OXPHOS complexes integrity and stability and is possible to evaluate its role on mitochondriopathies.

Our research aim is to determine changes on complex/supercomplexdistribution/array of mitochondriopathy dermal fibroblast primary cultures.

Native gel electrophoresis is a diagnostic tool formitochondriopathy diseases since unveils facts that are invisible to conventional assays. Through this gels is possible determine secondary damages when various OXPHOS complexes are damaged. However some diagnosis results show differences, suggesting a supercomplex participation. Since muscle tissue is limited, a good alternative is the primary dermal fibroblast culture, however since these results could be different according to mutation origin or biological sample, we focused on fibroblast phenotype verification.

Until now we got sixmitochondriopathy dermal primary cultures diagnosed by spectrophotometric activityassays on muscle tissue which express a complex IV related dysfunction.Since these cultures could not express disease, all of them are being analyzed by zymograms to phenotype corroboration. Once made this, mitochondriopathy fibroblast cultures are going to be analyzed by BN- PAGE and 2D electrophoresis to determine complex/supercomplex arrays.

Characterizationof proteolyticphenomenon inthestarchbinding domain(SBD) of the α-amylase of*Lactobacillusamylovorus*.

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α-amylases are glycoside-hydrolases that catalyze the hydrolysis of internal α-1,4glycosidic bonds in starch and glycogen, generating smaller oligosaccharides (1). They are multidomain proteins that contain a catalytic barrel $(\beta/\alpha)_8$,and sometimes, one or more non-catalytic domains whose function is generally described as carbohydrate binding domain (CBM) and particularly as a starch-binding domain (SBD).The SBD is a functional domain that can bind granular starch, increasing the local concentration of substrate at the active site of the enzyme, and that may also disrupt the structure of the starch surface (2).

The α-amylase of *Lactobacillus amylovorus* has a structure that consists of a catalytic domain (CD) and anunusual carboxy-terminal starch-binding domain with 5 identical carbohydrate binding modules (CBM family 26) in tandem (3). Each repeat acts as an independent fixing module with an additive or synergic effect between the units (4).

It has been observed that when we stored both pure SBD and α-amylase from L. amylovorus we found various forms of low molecular weight with a constant patron, which does not correspond to random degradation. So far there is little information on the proteolytic processing of amylases and the nature of it. Our first results suggest an autoproteolytic mechanism.

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Role of amino acid F763 in the function of *Saccharomyces cerevisiae* mitochondrial polymerase *MIP1*

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Mitochondria is a unique organelle that has its own DNA. Mitochondrial DNA (mDNA), encodes for proteins involved in ATP production, metabolism and signal transduction. It is believed thatmitochondria plays a key role during aging and the development of several pathologies. In each mitochondrion exist multiple copies of mDNA that are produced by mitocondrial DNA polymerase Pol gamma in humans. Mutations on Pol gamma cause a wide group of diseases, due to mitocondrial dysfunction. Yeast analogous polymerase Mip1 has been used as model to study the molecular mechanisms of mitochondrial disorders. Comparison between Mip1 and T7 polymerase sequences, showed that phenylalanine 763 residue could be involved in the fidelity of Mip1. To test our hyphotesis we performed punctual mutations on F763 of Mip1. *S. cerevisiaemip1*Δ/*sod2*Δ/PFL38-*MIP1*was transformed by electroporation withcentromeric plasmids PFL39-mip1 that carry the mutationsF763A, F763H, F763K, F763Y, F763V and F763W. Plasmid suffling was corroborated and the mutants were tested for oxidative growth and petite accumulation. Our data show, that only F763H and F763Y mutants were able to grow on ethanol and glycerol as carbon source. The petite frecuency was found to be very similar between wild type, F763H and F763Y. To support our results we perform an alingment including over 300 sequences identified as mitochondrial polymerases by the NCBI protein blast. We found that 18 organisms have an H residue instead F, and only 2 display a Y residue in that position. Our results show that F763 is a key residue to Mip1 activity, and it is very conserved among mitocondrialpolymerases. Experiments to determine mutation frecuency are being performed.

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Protein-ligand interactions to understand the role of the mutations in the Shwachman-Diamond Syndrome.

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High pH and salt affects growth and energetic metabolism in *Debaryomyceshansenii*

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Debaryomyceshansenii has been studied for its capability to adapt to saline stress. It was originally isolated from marine water, but it can also be found in salty foods. It can accumulate high Na^+ concentrations.Its adaptation has been attributed to some improved enzymes, different metabolic routes, or a combination of processes converging to cope with saline stress.However, its adaptation to elevated pH has not yet been studied.

*D. hansenii*is mainly respiratory and ferments less than *S. cerevisiae*,being phosphofructokinase the limiting step. Sodium and potassium chloride increase its respiratory capability and fermentation.

The pH of marine water is around 8, and to evaluate the effect of high pH, we used a pHStat tomaintain a constant pH of a yeast suspension (with or without 1 M NaCl) by adding known volumes of a NaOH solution. With this equipment we obtained the NaOH expenditure in mEq/ g of yeast, needed to maintain a pH of 6 (control) or 8.

Since the pH was maintained with NaOH, when incubating the cells, the internal Na^+ increased and K^+ decreased at pH 6, and more importantly to pH 8.This effect was more evident when incubating in the presence of 1 M NaCl, where the level of Na^+ reached 374 mM at pH 6, and 415 mM at pH 8, confirming that it is a “sodium includer” yeast.

High salt and pH affect yeast growth, measured as the increase ofO.D.at 600nm, by CFUs, or staining with FUN1, and also in growth curves in the different incubation conditions.

Both salt and high pH decreased the uncoupled / state 4 respiratory ratio. NaCl stimulated acidification, both at pH 6 and pH 8 (alkalinizing the cell interior). Rubidium transport and transmembrane potential were also increased at pH8 in the presence of 1M NaCl. Fermentation decreased at pH 8 and more at pH8 with 1M NaCl.

The data agree with a stimulation of the plasma membrane H^+-ATPase at pH 8 which in turn enhances the transmembrane potential, allowing the entry of Na^+. The energy expenditure of increasing H^+ pumping and of extruding excess Na^+, compromises the viability of the yeast.

It is worth mentioning that *S. cerevisiae* is not able to grow at the high pH and salt tested for *D. hansenii*.

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Characterization of the hydrophobic subunits of the peripheral arm of the ATP synthase from the colorless alga *Polytomella*sp.

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The F_OF1-ATP synthase (EC3.6.3.14) produces most of the cellular ATP in aerobic conditions. This enzyme complex is found in energy transducing membranes such as the mitochondrial inner membrane, the thylakoid membrane of the chloroplast, and the bacterial plasma membrane. The *Escherichia coli*ATP synthase exhibitsthe simplest known subunit composition, which consists of eight subunits $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1a_1b_2c_{10\text{-}12}$. Subunits -α, -β, -γ, -δ and -εform the F_1 domain, while subunits -a, -b and -c form the F_O domain. The mitochondrial F_OF_1-ATP synthase is more complex than the bacterial enzyme and is composed of at least 15 different subunits. Besides having orthologs of the eight essential subunits found in *E. coli*, it contains several supernumerary subunits (<20 KDa), which are required for the stability or the regulation of the enzymatic complex, while others are involved in its dimerization or oligomerization.In this regard, and in contrast to other known enzyme complexes, the ATPase from the algae *Chlamydomonas reinhardtii* and *Polytomella* sp. show unique structural characteristics. The algal enzyme was found to have orthodox subunits of the catalytic domain (-α and-β), as well as those that are part of the central rotarystalk (-γ, -δ, -ε and -c), however, with respect to the subunits which constitute the peripheral arm, only subunits –a and –OSCP were found. In addition, nine subunits of unknown evolutionary origin that do not have clear homologs in the databases were also found. These subunits were named ASA (Mitochondrial ATP Synthase Associated Protein) and were numbered successively as ASA1-9 subunits. It is thought that the ASA subunits replace those involved in the formation of the peripheral arm (-b, -d, -e,
-f, -g, -IF1, -A6L and -F6), the dimerization of the complex (-e, -g) and the regulation (-IF1) of the enzyme activity. Therefore, these ASA subunits could be the main components of the peripheral arm (ASA1, ASA2, ASA3, ASA4, and ASA7), others may participate in the dimerization of the complex (ASA6 and ASA9) and others could play a regulatory role (ASA?).Recent work in the laboratory using methodologies such as dissociation of the enzyme into sub-complexes induced by high temperatures, treatment with crosslinking agents or association studies employing recombinant proteins,have addressed the study of the interactions of the ASA subunits in the ATP synthase of*Polytomella* sp. However, still little is known about subunits -a, -c, ASA5, ASA6, ASA8 and ASA9 that form the membrane fraction of the complex. These subunits probably have a transmembrane domain or highly hydrophobic regions. Therefore, it is our interest to approach the study of the interactions of the hydrophobic subunits of the peripheral arm of the ATP synthase and to obtain more information about the membrane domain of this enzymatic complex.In this work we present the results obtained using recombinant proteins and their purification for the subsequent formation of *in vitro* sub-complexes, as well as qualitative information obtained from interaction assays using the two-hybrid system.For the use of recombinant proteins it is necessary to clone the genes that encode for the mature subunits:ASA6, ASA8 and ASA9 from the alga ATP synthase into vectors for further overexpression in *E. coli* and purifying them by fast protein liquid chromatography (FPLC). The recombinant proteins were used for interaction assays such as type Far-Western blotting and co-purification by immobilized metal affinity chromatography (IMAC) using nickel-NTA (nitrilotriacetic acid).The *in vivo* interaction assays were performed using yeast two-hybrid system. For this purpose, the constructions of the genes encoding the ASA6, ASA8 and ASA9 subunits vectors corresponding to the activation domain (pAD) and binding domain (pBD) were obtained. Subsequently, interaction assays were performed in the medium called "low stringency" (-Leu/-Trp/-His) and "high stringency" (-Leu/-Trp/-His/- Ade) observing interactions only in the former conditions. The results suggest an ASA6-ASA6 interaction, and in less degree the interactions ASA9-ASA8 and ASA9-ASA9. We will confirm these data using other methodologies used to determine protein-protein interactions.

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Structural Contributions to the Thermal Adaptation of the TATA-Binding Protein.

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The TATA-binding protein (TBP) is a monomeric transcriptional factor present in archaea and eukaryotes, which colonize niches with temperatures ranging from 10 to 100 ºC. The TBP DNA-binding domain shows structural and sequence conservation (~40% sequence identity)among different organisms [1]. Previous studies have shown that the optimal growth temperature (OGT) of a particular organism and the melting temperature of its TBP [2] are correlated, so thisindicates a selective pressure of temperature over the amino acid sequences of TBP. To assess the response of the DNA-binding domain of TBPto temperature, we carried out molecular dynamics simulations of a set of TBPs at five temperatures (273, 298, 323, 348 and 373K) in explicit solvent and 0.15M NaCl, during 50 ns for each protein, and measured the temperature responseof a collection of structural parameters. Regarding enthalpic contributions to stability, the amount of salt bridges increases with increasing temperature, and the rate at which salt bridges increase with temperature correlates with the OGT, while protein-water H-bondsdecreasein response to increasing the temperature.On other hand, the decreaseof total hydrophobic contactsshoweda slight temperature dependence in archaeal TBPs, while it was more marked in eukaryotic TBPs. Thearchaeal TBPs have more polar solvent accessiblearea (SAA), while eukaryotic TBPs have more non-polar SAA.As an approximation to evaluating the heat capacity of this DNA-binding domain, the total mean quadratic fluctuations of Hatomssuggest that the curvature of the minimum free energy well is similar for all of the analyzed TBPs. Finally, in a first approach to estimate the entropy of the domain, the temperature response of Cαfluctuationsofeach residuerevealzones with more pronounced thermal susceptibility in mesophilic TBPs compared with thermophilic variants.
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Heterologous Expression of an Immunogenic Peptide of *Helicobacter pylori* Vacuolating Cytotoxin A

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Background: *H. pylori* infection is the main cause of peptic ulcers and is a risk factor for the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer. In Mexico peptic ulcer is one of the main causes of hospital admissions and gastric cancer remains the third cause of cancer-related deaths. In this context, it is recommended to screen an eradicate *H. pylori* to improve public health and reduce expenses by the health system. To screen for *H. pylori* infections, the most reliable methods are biopsy-dependent (bacterial culture, rapid urease test, histological examination). However, those procedures are costly and are not widely available to primary care practitioners. Non-invasive procedures such as the urea breath test, stool test and serological assays are also dependable. Serology in particular is considered simple, minimally invasive, reproducible and cost-effective. Ideally, an antigen designed for serological detection of *H. pylori* should give some information on the risk of disease. Among the major immunogenic components of *H. pylori* that can be used as antigens in serology assays are surface-exposed components such as urease or secreted proteins like CagA (cytotoxin associated antigen) and VacA (vacuolating cytotoxin A). This work aimed at developing methodology for the expression of recombinant VacA peptides for their use as specific reagents to detect antibodies against *H. pylori.* Methods: A consensus nucleotide sequence of *vacA* was obtained through alignment of *vacA* sequences in the NCBI database using Codon Code Aligner 4. Subsequently, VacA antigenic regions were identified using Predicting Antigenic Peptides and IEDB Resource Analysis software. Results: One selected region of the *vacA* gene was cloned in-frame with the the histidine tail in plasmid pQE30 (Qiagen). The histidine-tagged VacA peptide was purified with Ni-nitrotriacetic acid resin (Qiagen) according to the manufacturer's instructions. The purified recombinant peptide was characterized by immunoblot with specific anti-VacA (Santa Cruz Biotechnology) and anti-histidine (Roche) antibodies. Thus, a first recombinant peptide of 19 kDa expressed in *E. coli* BL21 has been obtained and the purified peptide reacts with specific antibodies against VacA. The recombinant antigenic peptide is now being tested for serological diagnosis of *H. pylori* infection.

Analysis of changes in the transcription of genes involved in autophagy and apoptosis in aged cells of *Schizosacharomyces pombe*

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In recent decades, the study of the molecular basis of aging has allowed to elucidate some of the processes involved as oxidative stress-induced damage, the deregulation of apoptosis, telomere shortening, mitochondrial dysfunction and the proteotoxicidad, among others. However, none of these are considered the main trigger. Recently, it has been proposed autophagy and apoptosis as keys in aging. Autophagy is a catabolic process that made cells eukaryotes to remove proteins and damaged organelles. During aging, decreases the efficiency of autophagy and its regulation is altered. This work discussed changes in the transcription of genes involved in Autophagy and apoptosis in the chronological aging of Schizosaccharomyces pombe (S. pombe). For this purpose, it was cultured the 972 h^- strain *of S. pombe* by 1, 2, 3, 4, 5, 10 and 16 days in EMM medium. Cell viability was analyzed and assessed the integrity of the cell wall by the stain with calcofluor. Subsequently, the tRNA was purified, treated with DNase and used to assess the level of mRNA genes Atg5, Atg8, Atg4, metacaspase I (PcaI), caspase 3, 8 and 9 of *Homo sapiens*. Results indicate that gene ATG5 stays on until day 3. Given that the mRNA atg5 decreases to the 4 day when the yeast begins to aging, which suggests that it is a gene that could be turned off when the cell ages. Studies in rodents have shown that overexpression of this protein slows down the appearance of aging phenotype. In the yeast while the cell is young gene is on and is decreasing continues growing. In the yeast, while the cell is young, the gene Atg5 is turned on but decreased at continue the growing. With respect to the evaluation of the level of mRNA of the Atg8 gene, the assay showed that is turn on, in young as in aged cells. Moreover, since Atg5 also has a regulatory role of the process of apoptosis, a RT-PCR assay was perfomed to the 5 day using oligonucleotides for the metacaspase I and caspases 3, 8 and 9 of human.

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Interaction between the receptor for advanced glycation endproducts (RAGE) domains and quinolic acid (QUIN)

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The receptor for advanced glycation end products (RAGE) is a pattern -recognition receptor involved in neurodegenerative and inflammatory disorders. RAGE induces cellular signaling events upon binding to a variety of ligands. Recent evidence suggests that expression of RAGE is involved in amiloid-β25-35 and quinolic acid (QUIN)-induced toxicities in rodent models, where its early up-regulation can be observed. We are interested in the toxic events associated with early noxious responses, which might be linked to signaling cascades leading to cell death induced by neurotoxic metabolites such as QUIN. Therefore, with the aim to search for the mechanism of QUIN toxicity through a possible interaction with RAGE, in this work we explored the molecular recognition between RAGE domains and QUIN, determining the binding constant (K_b) of RAGE (VC1 domain) with QUIN by fluorimetric titration at pH 7.4 and 9.0, and ionic strength 0.15. In order to understand the interaction between RAGE domains and QUIN, we modeled by docking two conditions: possible binding on the whole receptor, and binding at a specific site, where the later has been described recently for the V domain (2L7U.pdb). For the docking studies we used the crystallographic structures of domains of RAGE, representative structures from molecular dynamics simulations, and a homology model to the complex between QUIN and the V domain of rat as this is a useful experimental model. Our docking studies suggest that RAGE residues Lys 31, Arg 77 and Lys 89 are important for the binding affinity of QUIN. We determined the polar/electrostatic (ΔG_p) and nonpolar (ΔG_{np}) contributions to the binding free energy in the RAGE domains-QUIN interaction under the same pH and salt conditions as the fluorimetric experiments using the Adaptive Poisson-Bolztmann program (APBS). These studies are complementary to previous reports employing the toxic model induced by QUIN in rats by unilateral striatal lesions. Likewise, we performed immunohistochemical assays for the identification of cellular damage caused by this receptor in the rat striatum over time. We supported these results with image processing methods to quantify areas and number of cells. This work contributes to understand the phenomenon of RAGE-QUIN recognition leading to the modulation of its function.

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Advances in protein-protein interface design: grafting, docking and *de novo* approaches

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Specific protein-protein interactions are essential for life; living organisms exploit such interactions as the basis for structural (e.g. Cytoskeleton), regulatory (e.g. G-proteins) and self-defense (e.g. Antibodies) systems, among many others. The field of Protein Design has two main targets, the first is to understand the molecular basis of protein structure and the second is to use such knowledge to create proteins with new functions. The aim of this work is to advance in our ability to design protein-protein interactions.Four approaches and the latest research advances on this problem are discussed: 1. Grafting known interface components into known protein scaffolds in order to create interaction partners that exploit natural occurring interfaces and scaffolds; 2. Grafting computationally designed interface components into known protein scaffolds with the aim of creating new protein interfaces that exploit natural occurring scaffolds; 3. *De novo* protein-protein interface design based on novel scaffolds; and 4. Computational design of both: a protein scaffold and its interface,for the purpose of binding a specific target. As proof of these concepts, we show the application of these techniques in two current scientific challenges: 1) the design of therapeutic inhibitors for two important threats to humans: the Botulinum neurotoxin (BoNT/B) and the Ebola Virus Glycoprotein (GP1,2); and 2) the development of novel protein-protein heterodimers based on human-made scaffolds. Altogether, the results of this research advance our capacity to design protein-protein interfaces, a knowledge that holds a great potential for itsnumerousplausibleapplications in biotechnological, pharmaceutical and bioremediation applications.

Isolation and characterization of bioactive peptides from conditioned media by vegetal cells of various species

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Plants due to their sessile nature, need to respond quickly to changes in their environment, so they have developed mechanisms that allow them to survive. These include the production of secondary metabolites. Due to its significant biological activity, these have been used for centuries in traditional medicine, today is a very important source of natural fragrances, insecticides, dyes, flavors, however they use as drugs is of primary interest.

The production of secondary metabolites is made from three main ways; I) Extraction from natural sources, II) Total or partial chemical synthesis, and III) heterologous production. However, they have disadvantages with each, including: the limited availability of active compounds which generally represent less than 1% of the dry weight of the plant, difficulties in synthesis by the presence of multiple chiral centers, the use of organic solvents aggressive with the environment and the lack of production pathways of secondary metabolites fully characterized to be introduced in other species.

As a solution of the drawbacks of the methods of production of secondary metabolites, plant tissue culture allows us to sustainable production from these in suspension cell culture through the use of bioreactors, however, research has to be addressed towards resolving the problems with their industrial level scaling, which include: the instability of the cell lines, low yields in the production of metabolites and slow cell growth, the latter problem is that by establishing a suspension cell culture requires a sufficient inoculum of cells to perform the cell proliferation. This problem can be solved with the help of the peptide with mitogenic activity in plants that promote cell proliferation in suspension cultures of low density.

To date, only been achieved the isolation of two peptides with mitogenic activity in conditioned media by plant cells; phytosulfokine (PSK) and PSY1 in *Asparragusofficinalis* and *Arabidopsis thaliana*respectively. And of these, has been tested only the effect of the addition of peptide phytosulfokine in two systems of production of secondary metabolites; *Atropa belladonna, Taxuscanadensis* and *Taxuscuspidata*, however, no conclusive results have been achieved.

Therefore, in this paper we try to isolate and characterize bioactive peptides that showingmitogenic activity in cell lines conditioned by *Tageteserecta*, *Taxusglobosa*and*Bouvardiaternifolia*, species which have not yet been searched this kind of composite media.

By the fractionation of conditioned media in a column of ion exchange, we have identified the fractions exhibiting mitogenic activity in all three species, protease treatment has shown that the active compounds have a peptide nature, the reverse HPLC spectra indicate that these compounds are highly hydrophilic, and finally with HPLC-mass analysis it was possible to know the sequence of the isolated proteins of the Tris-tricine gels.

Canine Tracheal Cartilage Cryopreservation: Freezing Injury is not related to Caspase-3 Expression.

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Introduction: Currently, there are no surgical strategies to treat tracheal lesions longer than 7 cm, which leaves these patients with recourse only to repeated palliative procedures to relieve their respiratory insufficiency, as they are not candidates for tracheal resection or end-to-end anastomosis. Experimental studies on cryopreserved trachea have produced contradictory results, limiting the clinical application of the technique. **Objective:** We evaluated caspase-3 expression and the histological integrity of canine tracheal cartilage preserved using two different solutions, two different cryopreservation temperatures and varying lengths of storage time. **Material and methods:** Thirty canine tracheal segments of 5 rings were studied. Group 1: Control without cryopreservation. Groups
2 and 4: F12K media cryopreserved for 48 hr in foetal bovine serum (FBS) at – 70ºC. Groups 3 and 5: Cryopreservation for 48 hr in FBS at – 70ºC. Groups 4 and 5 were stored for 15 days in liquid nitrogen. All of the segments were thawed, wax fixed and cut into rings. Three rings were selected for caspase-3 expression and histological evaluation. **Results:** The staining of the cartilage matrices was significantly modified in the tracheal segments of group 5. The central region of the cartilage ring is more vulnerable to the freezing effect than the edges. **Conclusions:** Under the same cryopreservation temperature and storage time, tracheal cartilage integrity is better preserved using F12K media. Caspase-3 expression is not related to cartilage injury from the cryopreservation process.

Effect of the proteicfraction G10P1.7.57 from the habanero chili (*Capsicum chinense* Jacq.) on growth of the tumoral cell lines Hep-2, SiHa, PC-3, and VERO.

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The family of DING proteins is included in the superfamily of proteins that bind to phosphate (Berna et al., 2009), they are distinguished from other members of the superfamily because in its N-terminal have preserved the sequence of amino acids: aspartic acid (D) Asparagine (N) and glycine (G). The DING proteins are involved in cellular events that regulate the health or disease in mammals, i.e., the transient heterologous expression of the protein DING p27sj from the *Hypericumperforatum* in cells of astrocytes inhibited the replication of the virus HIV-1. Also it has been shown that the DING protein called p38SJ inhibits the formation of glioblastomas, a type of malignant cancer (Bookland et al., 2012). For that reason, the DING proteins represent an opportunity for the development of alternative methods to the already existents for the treatment of HIV and cancer (Bernier, 2013).

In the working group, from seeds of the habanero chili, we isolated a protein fraction called G10P1.7.57, that showed antimicrobial activity (Brito-Argaez et al.,2009). The proteins sequencing showed that protein fraction G10P1.7.57 contained a DING protein as the main peptide. After the alignment of amino acids with other DING proteins, we selected the peptide that showed the highest conservation within the family of DING proteins and, against it, the polyclonal antibody COM2, was generated. By other hand, in this work, it was analyzed whether the fraction G10P1.7.57 also inhibits the growth of the tumoral cell lines Hep-2 (laryngeal carcinoma), SiHa (squamous cell carcinoma of the cervix) and PC-3 (cancer of human prostate) and as control, the VERO cell line (kidney epithelial cells of the African green monkey Chlorocebus) was used. It was found that the fraction G10P1.7.57 inhibited the growth of the differentscáncers cell lines with IC50s between 25 and 48 ug/mL; however, the selectivity index (IS) maintained values between 0.66 and 1.61. Those values suggest a low selectivity of the protein fraction. The biochemical characterization of the fraction has shown that some of their proteic components are glycosylated. Western blot experiments using the polyclonal antibody COM2 have shown that the DING protein contained in the fraction G10P1.7.57 is one of the proteins that present glycosylation. The biological meaning of such modifications on the fraction G10P1.7.57 and in the DING protein has not been established yet. In this work we will describe the results obtained until the moment.

Search S6 ribosomal protein not associated with ribosomes in nuclei of Saccharomyces cerevisiae

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The ribosomal Biogenesis is an event which begins in the nucleolus with ribosomal gene transcription generating the 35S rRNA. Subsequently forming the 90S pre-ribosomal particle composed by the 35S rRNA, snoRNA, some ribosomal proteins and non ribosomal. The pre-ribosomal subunits continue their processing and maturation along nucleoplasma to finally exported to the cytoplasm and end maturation. The mature rRNA are associated with ribosomal proteins forming 40S and 60S subunits of the ribosome. One of the proteins that form part of the 40S subunit is rpS6. rpS6 is an evolutionarily conserved protein in all eukaryotes and is found in the 40S subunit. In Saccharomyces cerevisiae, the rpS6 has two serine residues that are susceptible to phosphorylation in the C-terminal end. RpS6 phosphorylation has been associated with messengers translation increasedwith oligopirimidinas domain in the 5 'end. Recently reported in HEK293T cells a population of rpS6 in the cytoplasm that are not associated with ribosomes and that interacts with Hsp90 chaperone. Furthermore in the HeLa cell line was found rpS6 with proteins that interact with chromosomes (Nakagawa et. al. 2008). In our working group we are interested to identifying rpS6 in a of Saccharomyces cerevisiae nuclear fraction to know if rpS6 is only associated with pre-ribosomal subunits or rpS6 there are free populations too. So far we have managed to obtain a nuclear fraction enriched with pre-rRNA.

In vitro import of the cytochrome c oxidase COXIII subunit in *Polytomella* sp. mitochondria.

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Mitochondrial cytochrome *c* oxidase or complex IV is an oligomeric protein complex embedded in the inner mitochondrial membrane that oxidizes soluble cytochrome *c*and reduces O_2 to water. It is also a proton pump that contributes to the generationof the electrochemical gradient. In eukaryotic organisms it consists of 11 to 13 subunits. The largest polypeptides, subunits I, II and III are encoded in mitochondrial genes, whereas the rest of the subunits are encoded in the nuclear genome. Nevertheless, there are some organisms where subunit III (COX3) is encoded in the nuclear genome as is the case of some chlorophycean algae like*Polytomella* sp. and *Chlamydomonas reinhardtii* (1). Cytosol-synthesized mitochondrial proteins generally have a pre-sequence that targets them to mitochondria,where they are internalized, processed, and inserted into the corresponding complex. The expression of mitochondrial proteins from the nucleus is of great interest because of its potential applications in developing a gene therapy for the treatment of mitochondrial diseases. In our laboratory, we are interested in studying the mechanism through which the algal COX3, a highly hydrophobic protein containing seven transmembrane segments, is synthesized in the cytosol and imported into mitochondria. To study this, on one hand we obtained the COX3 protein synthesized *in vitro*and radio-labeled with sulfur^{35}, and on the other hand, we were able to isolatealgal mitochondria presenting respiratory control. We performed *in vitro* import assays and found that the protein is quickly imported into mitochondria, and that this import is sensitive to inhibitors of the respiratory chain and to uncoupling agents. We also found that the pre-sequence pf the COX3 precursor isedited in at least two stages, and that the mature protein is integrated into the membrane. Finally, using blue native gels, we found that the imported COXIII protein assembles into the mitochondrial complex IV.

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Native electrophoresis studies of different aggregation states of triosephosphate isomerase.

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Protein folding is a process mainly due to weak chemical interactions such as repelling of charges, hydrophobic interactions, and hydrogen bonds, which involve reversible disorder-to-order transitions of a polypeptide chain. Although changes between partially folded structures, in order to get a native functional protein, are highly efficient, proteins still may have transiently stable conformations, which yield amorphous oligomers and end in a state of aggregation that may or may not be reversible. Even though the amino acid sequence plays a key role in this process, the microenvironment has an important influence as well, given that chemical modifications by reactive oxygen and nitrogen species produce an inadequate folding.

Triosephosphate isomerase (TIM) is a protein susceptible to nitrotyrosination after which it becomes prone to aggregation; such aggregates have been observed in Alzheimer patients. This suggests that protein folding, oligomer formation and aggregation of this protein plays a key role in the complex biochemical pathway through its structural transitions, as well as in the development of diseases such as Alzheimer. Other research groups and ourselves have been able to provide evidence of protein aggregation *in vitro* using native electrophoresis and ionic exchange chromatography.

The present work focuses on the characterization of protein aggregates of TIMs from different species. Despite the fact that all TIM proteins described to date have a conserved $(\alpha/\beta)_8$ barrel structure, our results show a notable difference in their proclivity to generate one or several aggregation states, when their migration is analyzed using polyacrylamide gradient electrophoresis under native conditions.

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NaTrxh: its possible role in the cell-cell interaction that lead to pollen rejection in *Nicotiana*.

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Angiosperms display diverse reproductive strategies to maintain genetic diversity. To this end, many plant species evolved special mechanisms to enhance outcrossing, through a genetic self-fertilization barrier called the self-incompatibility (SI) system, which allow female reproductive cells to discriminate between "self" and "non-self" pollen and specifically reject self-pollen.

SI occurs in Rosaceae, SolanaceaeandPlantaginaceae and it is genetically controlled by a polymorphic*S* locus encoding two genes. The *S* locus products arespecifically expressed either in the pollen orpistil. The *S*-pollen encodesa F-box protein called SLF (S-locus F-box protein), while the *S*-pistil codes a ribonucleasa named S-RNase, which is secreted onto the pistil extracellular matrix. S-RNases are taken up by pollen tubes during pollination. Once in the pollen tube cytoplasm, S-RNases interact with SLF and if there is an *S* allele coincidence, RNA in pollen tubes is degraded leading to pollen tube growth inhibition and therefore, to pollen rejection. However, in spite of pollen rejection response depends on S-RNase-SLF interactions, numerous genetic studies show that other genes(modifiers genes, MG) not *S*locus linked,are also required for SI. To date, only three pistil MGs have been identified, which are: *120K, NaStEP* and *HT-B* in pistil and just two in pollen: *cullin 1* and *SBP*. Nevertheless, genetics predicts that there are more MGs playing a role in the genetic pathway of pollen rejection in *Nicotiana*.

We have identified another candidate to be a MG in *Nicotiana*, which we called*NaTrxh* and encodes a thioredoxin type h subgroup II. NaTrxh is abundantly expressed inpistils of SI*N. alata*, whit a low presence in pistils of self-compatible *Nicotiana* species. Biochemical and cell biology data show that NaTrxh is secreted onto the extracellular matrix of the style-transmitting tract. Additionally, NaTrxh reduces *in vitro* to S-RNase. These observations suggest that NaTrxh may be involved in the SI mechanism in *Nicotiana*.

The role of *NaTrxh* in pollen rejection in *Nicotiana* is being evaluated by lost and gain of function approaches. Likewise, we are determining if NaTrxh is taken up by pollen tubes and if this protein has its function in the pollen tube cytoplasm. To test it, we are developing transgenic plantsthat express to NaTrxhfused to GFP in the mature style.

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*IN VITRO*EVALUATION OF ANTIOXIDANT ACTIVITY OF ESSENTIAL OILOF*Satureja macrostema*

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ABSTRACT

Antioxidant activities of volatile oils obtained from aromatic plants is due to the presence of hydroxyl groups in their terpenes and phenolic compounds. However, the wide variety of chemical structures of these metabolites is a factor that influences their ability to capture radicals.Therefore, the aim of the present study was to investigate the *in vitro* antioxidant potential of essential oil of *Satureja macrostema* (Lam.), a medicinal plant known as nurhitinité or nurite, whose properties are mainly attributed to the volatile compounds from aerial part. Fresh aerial parts of *S. macrostema*micropopagated plants cultivated in the greenhouse for 3 months were collected andextracted by two different methods: hexanemaceration, 1g weight fresh/10 mL of solvent for 5 days at 4°C; and steam distillation, 100 g was subjected to two-hours of distillation using a Hydrodistiller-type apparatus. Subsequently extracts were filtered, evaporated with nitrogen to dryness and suspended in methanol (10 mg mL^{-1}). Volatile compounds were identified by Gas Chromatography coupled to Mass Spectrometry (GC-MS), finding that the major volatile compounds were terpenes (limonene, linalool, pulegone, menthone and thymol).Antioxidant effectiveness was examined by two different radical scavenging methods: DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid). The concentrations tested were 0.01, 0.1, 1 and 10 mg mL^{-1}.The essential oil from steam distillation showed the highest antioxidant capacity, reaching 53.10% capture of radicals in the DPPH and 92.13% in the ABTS methods. The essential oil from hexane extract just showed 16.36% antioxidant capacity in the DPPH and 57.16% in the ABTS. At the time, antioxidant testing with the major volatile compounds of essential oil by steam distillation are realizing.

Key words:Radical scavenging activity, *Satureja macrostema*, Terpenes, Volatilecompounds.

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Comparative analysis of the synthesis and accumulation of dhurrin in sorghum plants associated with mycorrhizal fungi under different water stress conditions.

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Sorghum (*Sorghum vulgare*L. Moench) is a cereal of socio-economic importance, with high drought tolerance.Itgrows in arid climates that are usually less suitable for the cultivation of other cereals (Rosenow*et al*., 1983). In addition to its socio-economic importance, sorghum is considered a model for studying the molecular mechanisms of drought tolerance in cereals. Besides the features sorghum has to deal with water stress,it can tolerate longer drought periods when associated with mycorrhizal fungi. It has been shown that the contribution of mycorrhiza to drought tolerance is the result of a combination of molecular, physical and nutritional effects. However, the mechanisms involved are still not fully understood (Ruiz-Lozano, 2003). In a recent work, our research group conducted a study of differential proteomics in leaves of sorghum plantsassociatedtomycorrhizal fungi underwater stress conditions, using 2D gel electrophoresis and mass spectrometry. The results indicated that most of the proteins that were up-regulated, were related to the antioxidant response and metabolism of cyanogen compounds. Considering thatdhurrin is the most abundant cyanogenic glucosidein sorghum, the aim of the present work was to evaluatedhurrin and transcripts levelsof some enzymes involved in the synthesis and degradation of dhurrin, in leaves of sorghum plants associated to mycorrhizal fungi and under water stress conditions. Dhurrinwas quantified in leaf extracts by GC-MS and the transcripts are being evaluated by digital PCR nano droplets. The aim of this project is to validate the data obtained in the proteomic analysis and determine the possible contribution of dhurrintotowater deficit tolerance in sorghum plants.

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Daily variations of liver 5-tryptophan hydroxylase in rats under daytime restricted feeding

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Serotonin or 5-hydroxytryptamine (5-HT) is synthesized in serotonergic neurons as well as in the enterochromaffin cells. It regulates a variety of physiologic states and behaviors. In the liver, 5-HT is known to modulate several key aspects of liver function, such as hepatic blood flow, innervations, wound healing and liver regeneration. The rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH, EC 1.14.16.4.) turns L-tryptophan into 5-hydroxytryptophan, an intermediate metabolite that is then converted to 5-HT. TPH exists in two isoforms, TPH1 found in the pineal gland and peripheral organsand TPH2 only in CNS.Biological rhythms are phenomena that occur in cycles of regular time. Rhythmicity associated to 24 h periods is known as circadian, and it is sustained by the master pace-maker the suprachiasmatic nucleus (SCN). Besides the SCN, it is now accepted that alternative clocks are located in peripheral tissues such as the liver. These peripheral oscillators can be synchronized by food access. The food entrained oscillator (FEO) allows an optimal management of nutrients, and is an important part of the timing system when food access is restricted. A key to understand the physiological mechanisms underlying these changes in the liver is the study of chemical transmitters such as serotonin. The aim of this study was to characterize the TPH1 in the rat liver during the expression of FEO. Our experimental protocol had 4 groups of male rats: 1) Food *ad libitum*, 2) Food access only 2 h per day (12:00 to 14:00 h), 3) Fasting for 24 and 48 h and 4) fasting for 24 and 48 h with further refeeding for 2 h. TPH 1 was studied by expression, presence and activity of protein synthesis .The results showed the presenceTPH1in liver with 24 h–variation in AL group, while expression of FEO changed rhythmicity and amplitude decrease of these parameters in food restriction group. In conclusion, exist the presence of the enzyme TPH in the liver with a rhythmic pattern of activity that is decreased with the FEO expression.

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Low pH induces changes in the dynamic properties of Ig light chain variable domain 6aJL2, impacting both unfolding pathways and fibril formation.

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Light chain amyloidosis (AL) is a systemic disease where immunoglobulin light chains (LCs) accumulate forming fibril aggregates[1].The lambda 6a family of LCs is frequent in AL patients.The thermodynamic stability and fibril formation efficiency of its germline protein (6aJL2) have been studied at neutral pH [2,3]. Acidic pH and the presence of residual secondary structure favor fibril formation in other members of this family [4].In order to gain understanding of the effect of low pH on the dynamics and unfolding of 6aJL2, we have performed molecular dynamics simulations at neutral and low pH at increasing temperatures (298, 398, 448 and 498K).Protonation of acidic groups reshapes the charge distribution of 6aJL2, eliminating salt bridges and leaving some basic residues without a counter ion, changing the pattern in hydrogen bonds and atomic contacts in the protein. At room temperature and low pH the most noticeableeffectis the destabilization of theloop connecting strands E and F,close to C-terminal of the protein, seen by an increase influctuations and side chain entropy of the residues in this zone. Upon raising the temperature we observed denaturation at both pHs, though with different features. We suggest that charge redistribution destabilizes primarily the loops opposite to the CDRs of the protein,allowing water access to the hydrophobic core. Finally, acidic pH may lead the protein to populate efficiently intermediates with residual secondary structure, able to aggregate.

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Transcriptomic analysis of two Arabidopsis mutants with altered expression of inorganic soluble pyrophosphatase isoforms under phosphate starvation.

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Soluble inorganic pyrophosphatases (PPa) are essential enzymes present in all living cells. In plants, there are several cytoplasmic and one chloroplastic isoforms, encoded by different genes. Two mutants of *Arabidopsis thaliana* affected in the expression of different PPa isoforms were selected up to the F2. Both mutants showed subtle but reproducible phenotypic alterations in the number of leaves and roots, the length of the floral stem and siliques, and the number of seeds. Despite being theoretically altered in the coding gen for only one isoform, there was a reduction in the mRNA levels of more than one PPa isoform (RT-PCR), and these changes in transcription correlated with changes in the activity peaks observed in extracts fractionated on phenyl-sepharose columns. These mutants display an increased tolerance to some forms of stress and we decided to explore more in detail their transcriptional (RNA-seq) and proteomic responses (2D-PAGE) under phosphate starvation. We observed a significant number of changes in transcription, and some changes in the 2D-PAGE patterns. These alterations were confirmed by RT-PCR of several transcripts involved in oxidative stress, primary metabolism and phosphate starvation responsive transcripts. The phosphate starvation also resulted in differential proteomic changes in comparison to wild-type control *vs.* phosphate-starved plants. From these data, the reduction in the expression of some PPa isoforms appears to be compensated by the remaining PPa isoforms, but the large number of changes observed in these mutants suggest a complex link between pyrophosphate metabolism and the primary metabolism under stress. From the quantitative analysis of the changes in transcription some possible explanations are discussed.

Analysis of storage proteins in native maizes of Oaxaca by electrophoresis

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ABSTRACT

Oaxaca is one of the centres of origin of maize, as shown by the archaeological remains found in the caves of GuiláNaquitz, located near Mitla, which are over 7000 years old (Benz, 2001), the carbonized plant remains and ears represented in clay figures. To this date, 35 maize races have been reported in Oaxaca, which represents approximately 70% of the diversity in Mexico (Aragon *et al*, 2006).In order to know the composition of four major groups of storage proteins in these maize races, we quantified albumins and globulins, prolamins or zeins, glutenins and reduced gluteninsin six samples of three different landraces of Oaxaca, by the method of Vasquez-Lara *et al* (2010). The samples were: *vandeño* (2), *arrocillo* (2) and *elotecónico* (2). The protein profile of the extracted fractions was performed by one-dimensional electrophoresis, of two selected samples of corn.

Concentration profiles of different protein groups in the races of maize analyzed were found. Albumins and globulins showed the highest proportion in six samples, while in the *elotecónico* race, prolamins concentration was the lowest. All samples tested showed the same protein profile, although there was a difference in the intensity of some bands, which may be indicative of the presence or absence of some storage proteins.

Keywords: Albumins and globulins, zeins, prolamins, glutenins and reduced glutenins.

Thermostability of *Phaseolus lunatus* Lectin

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Key words: Lectins, *Phaseoluslunatus*, thermostability.

The lectins are proteins or glycoproteins that bindmono or oligosaccharidesin a specific andreversible manner, they don´t keep catalytic activity and, unlike antibodies, they are not a product of an immune response.Lectins and carbohydrates join themselvesby relatively weak interactions as hydrogen bridges and Van der Waals strengths. The structure oflectins is diverse, only small changes in its aminoacid composition are necessary, especially on carbohydrate joining sites, for getting thatlectins with the same polypeptide fold have different specificities of glycan joining. Normally, each lectin contains two or more sites of carbohydrate recognition.

In some reports, we can find proofs of thermostability directed to plant lectins, one of them is the jamaica flower (*Hibiscus sabdariffa L.)* which is capable of withstanding temperatures up to 100 °C when it is in raw extracts.

The lectins of *P. lunatus* is a glycoprotein which contains more than one site of specific recognition to carbohydrates, moreover, it isspecific of one bloodgroup as it preferably agglutinatestype A human erythrocytes, which contain N-Acetylgalactosamine(GalNAc).In this work, the thermostability of *Phaseoluslunatus*lectinwas analyzed. For testing its resistance to temperature changes, the lectin was subjected to 25, 30, 40, 50, 60, 70 and 90 °C duringdifferent periods of time.For verifying that the lectin activity keepsactive, different haemagglutinationtestswere produced, and its activity withtype A human erythrocytes was confirmed.

DOMINANT NEGATIVE PHENOTYPE OF BACILLUS THURINGIENSISCRY1AB, CRY11AA AND CRY4BA MUTANTS SUGGEST HETERO-OLIGOMER FORMATION AMONG DIFFERENT CRY TOXINS

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Bacillus thuringiensis Cry toxins are used worldwide in the control of different insect pests important in agriculture or in human health. The Cry proteins are pore-forming toxins that affect the midgut cell of target insects. It was shown that non-toxic Cry1Ab helix □-4 mutants had a dominant negative (DN) phenotype inhibiting the toxicity of wildtype Cry1Ab when used in equimolar or sub-stoichiometric ratios (1:1, 0.5:1, mutant:wt) indicating that oligomer formation is a key step in toxicity of Cry toxins.

The DN Cry1Ab-D136N/T143D mutant that is able to block toxicity of Cry1Ab toxin, was used to analyze its capacity to block the activity against *Manducasexta* larvae of other Cry1 toxins, such as Cry1Aa, Cry1Ac, Cry1Ca, Cry1Da, Cry1Ea and Cry1Fa. Cry1Ab-DN mutant inhibited toxicity of Cry1Aa, Cry1Ac and Cry1Fa. In addition, we isolated mutants in helix □-4 of Cry4Ba and Cry11Aa, and demonstrate that Cry4Ba- E159K and Cry11Aa-V142D are inactive and completely block the toxicity against *Aedesaegypti* of both wildtype toxins, when used at sub-stoichiometric ratios, confirming a DN phenotype. As controls we analyzed Cry1Ab-R99A or Cry11Aa-E97A mutants that are located in helix □-3 and are affected in toxin oligomerization. These mutants do not show a DN phenotype but were able to block toxicity when used in 10:1 or 100:1 ratios (mutant:wt) probably by competition of binding with toxin receptors.

We show that DN phenotype can be observed among different Cry toxins suggesting that may interact in vivo forming hetero-oligomers. The DN phenotype cannot be observed in mutants affected in oligomerization, suggesting that this step is important to inhibit toxicity of other toxins.

Isolation and identification of thermophilic bacteria degrading hydrocarbons obtained from hot springs.

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The degradation of hydrocarbons is a process that can occur naturally by native microorganisms from contaminated areas using its metabolic pathways. For this reason, it is expected that microorganisms may offer this possibility based on their use in the remediation of environmental contamination by petroleum and petroleum technologies In the present investigation bacteria of hot springs from Atotonilco, municipality of San Luis Acatlán, Guerrero were isolated. For isolation, the samples were inoculated into culture media: nutrient agar, potato agar and tryptic soy agar and incubated at temperatures above 45 ° C, in order to isolate only thermophilic bacteria, thus checking the temperature optimal growth of the isolated strains is 50 ° C. Obtained by colonial morphology, Gram stain, and the conventional biochemical tests, isolation of seven different thermophilic strains was found, identifying the strain APB21 as *Bacillus stearothermopilus,* APF23 as *Bacillus cereus*, ASF11 as *Bacillus licheniformis*, ANF1 as *Bacillus megaterium* and ASB21 y ANF21 are reported as *Bacillus sp*. The only strain obtained that does not belong to the genus Bacillus is ASF21, which corresponds to the genus*Acinetobacter sp.*

The isolated bacteria were recovered on nutrient agar and degrading oil capacity of each of the strains by cultivation on agar Bushnell-Hass, using as carbon source 4 distinct hydrocarbons (gasoline, diesel, oil and burning oil) was verified, we observed growth of each strains in the presence of all hydrocarbon used, but with greater affinity for the medium added with gasoline.

Curtobacterium sp. strain MR2 exhibits two lead-resistance mechanisms dependent of cell density

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Introduction

Heavy metals are located in a natural way in the earth crust. These may be converted in contaminants, whether its distribution in the environment is altered due to anthropogenic activities. Lead is a persistent environmental contaminant which is accumulated in the tissues of organisms, generating biomagnification along the food chain. To help with this problematic, characterization of microorganisms able to tolerate heavy metals, that may be useful biological-tools in bioremediation of contaminated sites are needed (Naik & Dubey, 2013).

The goal of this research was the identification of microorganisms associated with the rhizosphere of plants that grow in mine wastes found in the Biosphere Reserve "Sierra de Huautla" and which could have a future application in the remediation of soils contaminated with heavy metals.

Methods

The growth and isolation of bacteria were made in mineral medium with low phosphorus and nitrogen,supplementedwith 200 ppm $Pb^{+2}$by microbiological standard methods. The resilience assays were made followingthemethoddescribed byJin ZM, S. W. (Jin ZM, 2013). PCR amplification of 16S rDNA was done using standard primer set 8F and 1492R and sequencing was done at IBT-UNAM, Morelos, Mexico. DNA sequence was compared with GenBank reference database using NCBI-BLAST search tools (Altchul *et al.*1997).

Results and discussions

Several aerobic rizospherical lead-resistant bacteria were isolated from mining wastes soil, associated with several vegetal species, located on the Biosphere Reserve “Sierra de Huautla”, Morelos, Mexico. These mining wastes contain an average of 1600 mg/Kg of Pb+2. The capacity of resistance of isolates were tested through resilience assays with concentrations from 0.6 to 3.6 mM of $Pb(NO_3)_2$, through these assays were isolated eight strains that tolerate above 2.4 mM of Pb^{2+}. One of the isolates, which can grow in presence of 3.6 mM of Pb^{2+}, in minimal salt medium, was identified as *Curtobacterium* sp. and designated as strain MR2. This strain was associated with *Dalea leporina* roots. Interestingly, this plant is one of those that present a lower concentration of lead in its tissues, only 87.4 mg/Kg. *Curtobacterium* sp. MR2 exhibits two lead-resistance mechanisms in solid mineral medium, dependent of cell density. In lower dilutions $1x10^{-1}$ to $1x10^{-4}$ (high cell density), colonies of this strain, produces an exopolysaccharide for extracellular sequestration of lead. In contrast, in higher dilutions $1x10^{-5}$ to $1x10^{-8}$ (low cell density), colonies take on a brown coloration, possibly by oxidation or precipitation of lead. Because to these phenotypes, this strain could play, an important role in bioremediation of lead contaminated soil.

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The role of Zn in the growth and development of *Phaseolus vulgaris* L.: anatomical and morphological effects.

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Zinc (Zn) is a lowmolecular weight heavy metal with a dual role inliving organisms. It is an essential nutrient, acting as a cofactor of enzymes through which it is involved in protein binding and regulation of enzyme activity, transcription, translation and signal transduction. However, in excess, it become toxic, causing membrane disintegration, ion leakage, lipid peroxidation, DNA/RNA degradation and eventually cell death. Zinc is the most common crop micronutrient deficiency, and the increased mining and industrial activities have raised its potential toxicity for crops. In this work, we are studying the effects of Zn deficiency and excess in the growth and development of the common bean *Phaseolus vulgaris* L. (cv. Negro Jamapa). Using a soil-free system, we treated 2 day-old emerged roots with 0, 0.5, 5.0, 50, 250, 500, 1000 and 2000 µM Zn for 1 week.Primary root length and root dry weight were significantly affected after 250 µM Zn. Hypocotyl length decreased in 50 µM Zn and was severely affected in 2000 µM Zn. Epicotyl length progressively decreased with increasing concentrations of Zn up to 50 µM Zn. From 50 to 1000 µM Zn epicotyl length was kept constant, however, epicotyl growth was completely inhibited in 2000 µM Zn. The dry weight of primary leaves significantly decreased after 250 µM Zn. Thus, *Phaseolus vulgaris* is able to grow and develop in up to 50 µM Zn, however, its growth and development is inhibited in 2000 µM Zn.

Alkaline phosphatase is a putative receptor of Cry1Ab and Cry1Ac toxins from *Bacillus thuringiensis* in stem borer *Diatraea magnifactella* Dyar

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Abstract

Bacillus thuringiensis is a gram positive bacterium that produces crystals during sporulation phase of grown. These crystals are constituted for toxins denominated Cry o Cyt. These toxins are toxic and specific against different insect pest, innocuous to humans and other mammalians. The *Diatraea* spp. is the most important group of the stem borers that principally attack corn and sugarcane. *Diatraea magnifactella* is the most economically important pest in Mexico, particularly is insect pest of sugarcane, with damage at 50% of crops. Unfortunately, commercially available insecticides are no effective for the control of this insect. The toxins Cry1Ab and Cry1Ac are specific against lepidopteran insects and in this research; we tested the virulence of this toxins in *D. magnifaltella* and for other side, we performed the immunolocalization of this toxins in the histological cuts of midgut of this insect. Finally, we performed the receptors identification by ligand blots assays. The results showed that Cry1Ab was more virulent that Cry1Ac with a LC50 of 19.347 µg/ml and 33.115 µg/ml respectively. The experiments of inmunolocalization showed that Cry1Ab bind exclusively on the apical tip microvilli and interesting the toxin Cry1Ac bind both basal mina and tip microvilli. We then analyzed the interaction between toxins and midgut BBMV. Determination of binding parameters indicated that the Cry1Ab bind to BBMV with an apparent binding affinity (*Kd*) of 34.62 nM while the Cry1Ac bind with an *Kd*= 109.22. These results correlated with the virulence (LC50) of these toxins. To determine possible receptors in the BBMV we performed ligand blot analysis and the results showed that both toxins bind to two bands of approximately 120 kDa and 60 kDa. The analysis of these proteins by zymograms with alkaline phosphatase substrate showed that the protein that binds Cry1Ab and Cry1Ac is an alkaline phosphatase. We think that the 120 kDa protein is a dimer of alkaline phosphatase and the 60 kDa is a monomer of this protein. This research is the first report of a receptor in the stem borer sugarcane *D. magnifactella*.

Role of the rhizosphere microbiome in the phytoremediation of the organochlorine pesticide endosulfan by Basil *(Ocimum basilicum)*

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Endosulfan is an organochlorine pesticide widely used worldwide for agricultural purposes. Based on studies about its toxicity, bioaccumulation and biomagnification, and persistence in the environment, it has been included in the list of persistent organic pollutants (POP's). Exposure to endosulfan could result in both acute and chronic risks of concern for terrestrial and aquatic organisms. Because of this, an increasing interest is being devoted to the search for effective remediation technologies for partial or total recovery of contaminated sites. The phytoremediation is an emerging technology that facilitates the removal or degradation of toxic chemicals in the environment by using plants. Phytoremediation technology is site specific due to the plethora of environmental variables that affect plant growth and pollutants mobility. The state of Nayarit, study site for this research, is among the top states with the highest pesticide use nationwide, where the organochlorine pesticide endosulfan is the mainly marketed. Previous phytoremediation works using Basil (*Ocimum basilucum*) plants showed a diminish in the endosulfan levels in planted soil related to unplanted soil, suggesting thus the potential of basil plants in the remediation of soils contaminated with endosulfan. However, so far the global mechanism involved in the phytoremediation of endosulfan by *Ocimum basilicum* is fully unknown. It is known that the success of phytoremediation of contaminated soils is connected with the ability of plants to enhance the microbial activity in the rhizosphere. Increased number of microorganisms and enzyme activities as well as an increase in the abundance of strains capable of metabolizing the contaminant are typically observed in the rhizosphere of plants during the phytoremediation process. For this reason, the aim of this work is to evaluate the role of the microorganisms of the rhizosphere of basil in the phytoremediation of endosulfan. The changes in the diversity of the microbiome of the rhizosphere were assessed by the enumeration of bacteria, fungi and actynomicetes and through the phenotypic characterization of the culturable microorganisms. Likewise, endosulfan-degrading bacteria were isolated and identified. The results of this work show the role of the microorganisms of the rhizosphere in the mechanism of phytoremediation of the organochlorine endosulfan by Basil (*Ocimum basilucum*) plants.

Insight of the active site of a fungal laccase: A molecular dynamic simulation.

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Laccases are multicopper oxidases that contain four copper atoms in the active site, classified according to their spectroscopic properties in three groups[1](T1, T2 and T3). In T1 site,one copper ionis coordinated by three ligands, one cysteine and two histidines.T2 is a mononuclear copper site which is coordinated by two histidines. Finally, T3 is a binuclear site where two copper ions are coordinated with three histidines each. These enzymes catalyze the oxidation of anorganic substrate concomitant with the reduction of molecular oxygen to water, thus are considered as green catalysts.For metalloproteins, modeling the active site is a crucial step for theoretical calculations, as the flexibility and electronic properties of the metal ions and coordinating groups modulate the redox potential and the catalytic power of the enzyme. The study of the dynamics in the active site of a fungal laccaseis interesting due to itsspecific characteristics. During catalysis, Cu ions are reduced from Cu(II) to Cu(I). The Cu coordination in the T1 site is similar in the oxidized and the reduced state, resulting in no conformational changes during the electron transfer and thus small reorganization energies and high electron transferrate are observed[2,3].

In this work we performed an investigation of the dynamics in the active site of a fungal laccaseby molecular simulations. Laccase from *Coriolopsisgallica* was selected as model due to its efficiency for pollutant removal and high redox potential[4]. In order to study the flexibility in the T1 site, we restrained the movement of the active site (both T1 and T2/3) varying the force field constant from 1000 to 1 kcal/molÅ^2. Simulations of 100ns were performed for each constrain. The Root-mean-square deviation (RMSD) of the first coordination sphere of copper at T1 site shows that there is no effect in the movement of these residues with the magnitude of the force constant, so that the coordination at this site is rigid. On the other hand, we studied the RMSD of the second coordination sphere in this site, and the results show that the movement in these residues, around 8Åof T1Cu,is dependent of the magnitude of the force field constant. On the other hand, for T2 and T3 site the RMSD of the first coordination sphere indicates that there is no significant change in the movement of the residues with the force field constant. However, there was an unexpected observation at T2 site. Whilst the force constant becomes smaller the CuT2 loose its coordination and leave the site through a water channel. This observation is in accordance with previous X-ray diffraction experiments, which show the loss of this Cu ion, suggesting this ion is more sensitive than the others[5].

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Study of Agave tequilana leaves an alternative biomass of sugars and cellulosic material for the production of second generation bioethanol.

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Second generation biofuels are developing as an attractive alternative to fossil fuels with the aggregate value that does not compete with the food chain production, and therefore is not affecting food security of a nation. Common source for second generation biofuels are the agricultural waste. The present work is focus on tequila industry in which agave leaf crop residue represents approximately 38% of the total weight of the plant. The agave leaves are not employed in the tequila production, however they represents an excellent source of biomass for bioethanol production. The objective of this workis to study the chemical composition of the agave leaf and its viability as biomass for bioethanol production. The leaves were dried and processed to obtaina flour to have a common starting point from which all the studies were carried out. Percent chemical analysis, and holocellulose (cellulose and hemicellulose) was performed in order to obtain the amount of source compounds fermentable sugars and lignin as a polymer that functions as a protective barrier for the cellulose-hemicellulose. Total sugar content (TSC) and direct reducing sugars (DRS) was determined by photocolorimetric method 3,5-dinitrosalicylic acid at 540 nm, the non-reducing sugars (NRS) is calculated by difference. High Performance Liquid Chromatography (HLPC) was used to identify the different sugars present in the samples. Agave leaf flour is mainly composed of carbohydrate and crude fiber by 53% and 31%, respectively. Soluble carbohydrates are composed TSC 520 mg / g, NRS 370 mg / g, DRS 150 mg / g. The NRS represent mainly Agavinas. The lignocellulosic material consists holocellulose 149.5 mg / g and lignin 162.4 mg / g. Sugars detected in agave leaf were fructose, glucose, sucrose and xylose. As a result is found that the high concentration of fermentable sugars present in the agave leaf flour supports is potential as a valuable biomaterial for second generation bioethanol. Proposing full use of the plant and generate added value to this biomaterial waste.

Study of acid-enzymatic hydrolysis of Agave tequilana Weber azul leaf for the production of second generation bioethanol.

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In the world many studies are conducted to develop a viable alternative for biofuel production without affecting food security, such as the second generation biofuels. The lignocellulosic materials are a potential source of fermentable sugars for conversion to ethanol. In order to obtaining sugars from lignocellulosic material various processes for the conversion of the structural polysaccharides sugars for subsequent fermentationare needed. This conversion can be performed by physical, chemical and/or enzymatic hydrolysis.In this work we study the acid and/or enzymatic hydrolysis of lignocellulosic biomass leaf agave previously dried and grounded to make flour samples, from which solutions are made. Acid hydrolysis of the samples were performed using sulfuric acid and hydrochloric acid at various concentrations (0.5, 1, 1.5 and 2%), at a temperature of 121 ° C, for 15 minutes. The enzymatic hydrolysis is performed using three commercial enzyme of Novozymes (Celluclast, and Viscozyme CTec2 Cellic) at a temperature of 50 ° C and pH 5.0 and agitated at 350rpm for 24 hrs. Reducing sugars (RS) obtained were determined by the method photocolorimetric 3,5-dinitrosalicylic acid (DNS) at 540 nm. Direct reducing sugars (DRS) on the samples without applying any hydrolysis was 6.7g / L. Theacid hydrolysis performed using H_2SO_4or HCL0.5% reported the higher content of sugars released, 8.2 ± 0.6 g / L and 6.7 ± 0.8g / L, respectively. The enzyme was Cellic CTec2 who released 22 ± 0.5 g / L RS with respect to Viscozymeand Celluclast. Hydrolysis was performed with the best acid-enzymatic conditions was 0.5% H_2SO_4 acid and enzyme CTec2 Cellic RS obtaining of 705 ± 0.5 mg/g~ 70% of reduced sugars released from the agave leaf flour. These results show that the acid hydrolysis yields significantly improves the enzymatic hydrolysis. These results show the attractive of studying the process of acid and/or enzymatic hydrolysis for evaluate the agave leaf flour as alternative biomass to convert them to fermentable sugars and produce second generation bioethanol.

Effect of fatty acids and derivativesfromavocado on the regulation of innate immunity in bovine mammary epithelial cells infected with *Staphylococcus aureus*

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Staphylococcus aureus is an opportunistic and pathogenic bacteriumresponsible ofbovine mastitis.*S.aureus* has the capability to internalizeinto professional (macrophages, neutrophils) and non-professional phagocytes (endothelial cells, epithelial cells, fibroblasts), which allows it to evade the immune system of the host and makes difficult the antimicrobial therapy. An alternative treatment to control this disease is the modulation of the host innate immune response. The Mexican native avocado*Perseaamericana*var. *drymifolia*has been used in theethnopharmacologyand it has been hypothesizedthat fatty acids and lipid compounds from this plant exhibit immunomodulatory properties.The aim of this work was to analyze the effect of fatty acids and derivativesfromMexican avocado seeds(*P.americana*var. *drymifolia*) on the internalization of *S. aureus*intobovine mammary epithelial cells (bMEC) andthe expression of innate immunitygenes.The fatty acids and derivativeswere extracted with hexane and cold crystallizationfrom avocado seeds. The composition of fatty acids and derivativesfrom crystals was analyzed by gas chromatography coupled to mass (GC-MS). The most abundant fatty acids and derivatives werethose of 17-21 carbons.Then, we tested by turbidimetry and MTT assay if these compounds affect the growth of *S. aureus*and bMEC viability. We did not detect any effect at the different concentrations (1ng/ml to 10 μg/ml)evaluated. To evaluate if these compounds are capable to modulate the immune response of bMEC, the cells were treated with different fatty acids and derivativesconcentrations for 24 h, then were infected with *S. aureus*(2 h)and the internalized CFU were recovered.It was observed that the concentrations of 1 ng/ml and 10 ng/ml decreasedthe bacteria internalization (50-60%). The correlation of this effect with the activation of genes of the innate immune response of bMEC, is actuallybeing conducted trough RT-PCRanalysis evaluating the expression of cytokines (TNF alpha, IL-beta, IL-10) and antimicrobial peptides (TAP, LAP, BNBD10).In conclusion, the fatty acids and derivatives from avocado seeds can inhibit the *S. aureus* internalization into bMEC.

"Molecular characterization of the acidic dehydrin OpsDHN1 from Opuntia streptacantha".

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Dehydrins are a group of the Late Embryogenesis Abundant (LEA) proteins that accumulate during the late stages of embryogenesis and in response to abiotic stresses; however, the molecular mechanisms by which their functions are carried out inside the cell are still unclear. We have previously reported that transgenic Arabidopsis plants overexpressing an Opuntia streptacantha SK3 dehydrin (OpsDHN1) show enhanced tolerance to freezing stress. In this study, we show protein dimerization of OpsDHN1using a yeast two-hybrid "split ubiquitin" system. In this regard, we show that deletion of regions containing K-segments and histidine-rich region of OpsDHN1 protein affects dimer formation. Not surprisingly, sequence analysis shows that OpsDHN1 is an intrinsically disordered protein, an observation that is confirmed by circular dichroism and gel filtration of the recombinant expressed protein. The addition of Zinc triggered the association of OpsDHN1 protein, likely through its histidine-rich motif. On the other hand, protein sequence analysis of OpsDHN1 protein revealed two putative PEST (a proteolytic degradation tag) motifs toward its C-terminal. The characterization was carried out by a translational fusion between the reporter gene β-glucuronidase (GUS) with 378 bp open reading frame of OpsDHN1 gene, coding for 126 amino acids of the C-terminus that includes the PEST sequences. We show that the fusion of the PEST sequences OpsDHN1 leads to degradation of the GUS protein reporter. These data brings new insights about the molecular mechanism of the OpsDHN1 SK3-dehydrin.

Characterization of cold-adapted digestive cathepsin D aspartic peptidase in American lobster (*Homarus americanus*)

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Cold-adapted enzymes are the main adaptive strategy of poikilothermic animals to achieve functional metabolic rates. These enzymes are of high biotechnological value, given their high catalytic efficiency at low temperature and low thermal stability, characteristics that are achieved byan increase in flexibility of its protein structure. American lobster (*Homarus americanus*) copes with environmental temperatures that vary on a seasonal basis over a range of 0–25 °C. We expected that the digestive proteolytic enzymes will exhibit the characteristics of cold-adapted enzymes. In our first approach, we investigated peptidase activity in the gastric juice of lobster using a novel strategy for multiplex substrate profiling by mass spectrometry (MSP-MS). A total of 140 cleavage sites were identified in the MSP- MS analysis of the gastric juice, about 60% of which are sensitive to E-64, which is a potent inhibitor of cysteine peptidases.About 20% of the cleavage sites are sensitive to pepstatin A, an inhibitor for aspartic peptidases. This result indicates that digestive peptidases in the gastric juice of American lobster is distinctive because, unlike decapod species studied so far, where protein digestion relies mainly on serine peptidases, most peptidase activity is accounted for by cysteine and aspartic peptidases. Then, we studied cathepsin D1, the first digestive aspartic peptidase ever reported in decapods. Lobster cathepsin D1 was analyzed for substrate specificity, thermodynamic activation, and stability; its homeothermic homologue,cathepsin D from bovine spleen and pepsin from porcine gastric pepsin, were included for comparison. The specificity of lobster cathepsin D1 is similar to that of its homeothermic counterparts, it is an endopeptidase hydrolyzing peptide bonds composed of bulky hydrophobic residues at P1′ and P1 (Phe, Trp, Tyr, Met, Leu). The catalytic constant (k_{cat}) of lobster cathepsin D1 is higher than its homeothermic counterparts over the range of temperature of 5–55 °C. The Gibbs energy of activation ($\Delta G^{\#}$) decreases with a decrease in the enthalpy of activation ($\Delta H^{\#}$) and/or by an increase in the entropy of activation($\Delta S^{\#}$), given that $\Delta G^{\#} = \Delta H^{\#} \Delta T \Delta S^{\#}$. The higher catalytic efficiency of lobster cathepsin D1 is also reflected in lower levels of $\Delta G^{\#}$: 64, 73.4, and 66.8 kJ/mol for lobster cathepsin D1, bovine cathepsin D, and porcine pepsin, respectively. As is the case for most cold-adapted enzymes, lobster cathepsin D1 relies on a lower enthalpy of activation, while entropy of activation is not optimized. Also, lobster cathepsin D1 loses its native conformation at 40 °C, while bovine cathepsin D and porcine pepsin lose their native conformation at 75 °C and 71 °C, respectively. The interaction with pepstatin A, a transition state substrate analog inhibitor, increases the melting temperature of lobster cathepsin D1 and porcine pepsin to 78 and 88 °C, respectively.The results demonstratethat cathepsin D1is a cold-adapted enzyme, with a more flexible structure, which is reflected in higher catalytic efficiency, lower thermal stability, and higher stabilization of protein structure by interaction with a transitionstate substrate analog inhibitor. The enzyme conserves the tridimensional structure of aspartic peptidases and the residues involved in the sub-sites of substrate interaction. Differences reside mainly in the amount of proline and glycine residues, a characteristic that enhances the flexibility of proteins.

Isolation of first genes from carotenoid biosynthesis pathway phytoene synthase, phytoenedesaturase and ζ-carotene isomerase from *Bixaorellana*.

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Annatto (*Bixiaorellana* L.) is a wild bushnativefromNeotropical region, annatto has become popular crop because produces the apocarotenoid (C_{24}) called bixin. Bixin is an organic red/orange pigment that is for human consumption, the pigment has great economic importance and is used in pharmaceutical, food and cosmetic industry. Despite bixin is globally quoted, the molecular mechanism for bixin production is poorly understood. For example, is known that the lycopene carotenoid is converted to bixin by the action of three enzymes, a carotene dioxygenase, an aldehyde dehydrogenase and a methyltransferase(Bouvier, et al.
2003). However, the carotenoid biosynthesis pathway genes from annatto arenot isolatedyet. Only partial gene sequences had been described (Jako, et al. 2002; Rodriguez-Avila, et al. 2011). In order to elucidated the carotenoid biosynthesis pathway from annatto and understand the molecular mechanism for bixin production, the aim of this research is isolate the three first genes of carotenoid biosynthesis pathway from annatto, phytoene synthase (*psy*), phytoenedesaturase (*pds*) and ζ-carotene isomerase (*z-iso*).

To isolate the three genes, total RNA was extracted from annatto leaf then cDNA was synthesized and this was used as template to amplify the open reading frame using PCR technique. To amplify the genes we designed specific primers for *psy,pds* and *z-iso* genes from sequences identified in annatto transcriptome. Amplicons was cloned and sequenced. We isolated four complete ORFs sequences, two ORFs for *psy*, and one ORF for *pds* and *z-iso*. One of the *psy* ORF is 1320bp long and the protein is 92% similar to PSY1 of *Carica papaya*. The second *psy* ORF is 1182bp long and the protein is 92% similar to PSY2 of *Theobroma cacao*. The *pds* and *z-iso* ORFs are 1698pb and 1092pb long respectively and theirs closest proteins homologous are PDS3 and Z-ISO form *T. cacao* which share 92% and 82% of similarity respectively.The isolation of the first genes of carotenoid biosynthesis pathway from annatto is the first effort to isolate all the genes of this pathway in order to understand the molecular mechanism of bixin production.

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Production of xylanasesfrom*Serratia sp.* isolated of the gut of bark beetle of*Dendroctonus*genera(Curculionidae:Scolytinae)

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Bark beetlesare insects engaged in symbioticrelationships with mainly bacteriaand yeast.Bark beetles and their associated microorganisms have narrow ecological and physiological relationships. Predominantly, the bacteria are important in the biology of these insects;for instance,may lower defenses of trees,may be involved in pheromone synthesis andthey may also provide protection from fungal competitors or fungal pathogens. Bacteria gut from bark beetlesplay an important role in the digestive process providingessential amino acids, B vitamins, nitrogen and sterols. Also, these bacteria are able to break the lignocellulose components; therefore, hydrolytic enzyme activity such as xylanases and cellulasesare present. These enzymes are of particular interest to understand their functional ecological role in the insect and evaluate their biotechnological potential. The aim of this work was to producexylanases from*Serratia sp*.isolatedof the gut of *Dendroctonus*species. *Serratia sp*. grows better in the medium containing xylan, gelatin, $KH_2PO_4$and $MgSO_4$, which was further modifiedby optimizing the concentrations of individual components. The optimum time for growth and enzyme production was 26 hours. Maximum xylanase activity was reported with 50mMcitrate phosphate buffer (pH 6.0) at 50°Cand 5 minutes incubation temperature. Finally, this is the first report of the production and activity of xylanase by a bacterium isolated gut of a bark beetle in liquid medium.

Degradation of antibiotics by basidiomicetes fungi (*Trametes versicolor, Lentinulae dodes, Lentinula boryana, Pleurotus djamor, Pleurotus djamor var roseus*)

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One of the most import problems in the world is the improved bacteria resistance to antibiotics. Its impact in human health in a near future will be a problem. In Mexico before 2006, there was not a government control on the use of antibiotics, neither a maximum permissible limit set to discharged them in water effluents, for instance from cleaning products, antibiotic production by industries or hospitals. Moreover, due to the tendency of people of antibiotics self medication without attending a doctor, or doctor prescription of a broad spectrum antibiotics without having a proper diagnosis of the pathogen involved, for instead, in ear, stomach, eyes, throat among other infections. Finally its uses specifically in the production of beef cattle, chickens, fish and agricultural products such as chili pepper and tomato allow them to be found in excess in the environment, meanly water and soil.For this reason it is important to look for an alternatives to degrade antibiotics from water using basidiomycetes fungi. In this work, we evaluated the use of five basidiomycetes fungi *Trametes versicolor, Lentinula edodes, Lentinula boryana, Pleurotus djamor* and *Pleurotus djamor* var *roseus* to degrade antibiotics such as chloramphenico land amoxicillinus. first in malt extract agar in Petri dishes evaluating its radial growth velocity and mycelia production per day. Secondly the best basidiomicete fungi mycelia was cultured in minimum media in flasks, samples were evaluated by HPLC to follow antibiotic degradation. In agar medium on Petri dishes concentrations of 5, 10, 15 ppm of each antibiotic were employed and water as a negative control. Results were analyzed by ANOVA program, to select the concentration to be used and the best strains. Then biomass from agar growth in Petri dish was cultivated in liquid medium supplemented with both antibiotics separately in mineral medium with a vitamin solution, weekly samples from supernatant were analyzed by HPLC. Results showed that *Trametes versicolor* is able to degrade both antibiotics efficiently. Since a higher growth rate with both antibiotics treatments were registered, mycelia covered the entire Petri dish between days 8 to 10 as compared with positive or negative control. In liquid media samples are being evaluated.

Biomimetic coats for improving the interaction and stability of DNA-delivering nanoparticles

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The use of nanoparticles is a promising way of improving drug delivery systems. Moreover, there is a wide variety of applications of this chemical method, which has allowed the development of different types of trials that lead us to analyze the most efficient method for gene therapy. In this project, mesoporous silica nanoparticles were prepared for the encapsulation of nucleic acids, either alone or coated by cationic liposomes as a biomimetic surface. The fluorescent dye calcein was used as a model for testing the encapsulation of acidic molecules into nanoparticles. Structural analyses of the systems included transmision electron microscopy (TEM) and Dispersive Raman spectroscopy. Our results indicate that both encapsulation and stability of calcein-loaded nanoparticles was increased by the addition of the lipid bilayer onto nanoparticles. Moreover, the presence of this bioinspired coat significantly improved the attachment of particles to HEK 293 cells, as revealed by confocal microscopy. Current work is underway for inducing the entry of these hybrid nanoparticles with a GFP-encoding plasmid into cells through shock waves a method based on the principle of acoustic cavitation. As a future perspective, we expect to use this vector as a vehicle for an anticancer drug.

Functionalization of iron nanoparticles (FeNP´s) with proteins secreted by*Streptococcus pneumonia,*which bind haem and haemoglobin.

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The usage of iron nanoparticles (FeNP´s)with magnetic properties, which carrying anticancer drugs,has been a prominent strategy, because their magnetism could be useful to direct the NP´s towards a specific tissue. Interestingly, FeNP´sdo nothave a toxic effect over the cells. Therefore, FeNP´s can be potential candidates to transport drugs in the human body. In this work, we are interested in functionalizing FeNP´swith proteins secreted by a bacterium in order to direct them towards its respective receptor located in *Streptococcus pneumoniae*membrane. We purified proteins secreted by *S. pneumoniae* by affinity-chromatography. One of the proteins was identified by Mass spectrometry asglucose-6-phosphate dehydrogenase (GAPDH). This protein was utilized to functionalize the FeNP´sby sonication.The functionalization was demonstrated by quantification of the protein (Bradford method) in the supernatant of NP´s after of centrifugation. The FeNP´s functionalized with GAPDH were tested in bacterial cultures of *S. pneumoniae*. These results showed that the FeNP´s were not toxicfor *S. pneumoniae*,therefore, could be used to carrying any antibiotics and directed them towards a specific tissue. These results are the first approach in order to develop a new strategy to eliminate bacteria of the human host, which be specific and with less secondary effects for the patient.

Regulation of morricinsynthesis

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Bacillus thuringiensis (*Bt*) is a gram positive bacteria widely studied by its capacity of Cry proteins production. However, *Bt*is also a producer of antibacterial peptides ribosomallysynthesized (bacteriocins). Eighteen different bacteriocins have been found in different *Bt* strains. The genetic clusters involved in bacteriocin production in two of strains areknown. These bacteriocins are designed as thuricin CD and thurincin H. In our laboratory we found that*Bacillus thuringiensis* subsp*. morrisoni* contains the same genetic cluster as thurincin H. Cluster is composed of three tandem thurincin Hgenes, as well as the thnP, thnD, thnE, thnR, thnB,thnT, and thnI genes. All of these are required for prepeptide processing,regulation, modification, exportation, and immunity. Thurincin H cluster contain a putative transcriptional regulator (thnR), this regulator has homology with GntR superfamily and has not been related with bacteriocins regulation. The most studied bacteriocins belong to lactic acid bacteria which are regulated by two component system including a kinase and a transcriptional regulator. There are no reports about bacteriocins regulation in *Bacillus thuringiensis* and this is the goal of our work. We want to understand the regulation of bacteriocin cluster.We observed that the producer strain transformed with different constructions that contain some genes of the cluster with the structural genes lose the bacteriocin activity. It is important to highlight because the transformed strain was capable of bacteriocin production before the transformation. This fact could be due to that the regulation mechanism is affected by over expression of the structural gene. The objective of this work is to understand the mechanism of regulation of bacteriocinmorricin.

PRELIMINARY ANALYSIS OF PROTEIN CONTENT IN HUITLACOCHE

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The edible mushrooms are source of food with impotant nutritional properties and Mexico has at least 200 species. Huitlacoche (*Ustilago maydis*) is one of these species and is known as the “Mexican truffle”; it is a corn smut and it causes severe losses worldwide. The Huitlacoche is considered the King of all edible mushrooms for its characteristic flavor and it is a legacy of our prehispanic ancestors. Many studies have recognized its nutritional value and it has been determined that it is a good source of fiber, carbohydrates, proteins, vitamins, minerals and bioactive substances with several biological properties like hyperlipidaemic, antitumoral, immunomodulation, antiinflammatory, antimutagenic, antiatherogenic and hypoglycaemic, being the primary bioactive compounds carbohydrates and glycoproteins.

Due to the growing interest in healthier natural foods and their capacity of helping against certain pathologies it is consider of great importance to know the biochemistry and general characteristics of the proteins contained in huitlacoche that can be included in human diet and that have not been fully studied. In this work, is proposed the isolation and identification of Huitlacoche´s proteins which have potential use in biotechnological research as therapeutics.

For instance, it has been started to research with the teliospores from *U. maydis*. Infected cobs were used for this purpose. They were collected in Ixtlahuaca, Estado de México during August and September. 5 g of teliospores were washed and broken by mechanical methods. It was used several buffers for protein extraction. The extracts were concentrated and desalted. A gel filtration chromatography was used as first step of purification. Protein concentration was determined in each extract and fractions and they were analyzed by SDS-PAGE.

It was obtained five extracts. The cromatographic profiles were have differents retention times, we found several proteins in each extract, the more complex is the extract five (NaOH 0.1M) with 8 different putative proteins. The extracts with major concentration of protein were 1.6 mg/ml from extract 2, 1.3 mg/ml from 3 and 1.6 mg/ml of extract 5. The extract 4 show compound with lipid character.

Pycnoporus sanguineus acetonic extract insecticidal effect on *Spodoptera frugiperda* larvae

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Pycnoporus sanguineus has been investigated because it produces various metabolites of biotechnological interest, such as enzymes and pigments. Among the enzymes reported in Pycnoporus spp are laccases, invertases, carboxipeptidas, alpha and beta D-mannosidase, exo-poligaracturonasas, alpha amylase, celulasas, xylanase, tiranasas, beta-glucosidase, pectinase and lipase with industrial applications such as, paper pulp bleaching papel and bioremediation of aromatic compounds. Among the pigments that *Pycnoporus sanguineus* is able to produce cinabarina, tramesanguina, cinabarínico acid picnosanguina and several derivatives of 2-amino-phenoxazine-3-one have antimicrobial activity and medicinal potential to be uses in humans and animals. It has been reported its potential uses in the pigments industry for food and cosmetics products, however *Pycnoporus sanguineus* pigments have been little studied for its insecticidal activity. Cinnabarine is a pigment with a 2-amino-phenoxazine-3-one structure, a flavonoide which may act on para typE voltage gate sodium channel in insects pest as pyrethroids does. In this work we reported the effect and the potential use of *Pycnoporus sanguineus acetonic extract on Spodoptera frugiperda* a pest insect as an alternative for its control. Acetonic extracts were obtained from P. sanguineus fruty bodies growth on solid state fermentation over pine sawdus. The fruty bodies were dried, cuted and milled and tne placed in a Sohxlet system using 40 g of mooshroom and 500ml of acetone and obtaining 1.12 g of dry extract. The extract was given in a mmeridic diet and on maize leaves to the third instar larvae of *Spodoptera frugiperda*. Using the Probit analysis program the LD_{50} dose of *Pycnoporus sanguineus* extract were determined to be 0.99 m mg/mg-1 larvae. An interesting chronic response was obtained with noticeable morphological changes. HPLC cinnabarine purification is being performed to obtain cinnabarine concentration in each extract sample used and to determine pure cinnabarine effect in *Spodoptera frugiperda larvae.*

Expression of the hemagglutinin-neuraminidase´s ectodomain from *Rubulavirus porcine* in the yeast *Pichia pastoris*

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The viral blue-eye disease of pigs (EOA) was detected in porcine farms of La Piedad, Michoacán, México in 1980. The etiological agent is a *paramyxovirus* classified like *Rubulavirus porcine* (**RVP**), which causes neurological problems, reproductive, respiratory and opacity corneal in 1-10% of the cases. The hemagglutinin-neuraminidase (HN) is a protein that plays an important role for the RVP´s biological cycle and is the most immunogenic as reported by Hernández Jauregui (1998). In the hemagglutinin-neuraminidase's ectodomain (**eHN**) we found the immunogenic sites, glycosylation, active site and is the most interesting for develop a vaccine. Therefore, the main objective of this work is obtain yeast strains *Pichia pastoris* that produce the eHN of RVP and characterize the protein´s antigenicity.

The HN gene of PAC1-RVP strain was amplified from PCR and cloned into expression vector pPICZaB to obtain the pPICZaB-HN plasmid. The plasmidic DNA was inserted into chromosome of the *Pichia pastoris* in competents cells X33. Thereby, were generated clones of overproducing of the eHN named X33-HN. By PCR was determined the phenotype Mut^+ and Mut^S, therefore the process to express the protein was different for each one. The recombinant protein was detected with western blot in the extracellular fraction and the production in the Mut^+ strain was improved significantly after 8 days in methanol medium and with using high density of cells. The activity enzymatic was determined by using the thiobarbituric acid method (ATB, Aminof method with some modifications), the eHN showed neuraminidase activity similar to the Avian virus influenza (A/H5N1) and to Puerto Rico´s virus influenza (1934). Finally, the recombinant eHN was detected by antibodies from infected pigs serum with *RVP* using western blot and these assays are good indicators of the possible antigenicity of the recombinant eHN.

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Partialcharacterization of a lectin-proteaseinhibitorfractionfromTeparybean (*Phaseolusacutifolius*)

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Tepary bean (*Phaseolusacutifolius*) is distributed from arid and semi-arid regions of Mexico and the United States. This bean presents antinutritional compounds as phytates, lectins, and protease inhibitors. Regarding the common bean, Tepary has lower concentrations of lectins however they possess higher biological activity. Lectins (L) are proteins that have affinity for inducing cancer cell death by apoptosis. Meanwhile, the protease inhibitors (PIs) are peptides having an inhibitory activity against proteolytic enzymes, some of which exhibit anticancer properties. Our working group has conducted studies using a protein fraction rich in Tepary bean lectins (TBLF), which has shown cytotoxic and antitumorigenic effects on colon cancer. Meanwhile, the IP has shown to decrease *in vitro* invasive capacity related to its protease inhibitory activity. The aim of this work is to characterize a protein fraction from Teparybean containing both, L and IP (LIP-60). The fraction was obtained by sequential precipitation with ammonium sulfate from crude protein extract. The electrophoretic profile of LIP-60 showed the presence of L (apparent MW 31 kDa) and IP (apparent MW 8 kDa) as well as other uncharacterized proteins. The agglutination activity was determined(2,782 AU/mg protein against type A + erythrocytes), the half of that found for the FCL. The IP fraction activity against trypsin was, with BAEE substrate (168 IU/mg protein) with BApNA substrate (4,100 IU/mg protein) and against chymotrypsin using SAAAPFpNAsubstrate (680 IU/mg protein). The cytotoxic effect on HT-29 colon cancer cells found an LC_{50} of 0.804 mg/mL, twice that observed for the FCL. This result suggests that the cytotoxic effect is directly related to the presence of lectins. Currently, the separation of the uncharacterizedproteinsisin progress in orderto determine iftheyhaveanycytotoxiceffect.

Palabras clave: Lectins, *Phaseolus acutifolius*,protease inhibitors, Tepary bean.

Transcriptional profile of leaves in *Solanumlycopersicum*mycorrhizal plants using RNA-seq

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ArbuscularMycorrhizal Symbiosis (AMS) is an ancient beneficial association that occurs in about 80% of terrestrial plants, and confers different benefits like mineral nutrient acquisition and enhanced defense capacity. Although mycorrhiza colonization takes place in roots, the symbiosis establishment has systemic effects in other parts of the plant, in processes such as nutrient translocation, secondary metabolism, and systemic induced resistance, among others.
In order to understand the transcriptional changes that occur in leaves of mycorrhizal plants, we grew tomato plants fertilized with Hoagland's solution containing low (0.05M) phosphate concentration to favor the symbiosis establishment.Half of plants were colonized with the arbuscularmycorrhizal fungus (AMF)*Rizophagusirregularis* and the other half was set as non-colonized controls. Plants were harvest four weeks after mycorrhization with no differences in fresh weight. The colonization percentage was above 70%. The transcriptome of leaves of mycorrhizal(myc) and non-mycorrhizal (no-myc) tomato plants was obtained using RNA-seq technology (Illumina). CLC genomics workbench was used for quality filtrate (Phred +33 $\geq$20) and to determinate gene expression abundance between both conditions according to unique gene reads (UGR) comparison. Under our experimental conditions, 17, 698 genes showed expression in tomato leaves, and 980 of them showed differential expression between myc and non-myc conditions, so they were called mycorrhizal-responsive genes. According to MAPMAN classification, the main regulated functional categories were "protein processing" "RNA processing", and "Signaling", as well as "Transport", "Hormone metabolism" and "Biotic and abiotic stress".Mycorrhiza-responsive genes includevarious transcription factors, genes involved in post-translational modifications and signaling, among many others.Some of these genes may play important roles in the regulation of processes such as gene expression, cell organization and systemic defense priming, which occurs in mycorrhizal shoots previous to the encounter with a pathogen. A q-PCR strategy was used to validate RNA-seq data.

Physiological and molecular characterization of the response to drought in four *Brachypodium distachyon* natural variants

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Brachypodium distachyon is a new monocotyledon plant model with a high potential to study response to drought stress because of the great diversity of natural variants with different levels of tolerance. In addition, natural variants of *Brachypodium* also display a broad diversity in flowering time.

During the course of plant life there is a delicate process where the transition from vegetative to the reproductive phase occurs. This phase change can be affected by drought stress, in some cases delaying, and in others, inducing early flowering. The last is also known as the drought escape response.

In this work we performed the study of four *Brachypodium distachyon* natural variants with contrasting tolerance to drought and different flowering time (early or delayed flowering): Bd21 and Bd2-3 (early flowering and low tolerance to drought) and Bd1-1 and Tek-10 (late flowering and high tolerance to drought). The four natural variants were grown under controlled conditions (23ºC, 20h/4h light/dark photoperiod). Plants were grown in pots with substrate at normal irrigation (100% water retention capacity) and three stress levels: Moderate (60%), intermediate (40%), and severe (30%). In order to induce a chronic state of drought, water was maintained to these levels along the plant life cycle.

It was possible to observe different developmental patterns and time to reach maturity, measured as flowering time, in the four natural accessions. We found that drought stress modifies physiological and morphological characteristics in all the *Brachypodium* accessions tested, in function of the level of stress applied, such as number of leaves and tillers, height and time to flower. Moderate and intermediate drought stress induced the escape response in variant Bd2-3, while variants Bd21, Bd1-1 and Tek-10 did not show significant changes in flowering time. In most cases, severe drought stress caused late flowering, or even irreversible damage and death. Using a quantitative RT-PCR approach, we also investigated the expression pattern of *GIGANTEA* mRNA in a 48h-kinetic and found upregulation of this gene in response to drought, when compared to its level in control plants. The expression patterns of other key genes in flowering pathways will be also shown.

Diazotrophic potential among bacterial communities associated with wild and cultivated agaves

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The *Agave* genus includes almost 200 species, from which 150 species grow in Mexico, and 104 are endemic. *Agave* species are ecologically, economically and culturally important due to their distribution in arid and semiarid regions, and their use in the production of mezcal and tequila. Due to its relevance, we were interested in investigating: 1. which microorganisms are associated to these plants, 2.if management histories affect the diversity of their bacterial communities; and 3. if diazotrophic associated bacteria influence agaves´ fitness. Therefore, in this study we determined the external and internal bacterial communities associated with wild *A. salmiana* and cultivated *A. tequilana* plants in two sites and two seasons using 16s-rDNA-denaturing gradient gel electrophoresis (DGGE)-fingerprinting and sequencing. In parallel, we also used culture-dependant methods to specifically isolate diazotrophic bacteria associated to agaves, as nitrogen is the most limiting nutrient in *Agave* soils. DGGE profiles allowed us the identification of 65 bacterial operational taxonomic units (OTUs) associated with *A. salmiana* and *A. tequilana*, where Proteobacteria, Actinobacteria and Acidobacteria were the major bacterial phyla. Statistical analyses indicated that bacterial communities associated with agaves were mainly influenced by community type and plant species. Bacterial communities externally associated to the plants (e.g. rhizosphere and phyllosphere) were particularly affected by their host species, while endosphere communities were influenced by the season. As expected, species richness was higher in the wild *A. salmiana*, although its communities were less even than those of cultivated *A. tequilana*. In addition, we detected 11 bacterial endophytic OTUs during the dry season in both *Agave* species, which we postulate as the endophytic dry core of agaves. Notably, some of the characterized diazotrophic bacteria belong to this group and represent promising candidates for further functional studies *in planta*.

Expressed genes in *Capsicum annum* transformed roots in interaction with *Rhizoctonia sp.* binucleate

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Damping-off is caused by a fungus consortium that including *Rhizoctonia solani*. This infection is the most lost economic disease of the important crop *Capsicum annum*. Biological control with friendly *R. sp.* binucleate has been proposed to fight *R. solani*-caused illness. *C. annuum* transformed roots with *Rhizobium rhizogenes* is a useful strategy to analyze gene function. However this strategy alters gene expression. To understand the benefit relation *C. annum-R. sp.* binucleate, a bioinformatic analysis searching genes related to these biological processes in a subtracted cDNA library was done. It was found two candidate genes, a WRKY-like transcription factor (WRKY; pfam03106) and a putative LRR receptor protein kinase (PK; cd00180). Molecular modeling of WRKY domain and catalytic kinase domain of PK protein suggested that those proteins can be functional. To probe gene functionality, qRT-PCR analysis from *C. annuum* transformed roots inoculated with an avirulent isolate of *R. sp* binucleate was done. WRKY-like gene expression showed a 50% decrement at 8 to 16 hours post-inoculation (hpi) in transformed roots and *R. sp* binucleate interaction respectively. At 16 hpi, PK gene showed equal gene expression in all conditions. However, at 8 hpi, PK gene has 4.3 fold increase in transformed roots and 7.3 fold increase in *R. sp* binucleate interaction. These findings suggest that at least PK-like protein has a predominant role in the protective effect of *C. annum* triggers by *R. sp* binucleate against *R. solani*, and *R. rhizogenes* transformed root lead to important changes at the molecular level in plant-*R.sp.* binucleate interaction.

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Isolation and Characterization of Denitrifyingrhizobacteria from*Portulacaoleracea* L.in Chinampa: an Anthropogenic Crop Soil and Wetland in the Valley of Mexico.

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ABSTRACT

Denitrification is an important microbial process where oxidized nitrogen forms (NO_3^- and NO_2^-) are reduced to NO, N_2O and N_2 [1]. However this process promotes the loss of nitrogen from crop soils and enhances the emission nitrous oxide, a potent greenhouse gas and destructive compound of ozone layer. Chinampa soils represents an artificial wetland built with aquatic vegetation, woods and sediments from the lakes of Valley of Mexico employed since pre-Columbian era for the culture of grains, flower and vegetables, where the soil is alkaline, slightly saline, continuous humidity and high amount of organic matter. [2] Also it has been observed that denitrification is enhanced by humidity, alkaline pH and rhizosphere influence.To assess the capacity and diversity of denitrifying heterotrophic bacteria first we quantified the populations by Most Probable Number (MPN), and develop the enrichment and isolation of these microorganisms in bulk soil and rhizosphere of purslane (*Portulacaoleracea* L), a traditional weed consumed in traditional cuisine.Enrichments yielded 35 isolates that were identified by 16S rRNA sequencing and characterizedby observation of denitrification activity in nitrate broth medium with Durham inserts [3]. We tested the strains for develop PGPR (plant growth promoting rhizobacteria) activities in solid media: siderophores production, phosphate solubilisation, indol-acetic production, chitin, xylan and cellulose degradation also polyamines production. By MPN method, we observed that the rhizosphere had 100 times more denitrifying bacteria than bulk soil. The majority of isolates have been classified into species *Pseudomonas aeruginosa*, *P. stutzeri*, *P.putida, Stenotrophomonasmaltophilia*,*Agrobacteriumtumefaciens,Bacilluscereus,*among others. The main PGPR activities were the siderophores production and phosphate solubilisation. This research will permit a best knowledge about this physiological group in chinampa soils, a sustainable ecosystem poorly studied.

Keywords: Denitrifiers, rhizosphere, chinampa, 16S rRNA gene, PGPR, nitrite reductase gene.

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Intra and extracellular metabolite analyses on the accumulation of aromatic intermediates as an effect of carbon flux increase due to the presence of the Plasmid pJLB*aroGfbrtktA* in a ΔaroC *E. coli* strain. Magda Karina Espíndola Martínez, Luz María Martínez-Mejía, Guillermo Gosset Lagarda, and Georgina Teresa Hernández-Chávez
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As a part of systems biology, metabolomics allows us, under certain growth conditions, to determine qualitatively as well as quantitatively, the total metabolites in and outside of the cell.

In this work we characterized an *Escherichia coli* strain BW25113ΔaroC. This strain has an interruption by a kanamycin resistance cassette in the *aroC* gene, coding for chorismate synthase from the common aromatic aminoacid pathway (CAP). We also used the BW25113ΔaroC/pJLB*aroGfbrtktA* strain, transformed with the plasmid pJLB*aroG*^{fbr}*tktA*, which has an *aroG*^{fbr} gene, coding for a feedback
resistant version of the 3-Deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase and the *tktA* gene, which encodes a transketolase. This gene combination redirects precursors from central metabolism to CAP.

In this work we have applied a sampling protocol, utilizing 60% methanol al -48ºC to arrest cellular activity and 100% methanol at 90ºC to ultimately extract intracellular metabolites. Detection and quantification of intra and extracellular metabolites from CAP, were performed by an HPLC system equipped with refraction index and photodiode array detection.

We performed resting cells experiments and the subsequent processing and analysis of the produced intermediates in the pathway. Our results showed that, as expected, carbon directed to aromatic pathway biosynthesis increased 9.23-fold the measured DAHP and 4-fold the shikimate concentration for the strain transformed with the pJLB*aroG*^{fbr}*tktA* plasmid, compared to the no plasmid control. However, the specific production rate for the intermediate shikimate-3-phosphate was 3.75 times higher in the control strain, compared to BW25113ΔaroC/pJLB*aroGfbrtktA*.

This study allows us a tool to obtain intracellular information in overproducing strains, under standard analytical conditions. This, added to the supernatant information, would help us to obtain a more complete metabolic scenario of the studied mutants

Detection of anti-cancer proteins in the atypical crystalline inclusions of two*Bacillus thuringiensis*isolates

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ABSTRACT

Background.*Bacillus thuringiensis* synthesizes toxic proteins currently used for biological control of agricultural insect pests, and recently some strains have displayed toxic action against certain cancer cell lines. The parasporal crystal proteins with anti-cancer activity are best known as Parasporins (PS), which are produced by a few *B.thuringiensis* strains. The main feature of the PS is their natural ability to discriminate between cancer and normal cells, compromising the plasmatic membrane integrity or inducing apoptosis.

Results. Crystal proteins from native *B. thuringiensis* IB79 and GM18 strains showed toxic action against a human acute lymphoblastic leukemia cell line. Both strains synthesize peculiar irregular concave crystals with irregular shape. Those toxins also had cytotoxic effect over six cancer cell lines from very distant origins, such as human melanoma, carcinoma, two leukemia types and two murine hepatomas. Biocide effect was estimated as effective medium concentrations (EC_{50}) and it was established from 0.08 to 20.72 µg ml^{-1}. No toxic effect was observed when they were tested against normal cells, even at the highest dose of 100 µg ml^{-1}.Analysis of mode of action suggested that the IB79 strain produces membrane cell damage, similar to Pore Forming Toxins (PFT). On the other hand, the GM18 crystals do not seem to compromise the membrane integrity but increase the Caspases 3/7 activity, suggesting the induction of a programed cell death.

Conclusions. The native Mexican B. thuringiensis GM18 and IB79 isolates synthesize irregular parasporal crystals with anti-cancer activity against several cancer cell lines. The principal advantages are the rapid cytotoxicity and specific mechanisms of action over diverse target cell spectrum, and their nontoxicity towards normal human cells. Therefore, the isolation and characterization of new bacterial toxins could contribute to the improvement of anti-cancer therapies.

***Keywords*:** *Bacillus thuringiensis*, Cry proteins, Parasporins, anti-cancer activity.

Evaluation of soil *Streptomyces* strains with antagonist activity againstdiverse fungal plant pathogens.

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Fungi are the major disease causingagents for the economically important plants in the field, especially in the subtropical and tropical regions.The use of antagonist microorganisms to control fungal pathogens affecting plants is an ecological alternative to the use of chemical pesticides. This study reports the isolation and evaluation of antifungal activity of soil streptomycetes isolated from chilli pepper fields at Aguascalientes, Mexico. Culture-dependent isolations were characterized by morphological and physiological features. A total of 44 isolates were obtained andinitially screened in a dual culture plate-assay for antagonistic activity towards *F. oxysporum, F. solani, and P. capsici.* Four isolates with the higher antagonist activity were selected for further evaluation against *Aspergillusniger, Bipolaris sp.* and*Rhizoctoniasolani*. The inhibitory activity of selected strains was compared with the antagonism exerted by the commercial streptomycete, *Streptomyces lydicus*. Results showed a higher reduction of fungal pathogens growth by the native strains than *S. lydicus*. Production of extracellular chitinase and cellulase enzymes was observed in the selected strains that could suggest a synergy with the antifungal activity produced by secondary metabolites. Cultural and molecular results showed that strains be membersof the genus *Streptomyces*. These selected strainsshowed potent antagonistic activity against all tested fungal pathogens. This study identified novel streptomycete strains that could be used for biocontrol of plant pathogenic fungi.

Study of the hydrodynamic effects and dissolved oxygen on the growth, morphology and laccase production by *Pleurotusostreatus*CP50 in submerged cultures

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Introduction: Laccases are multicopper enzymes that catalyze the oxidation of a broad variety of substrates like phenolic compounds and aromatic amines. Laccases have great potential for industrial applications. In submerged cultures, laccase production by fungal strains is frequently evaluated as a function of the agitation rate in the reactor; nevertheless there are not appropriate studies about the effect of that parameter. It is known that agitation, for one way, determines oxygen transfer rate in the growth media, and in another, the energy dissipation from the turbines causes a hydrodynamic stress that could cause several damages in fungal cell wall.

The aim of this study was, for the first time, to independently evaluate the effect of the hydrodynamic stress and the dissolved oxygen tension on the growth, morphology and laccase production by *Pleurotusostreatus*CP50 in stirred tank reactors.

Method: Cultures were performed in a 14L fermenter with 10 L of work volume keeping the dissolved oxygen tension (DOT) controlled between 8 and 22% of the saturation (via gas mixture) and the energy dissipation/circulation function (EDCF) between 1 and 21 kW/m^3s. Effects of the factors were evaluated through a factorial experimental design 3^2. The responses, evaluated by ANOVA, were the specific growth rate, laccase yield and the pellet size of mycelia.

Results: EDCF wasthe most significant factor affecting growth and laccase production by *P. ostreatus*. In the case of mycelial growth, the effect was quadratic with an optimal value at the medium level of EDCF; mycelial growth increased from 1 to 11 kW/m^3s while pellet size decreased and a negative effect on mycelial growth was observed as EDCF increased from 11 to 21 kW/m^3s. Nevertheless, such increase in EDCF caused an increase in laccase yield. Besides that, laccase production is not only affected by hydrodynamic stress but also by oxygen concentration, that has an important negative effect. Oxygen consumption and metabolic activity of the pellets by fluorescence analysis are evidence of the existence of different levels of oxygen limitations in the cultures.

Conclusion: EDCF is the factor with the greatest impact in the process; at low level, the phenomenon that determines the specific growth rate is the oxygen limitation due to the pellet size. At high level, the improvement of laccase production from hydrodynamic stress could be explained as a defensive mechanism against oxidative stress. In addition, the negative effect of the oxygen concentration on laccase production could be due to the decrease in the ROS generation as a part of an alternative respiratory pathway at high oxygen levels.

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IMMOBILIZATION OF *Burkolderia* sp. ON DIFFERENT SUPPORTS FOR BIODEGRADATION OF METHYL PARATHION

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Keywords: cell immobilization, methyl parathion, p-nitrophenol, *Burkholderia*.

Organophosphate pesticides are of great interest because they are the most currently used pesticides worldwide. Although several microorganismscapable of degrading these pesticideshave been isolated, reports of bacteria that mineralize methyl parathion (MP) are scarce. This is mainly due to the p-nitrophenol (PNF), the hydrolysis product of PM is highly toxic to bacteria. This hydrolysis is the first step in the degradation pathway of this pesticide to result dimethyl thiophosphoric acid and PNF. Strain *Burkholderia* sp. S5-2, isolated from agricultural soils of the state of Morelos, México, is capable of hydrolyzing the PM and uses the PNF as sole carbon source. To increase yields of degradation, cells were immobilized on three supports: powdered zeolite, pieces of *Opuntia* sp. and *Agave*spfibers. The immobilized cells were inoculated in mineral medium supplemented with PM as a sole carbon source. Hydrolysis of the pesticide and the production and degradation of PNF by HPLC were measured with suspended and immobilized cells. The behavior of the cells with different concentrations of PNFwas also evaluated. The results showed a significant increase in the rate of PMhydrolysis and PNFdegradation using immobilized cells compared with suspended cells. Furthermore, the immobilized cells survive and are capable of degrading higher concentrations of PNF compared with suspended cells.

Isolation and identification of entomopathogenic bacteria from *Diatraea magnifactella* in sugarcane soils from Morelos state

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The increase in the use of chemical insecticides in pest control of sugarcane have been generating larvae resistance, toxicity in humans and increased environmental pollution. These issues give raises to the search for new alternatives less aggressive or harmful to human health and to the environment. *Diatraea magnifactella* is an insect plague of sugar cane endemic and important in the central region of Mexico, specifically in Morelos state, it is best known by the name of sugarcane borer, causing losses of 70% of sugar cane crops, It devours the leaves of sugarcane plants in its larvae instars and once the larvae gets stronger, it borer a hole to penetrate the cane eating causing multiple damage to the whole production in. There is a need to find out an alternative source of natural enemies of *Diatraea magnifactella* to be used as biological control. It have been reports the isolation of bacteria of the genera *Photorhabdus* spp. from *Diatraea magnifactella* larvae able to cause death, it producea different toxin complex such as: TcdA1 of 280 kDa, TcdB1 of 170 kDa and TccC1 of 110 kDa, among others that are responsible of it death. In this work we searched for entomopathogenic bacteria that naturally can kill *Diatraea magnifactella*. The main objective of this work, was to isolate, identify entomopathogenic bacteria of *Diatraea magnifactella* from sugarcane soil. Soil samples were collected from different sugar cane fields in Morelos state. Soil samples were sieving to 0.75 mm, and an antifungic was added to eliminate yeast, molds and fungi growth. Larva of 5th instar were used as a bait to trap nematodes. Once nematodes appear they were macerated or/and hemolymph was extracted from larva to innoculate both on MacConkey and NBTA agar media: Nematodes were kept in infective stage by growing in *D. magnifactella* larva. Pathogenicity bioassays were conducted to evaluate both nematodes and bacteria on larva. Results, first showed the isolation and characterization of *Serratia, Pseudomon*a and *Klebsiella* spp, however were interested in *Photorhabdus* and *Xenorhabdu*s bacteria genres. Fortunately, three different type of nematodes were isolated from sugar cane soils and their symbiotic bacteria are being identified. Further studies of all of them will be conducted to determine bacteria and nematodes genres at the same time by molecular biology assays.

Analysis of the endophytic bacterial communities associatedwith two wild sympatric species of Cacti

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In plants, the symbiotic associations with endophytic microorganisms, mainly bacteria and fungi, have shown to confer multiple benefits when coping with adverse environmental conditions. Cacti are plants that have undergone profound morphological and metabolic changes to adapt to harsh habitats characterized by nutrient scarcity and severe drought. We wonder if the interaction of cacti with bacteria plays a role in the adaptation of these plants to their environment. Therefore, the goals of this work are: 1. To analyze the composition and diversity of endophytic bacterial communities associated with two wild sympatric species of cacti; 2. To investigate if biotic or abiotic factors play a major role in their conformation; and 3. To explore their role in the fitness of the host plant.

Our experimental design consisted in selecting two sympatric cacti species in Guanajuato: *Opuntiarobusta (Opuntioideae)* and *Myrtillocactus geometrizans (Cactoideae).* We then sampled two different communities (root and stem endosphere) in two sites (Magueyal and San Francisco), at two different seasons (dry and rainy), during two years (2012 and 2013) in these two cacti species. We investigated the composition of the prokaryotic endophytic communities by 16s-rDNA-DGGE fingerprinting and sequencing. At the same time, we established *in vitro* cultures of these cacti species from seeds gathered in the field, and isolated and partially characterized some of the endophytic bacteria associated with them.

Our molecular analyses revealed that endophytic bacterial communities of *O. robusta*and *M. geometrizans*are mainly composed of51.6% Proteobacteria, among which members of the Enterobacteriaceaefamily are predominant,21.2% Actinobacteria, 6% Firmicutes and 3% Acidobacteria. Despite similar phyla composition, Rényi diversity profiles showed that endophytic bacterial diversity was higher in *O. robusta* than in*M. geometrizans*. PERMANOVA together with non-metric-multidimensional scaling (NMDS) analyses revealed that all factors evaluated and their interactions were significant, but the factor cacti species followed by the sampling year explained 18 and 11% of the total variance, respectively. This result suggests that endophytic bacterial communities are most influenced by the host plant and vary along time.Interestingly, we recovered 17 bacterial isolates from *in vitro* plants developing from seeds. These likely vertically transmitted bacteria belonging to the Firmicutes, Actinobacteria and Proteobacteria are promising candidates for further functional analyses both *in vitro* and *in planta*.

Determination of carbon flow to the common aromatic pathway in *Escherichia coli* mutants lacking glucose phosphoenol pyruvate: phosphotranspherase system (PTS) and non-PTS transporters

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Escherichia coli is a microorganism widely used in the industry for the production of several compounds including the aromatics. The pathway of synthesis of aromatic compounds in *E. coli* begins with condensation of phosphoenol pyruvate (PEP) and erythrose 4 phosphate (E4P), generating 3-deoxy heptulosonate-7-phosphate (DAHP) produced for three isoenzymes (DAHP synthases) encoded in the genes *aroF, aroG* and *aroH*. The next step in the aromatics pathway is the reduction of DAHP to dihydrokinate by the enzyme dehidrokinate synthase codified in the *aroB* gene. Performing the inactivation of this gene causes accumulation of DAHP. The PTS transport glucose and consumes the 50% of the PEP produced in the glycolytic pathway (EMP). The PTS system is constituted by the enzyme I (EI), the phosphohystidine carrier protein (Hpr) and the enzyme II (EII) coded by the operon *ptsHIcrr* respectively. Fuentes et al 2013, constructed a strains collection with a wide variety of rates of glucose consumption (Qs), this was achieved by the inactivation of different PTS complexes; for example the glucose, mannose, maltose, the PTS general components (EI,EII and Hpr), and the non-PTS transporters like the symporter H^+:glucose/galactose GalP and the high affinity glucose transporter MglABC.Were constructed a collection of $aroB^-$ strains transformed with the pJLBaroG^{fbr}tkt plasmid, that contains genes encoding a feedback resistant version of DAHP synthase and transketolase. This study was performed with resting cells cultures with 10 g/L of glucose. The objective of this study was determinate the effect of the Qs over the specific rate of DAHP production (Qp). The strains used in this study were named W3110bg, WGbg, WGXbg, WGMbg, WGMC and WHIbg, they lacks one, two or more PTS transporters as glucose (G), maltose (X), mannose (M) and in the case of the strain WGMC it lacks of the MglABC transport system (C), for other hand. These strains displayed Qs of 2.02±0.16, 1.74±0.08, 0.93±0.11, 0.28±0.02, 0.26±0.05, 0.15±0.02 g glucose h^{-1}/g Dry Cell Weight (DCW) and Qp of 0.90±0.05, 0.80±0.02, 0.46±0.05, 0.18±0.01, 0.19±0.01, 0.070±0.00 g DAHP h^{-1}/g DCW respectively. These strains displayed a yield of DAHP from glucose of 0.47±0.07, 0.46±0.03, 0.45±0.02, 0.64±0.01, 0.58±0.06, 0.70±0.04 mol DAHP/mol glucose. In the strains WGMbg and WGMCbg the evidence indicates that in these strains there is a contribution of non-PTS glucose transporters that not consume PEP. The Qp of DAHP was proportional to the Qs in a ratio of Qp=0.4271Qs + 0.0521.

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Same virus, new targets: Parvovirus B19 meets hepatocytes.

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Virus-like particles (VLPs) are biological nanoparticles identical to the natural virions, but without genetic material. VLPs are suitable for the analysis of viral infection mechanisms, vaccine production, tissue-specific drug delivery, and as biological nanomaterials.Human parvovirus B19 (PB19) infects humans; therefore VLPs derived from this virus have enormous potential in medicine and diagnostics. It has been demonstrated that Human Hepatitis B Virus (HBV) binds to a receptor on the plasma membrane of human hepatocytes via the preS (21-47) domain of the large envelope protein as an initial step in HBV infection.Experimental evidence has shown that the presence of this peptide on the surface of different particles is sufficient to generate tropism for liver cells.

The PB19 normally infects erythroid progenitor cells. To generate tropism for hepatocytes, the peptide, preS(21-47) from HBV was introduced at the N-terminus of the PB19-VP2 protein, generating the chimeric protein PSVP2.

PSVP2chimera was purified under denaturing conditionsand retained the competence of VP2 to assembly VLPs *in vitro*, either alone or in combination withVP2.VLPs assembled by a mixture of VP2 and PSVP2 (ratio 9:1) werechemicallylabeled with monobromobimaneat mild conditions. The bimane-labeled VLPswere usedas fluorescent tracerin cellular tropism assays. Our results demonstrate that VP2:PSVP2 particles clearly showtropismfor hepatocytes in cell culture.

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Un-targeted metabolic profiling for the discovery of highly heritable metabolites linked to coffee (*C. canephora*) cup quality

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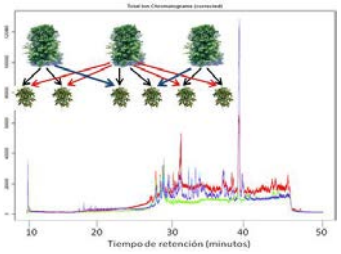

Coffee is the second most valuable traded commodity around the world (after crude oil), with Mexico being one of the main exporters and the globally leading producer of organic coffee. *Coffea arabica* and *C. canephora* are the two main cultivated species, *C. arabica* is of excellent cup quality, but it is more susceptible to diseases and pests. In contrast, *C. canephora* is more adaptable to several biotic and abiotic stresses and it has attractive characteristics for industry, such as lower price in the market and higher yields. The high content of soluble solids makes it the main material for lyophilized coffee [1].

Currently, there is a great interest in genetic improvement of coffee plants. Conventional methods of plant selection take long time, since the coffee trees need 3-5 years to the first harvest. Current selection tools assisted by genetic markers are limited, because the correlation between genetic markers, plant phenotype, and final product quality is poor [2].

For this reason, we propose an un-targeted metabolomics approach for the identification of highly heritable metabolites linked to coffee cup quality. Identified metabolic features subsequently could be employed for the marker-assisted selection and improvement of *C. canephora*. First, we designed a maternal half sibs approach in order to estimate the heritability of metabolites. We included one hundred and twenty *C. canephor*a plants (40 mother plants and eighty half sibs). Coffee plant leaves were collected from an experimental farm in Tapachula, Chiapas, México. We also subjected roasted coffee samples to a sensorial evaluation, employing a trained panel, who evaluated ten sensorial characteristics related to coffee cup quality.

Extracts of both leaves and roasted coffee samples were analyzed by LC-MS/MS. The data were pre-processed automatically through a bio-informatic pipeline. MS/MS spectra and normalized retention times, as well as a custom database of coffee metabolites were employed to identify and quantify authentic metabolites. To estimate the heritability of every metabolite, we calculated the genetic variances progeny-progenitor. Employing statistical tools such as metabolic network analysis, principal component analysis (PCA) and self- organizing maps (SOMs) we identified highly heritable metabolites and metabolic markers of coffee cup quality. Our results allow new insights into the heritability of metabolites in *C. canephora* and highlight new alternatives to identify cup quality-related markers, which supports process optimization and early plant selection.

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Study of RsmA over-expression effects on the expression of the *alg*D gene in *A. vinelandii*

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Azotobacter vinelandii is a Gram negative, this is a free living soil bacterium which includes several interesting characteristics, including the ability of growing within a wide range of carbohydrates, alcohols and organic acids. It is a polyploidy bacteria getting to have 80 copies of its chromosome. Besides, it is capable of fixing atmospheric nitrogen in aerobic conditions.

A.vinelandii has the ability to produce two polymers of industrial interest: the extracellular polysaccharide alginate and the intracellular polyester poly beta hidroxibutirate (PHB). The alginate is an important polymer because of its viscosifier and gelling ability, and the PHB is a biodegradable plastic accumulated by the bacteria as a result of carbon and energy reserves.

GDP-mannose dehydrogenase is an important key enzyme for the alginate biosynthetic pathway, the gene encoding for this enzyme is *alg*D. The expression from the *alg*D promoters are under control of the two-component system GacS/GacA.

In many ɣ-proteobacteria, GacA homologs control the expression of small regulatory RNAs of the RsmZ/Y/X (CsrB/CsrC) family which interacts with RsmA (CsrA) proteins. This protein binds their target mRNAs by acting as translational repressors. The interaction of Rsm/Csr small RNAs with RsmA/CsrA counteracts its repressor activity. In *A.vinelandii* mutations in the *gac*S/*gac*A genes appeals the alginate synthesis, this can be explained by the control that GacS/A has on the expression of the *alg*D gene.

Our research group has demonstrated that RsmA protein binds the non-coding region of the messenger RNAs *alg*D gene; this suggests that the expression of these genes is regulated at the post transcriptional level by RsmA.

The present work studies the regulatory effect that RsmA has on the production of alginate. To achieve these results, we are determining the *rsm*A gene overexpression effect in the expression of the *alg*D gene. On the other hand we are studying the effect of the *rsm*A gene mutation on the expression of the *algD* gene.

Redesign of the Shikimate Dehydrogenaese Enzyme from *E. coli*. Inversion on the cofactor specificity

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The fourth step in the common pathway of aromatic amino acid biosynthesis is catalyzed by the Shikimate Dehydrgenase (SDH) enzyme, this enzyme converts one molecule of 3-dehydroshikimate and NADPH into shikimate and NADP+ in a reversible reaction. This enzyme in *Escherichia coli* has been reported to be NADP specific, without the ability to recognize nor react with the analog molecule NAD[1]. This happens to be an unusual fact because most of the NAD/NADP dependent dehydrogenases recognize both molecules preferring one of the coenzymes over the other. The SDH enzyme from *E. coli* was cloned into an expression vector with a poly histidine sequence at the C-terminal end of the protein and enzymatic activity was found using NAD+ and shikimate as substrates, demonstrating that the SDH enzyme is able to recognize both cofactors, although it still prefers NADP+ over NAD+ about one thousand times.

The residues in contact with the adenosine moiety, responsible for the recognition and specificity, were redesigned toward the preference of NAD over NADP. To pursuit the specificity inversion the Rosetta Software for Enzyme Design[2] was used. A set of designs were done using NAD+ as the new cofactor and in parallel a second set of designs were done using the original NADP+ cofactor. The sequence of the best scored designs of each set were extracted and compared against the other set of designs, a couple of mutations were identified exclusively among designs using NAD as substrate. One of the two proposed mutations inverted de NAD/NADP specificity while the double mutation just diminished the activity about a hundred times. The switch of cofactor specificity from using NADP to NAD could permit to study the effect on aromatic amino acid production.

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Kinetic and structural characterization of mutants of the interactions surface of the C-terminal tail of nitrilase from *R. pyridinovorans.* Agustin Gomez Aguilar, Georgina Garza-Ramos Martinez. Lab. Fisicoquimica e lngenieria de Protefnas. Departamento de Bioquimica, Facultad de Medicina, Universidad Nacional Aut6noma de Mexico. Circuito Interior, Ciudad Universitaria, Av. Universidad 3000, CP 04510. Tel 56232259, Mexico, D.F ggarza@bq.unm.mx.

Nitrilases catalyze the direct conversion of nitriles to the corresponding carboxylic acids and ammonia. The structure-activity relationships in nitrilases remain unknown due to missing crystal structures; even if the first crystal structure for a dimeric nitrilase from *Pyrococcus abyssi* was recently obtained'. Nitrilases are formed by monomers of 30-40 kDa which self-associate to form active oligomers having between 4-22 subunits. In addition, it has been shown that some nitrilases could form long regular helices of variable length. It is proposed that C-terminal segment produced a steric hindrance which prevents the formation of helical structures[2] Previous results of our group showed a dependence of activity and stability of nitrilase from *Rhodococcus pyridinovorans* on quaternary structure. The *WT* nitrilase was shown to form active dodecamers of 460 kDa. Two mutant enzymes were prepared in which stop codons were inserted after positions R340 and T328 with 27 and 39 amino acids missing, respectively. Structural studies shown that T328 mutant form long helical fibers with improved activity and thermostability, while R340 mutant form shorter oligomers that tends to dissociate with the loss of its catalytic activity In this study we further explore the turning point that triggers the oligomeric equilibrium of the nitrilase from *R. pyridinovorans* by the construction of mutants with stop codons between T328 and R340 positions. In addition we generated site-specific mutants along this region to induce electrostatic interactions and obtain more stable truncated mutants This will help to understand the role of the C-terminal end in the protein-protein interactions which lead to the formation of functional helical filaments.

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Lipid bilayer disruption originated by a parasporal protein of *Bacillus thuringiensis subspecies neoleonensis,* GM18

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Bacillus thuringiensis (Bt) is a gram-positive microorganism characterized by production of parasporal crystals during sporulation. These inclusions contain proteins called delta-endotoxins, which exhibit strong insecticidal activity againts several orders of insects making Bt a reliable agent for microbial control of insects pest of agricultural and medical importance. However, earlier studies have demonstrated that Bt strains also produce non-insecticidal parasporal inclusions with a unique activity: a cytocydal toxicity *in vitro* for human cancer cells, this proteins were named parasporins. GM18 is a new subspecies of Bt discovered on Mexican soils, its parasporal protein was not active against dipterant and lepidopteran insects, but was highly cytocidal against leukaemia T cells. However the mode of action of the parasporal protein of GM18 toward the cell membrane and the factors that affect the lipid-protein interaction remains unknown. In this work we evaluated the membrane disruptive effect of this protein on lipid vesicles of different headgroup composition using the ANTS/DPX assay. This research shows that the extent of dye leakage from each kind of vesicles was clearly dependent on protein concentration and also that membrane permeabilization is mainly determined by physical membrane properties suggesting that hydrophobic interactions contribute to lipid-protein binding. These data shed important insights into the driving forces governing the process of parasporin-membrane interactions.

The nanoparticles of gold destroy the capsule of *Streptococcus pneumoniae*; the principal virulence factor that this pathogen has been developing to evade the immune system.

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Streptococcus pneumoniae is a gram positive bacteriumwhich has form of coccus. This pathogen causes otitis, sinusitis or infections more severe like pneumonia, meningitis or even worsesepticaemia, resulting lethal in many occasions for children and old people. The principal treatment is based in antibiotics as beta-lactams, trimethoprim-sulfamethoxazole, or inclusive in some cases itis necessary to give antibiotics of third generation like vancomycin.Since the excessive use of antibiotics produces resistance and even worse multi-resistance,itis therefore imperativeto develop new alternatives of the treatment. One of them could be gold nanoparticles(AuPN´s). TheAuNP´stested in a non-pathogen laboratory strain, have shown tobe a good alternative if they are utilized as antibacterial. In the case of *S. pneumoniae,* a bacterium capsulated has not been tested the antibacterial effect of AuNP´s.In this work, the antibacterial effect of AuNP´s was demonstrated in two strains: TIGR 4 (wild type) and R-6 (a mutant, without capsule). We observed that AuNP´sinhibitedthe bacterial growth and the viability in both strains. A hypothesis proposed in order to explain the antibacterial effect of AuNP´swas by modifications in the pH or the concentration of reactive oxygen species (ROS). In the case of *S. pneumoniae* cultures, the presence of AuNP´sshowed that the media was acidified for around of onepH unit. Interestingly, ROS quantificationalso showed an increasing.Finally,we attempted to explain the molecular mechanism by Transmission electronic microscopy (TEM) and we found that the NP´s entered in both strains with or without capsule, in fact, we thought that the internalization was favoured by the presence of capsule, perhaps the presence of capsule attractedbetter the NP´s, then they were grouped in a particular place of the bacterium and thus they were introduced in the bacterium forming a circular structure. Probably, this circular structure was involved in the bacterial lysis. Our overall results are showing the first approach in order to explain the mechanism by which the AuNP´sdestroy the bacteria. Additionally, we are proposing that these NP´s are more effective to counteractinfections caused by bacteria capsulated as *S.pneumoniae*, giving a better alternative in infections caused by this pathogen.

Expression and chromatographic purification of full-length and a truncated form of the GTPase Gpn1 in bacteria for structural and functional studies.

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Gpn1 is a GTPase conserved in all eukaryotes and essential for life, which is explained at least in part by the fact that Gpn1 is a protein necessary for the nuclear accumulation of RNA polymerase II (RNAPII). RNAPII has 12 core subunits and is stably associated with other proteins to generate the messenger RNAs that subsequently will direct the synthesis of all proteins in eukaryotic cells. Although details of the molecular mechanisms controlled by Gpn1 are unknown, this GTPase is transported between the nucleus and cytoplasm in the cell. We recently described a nuclear export signal in Gpn1 (Reyes-Pardo et al., 2012, BBA 1823:1756). This signal is required for the mobilization of Gpn1 from the nucleus to the cytoplasm of the cell, and is sufficient to mediate nuclear export of the fluorescent protein EYFP. Molecular modeling carried out based on the crystallographic structure of the single Gpn protein in *Pyrococcus abyssi* revealed that the nuclear export sequence is located in a carboxy-terminal acidic domain, which is unique to the eukaryotic version of this protein. The molecular modeling also allowed us to establish the reason why only one of six possible sequences of nuclear export is functional, since the latter corresponds to the one predicted to have a greater exposure to the solvent, and therefore most likely to Crm1, the nuclear export factor that directly recognizes nuclear export signals.

The aim of this study was to establish the conditions to purify Gpn1 with the goal to generate protein crystals to determine, in the near future, the three-dimensional structure of this protein by x-ray diffraction. We determined the conditions to efficiently produce a recombinant form of Gpn1 using a bacterial expression system. Recombinant Gpn1 was expressed with an N-terminal His6 tag, and purified using a combination of immobilized metal affinity chromatography and size exclusion chromatography. This procedure resulted in the production of milligram quantities of high purity full Gpn1 and a truncated version of Gpn1 devoid of the acidic C-terminal domain. Both Gpn1 versions were eluted from the nickel column, concentrated and further fractionated on a Highload Superdex 200 column, where full Gpn1 migrated in the void volume. However, truncated Gpn1 migrated with an apparent molecular weight of 30 kDa, a size consistent with this protein being a monomer in solution. With the use of additives, part of full-length Gpn1 changed its oligomeric state to fractions corresponding to the monomer. The high quantity, purity and monodispersity of the protein make it suitable for future structural and functional studies.

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Evaluation of extraction techniques of grain amaranth albumin (*Amaranthus hypochondriacus* L.) for increased efficiency

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Amaranthus hypochondriacus L. (Amaranth) is an excepcional pseudocereal, due to its high content of the albumin fraction (AmA1), of high nutritional value (between 17-19%) and a rich composition in essential amino acids (threonine, isoleucine, valine, lysine, phenylalanine and tyrosine), so satisfactorily meets the recommendations of the FAO/WHO (approximately 14%) and is considered suitable for human nutrition (FAO, 1970). In the amaranth, albumins represent a significant fraction of storage proteins, which makes them good indicators of the nutritional quality of pseudocereals. The aim of this study is to evaluate the conventional extraction methods of the albumin fraction through the application of proteomic techniques for separation and analysis, with the goal of optimize the extraction method for further biochemical and molecular analysis of the albumin fraction. The yield of the albumin fraction obtained per gram of seed flour by three different extraction methods; deionized water, and precipitating agents (acetone and ammonium sulfate) was determined. The protein patterns were obtained in polyacrylamide gels. The next step is to obtain two-dimensional gels for analysis.

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Development of recombinant strains of *Bacillus thuringiensis* expressing the endochitinase ChiA74 as inclusion bodies or chimeric crystals with Cry1Ac

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Bacillus thuringiensis is the most important entomopathogenic microorganism due to its insecticidal proteins (Cry, Cyt) that synthesize crystals of different morphology and represent the hallmark of this bacterium. Recently it has been shown that chitinolytic enzymes producing inclusion bodies with the Cry crystal are able to increase the insecticidal activity of *B. thuringiensis* (Driss et al., 2011; Barboza-Corona et al., 2014). In this work, our objective was the development of recombinant strains that express the endochitinase ChiA74 along with Cry1Ac or as chimeric crystals formed by Cry1Ac-ChiA74. Two recombinant strains were obtained. The first strain (*B. thuringiensis 4Q7-chiA74*Δps-*cry1Ac*) has a transcriptional fusion of *chiA74Δsp* under the regulation of pcytA promoter and the wild transcriptional terminator with the cry1Ac gen under the regulation of its own promoter (BtI-BtII) and transcriptional terminator. The second strain (*B. thuringiensis* HD73-*chiA74*Δ*ps*Δ*tt-ctcry1Ac*) harbor the *chiA74Δsp* without the stop codon (*chiA74*Δ*ps*Δ*tt*) fused to region the encode the C-terminal half of Cry1Ac. Both strains produced an enzyme with endochitinase activity of ~ 74 kDa of molecular mass. When *B. thuringiensis 4Q7-chiA74*Δps-*cry1Ac* was compared with *B. thuringiensis 4Q7-cry1A* it was observed that the first strain had an endochitinase activity, crystal area, and spores/mL of 107 mU/mL, 0.59 μm^2 and 11 $x10^7$ esporas/mL, respectively, whereas the second strain had values of 3.14 mU/mL, 0.80 μm^2 and 7.33 esporas/mL. Alternatively, *B. thuringiensis* HD73-*chiA74*Δ*ps*Δ*tt-ctcry1Ac* had 74 times more endochitinase activity than *B. thuringiensis* HD73, but showed a decrement of ~ 30% in the crystal size. Our data suggest that endochitinase ChiA74 can be produced as inclusion bodies o be integrated into the Cry crystals. The potential of the recombinant strainss in the control of lepidopteran larvae will be discussed.

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Solid Phase Microextraction of Volatile Compounds in Basil (*Ocimum basilicum* L.)

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Volatile compounds of basil (*Ocimum basilicum* L.) were extracted with solid phase microextraction (SPME) and analyzed with gas chromatography-mass spectrometry (GC-MS). Two SPME fiber coatings, Polydimethylsiloxane/Divinylbenzene (PDMS/DVB, 65 µm) and Carbowax/Divinylbenzene (CW/DVB, 65 µm) were evaluated (1), in order to compare the extraction of components (Figure 1).

Figure 1. SPME in Basil (*Ocimum basilicum* L.).

Among the 25 volatile compounds detected were henylpropanoids, monoterpenes, sesquiterpenes, esters, and aldehydes. There were significant ($P < 0,05$) differences between the two analyzed fibers: with CW/DVB fiber is apparently superior with respect to the number of components isolated as well as the total concentration of compounds. Quantitatively, the most important component was methyl cinnamate, followed by linalool.

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Mechanical properties of thermoplastic sorghum starch films reinforced with lignin from *Agave tequilana* Weber

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There has been in recent years, a growing interest in preserving the environment and decrease the negative impact that the waste accumulation of package materials (usually produced from fuel derived synthetic polymers), has on such an environment. One of the most promising alternatives is the development of biopolymers, which come from renewable and commonly biodegradable natural resources. Among the most promising natural source of polymers, stands out starch, because of itslow cost, world-wide availability and easy handling during films production, with some reports on films produced from this polysaccharide. However, films from starch still have limitations, because of their poor strength and rigidity, towithstand the stresses the packing materials are subjected to. That is why it is currently studied the addition of compounds that favor the cross-linking among polymeric chains, in order to reinforce the mechanical and barrier properties of starch films. Lignin is a component of plant cell walls that provides rigidity to plants and that might be obtained from waste material prevenient from *Agave tequilana* Weber in the Tequila production process.Lignin has been used to blend with various synthetic polymers such as propylene, PET, poly (vinyl alcohol) and poly (vinyl chloride) among others and it have been found that improve several characteristic of films made of such materials.However, there are only a few reports of lignin being used as a reinforcement material for starch based materials. Mechanical properties and solubility of sorghum (*Sorghum bicolor*L.Moench) starch films, with lignin from *Agave tequilana* Weber waste material addition were evaluated. Films of thermoplastic starch (TPS) and mixtures with lignin addition at 0.03 and 0.06%w/w were prepared by casting technique with glycerol as plastificant agent. A significant increase ontensile strength and Young modulus of TPS films with lignin addition were observed. Lignin addition doubled tensile strength and tripled young modulus at the highest lignin concentration. On the other hand, relative elongation of the films were diminished with lignin addition but an important parameter, the solubility were significantly improved, achieving values of almost a half of that found for TPS alone films.

Overexpression of fatty acid synthesis and its effect over the biosynthesis of medium-chain-length polyhydroxyalkanoates (mcl-PHAs) in *Azotobacter vinelandii*

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Azotobacter vinelandii is a bacterium able to synthesize polyhydroxyalkanoates (PHAs), a large family of biodegradable polyesters of industrial interest. This bacterium mainly produces poly-3-hydroxybutyrate (PHB) when is grown in carbohydrates, and is also capable to synthesize a copolymer of hydroxybutyrate and hydroxyvalerate (PHB-co-PHV) when is grown in pentanoate or heptanoate. However, its ability to produce other kinds of PHAs is limited. Conversely, *Pseudomonas putida* synthesizes a greater diversity of PHAs from carbohydrates. This is due to the presence of a β-hydroxyacyl-ACP:CoA acyltransferase, an ezyme wich provides the link between *de novo* fatty acids synthesis and the synthesis of PHAs. A recombinant strain of *A. vinelandii* (named JGF) was constructed, expressing the *phaG* gene (coding for the transferase enzyme) of *P. putida*, under control of the *scrX* promoter (inducible by sucrose) of *A. vinelandii*, in order to produce different PHAs with specific monomer composition. After 12 h induction of *phaG* gene, there is a change in the monomeric composition of the PHAs in this strain, which is now a copolymer composed of a PHB and small percentage of hydroxyhexanoate, in a 90%-10% rate, respectively. Since the enzymatic activity of the transacylase directly connects the fatty acids metabolism and the synthesis of PHAs, our work group is interested in the study of fatty acids synthesis in *A. vinelandii*, and the contribution of this metabolic pathway in the composition of the polymer. The aim of this study was to construct a fatty acids overproduction model, in order to contribute to fatty acids synthesis and to change the composition of the PHAs, in the *A. vinelandii* JGF recombinant strain. Increasing the production of fatty acids was accomplished by the overexpression of a two different fragments consisting of one or many catabolic enzymatic activities. This two models, named Y2 (overexpression of FabY, a fatty acids keto-synthase) and DGF3 (overexpression of four enzymatic activities of the fatty acids anabolism) are being tested and their capacity of growth and PHAs production and composition, will be discussed.

Selection and Characterization of a Native *Psathyrella* Strain as a Producer of Laccases Exhibiting Significant Activity under Near-Neutral pH

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Background: Fungal laccases are multicopper oxidases capable of oxidizing a broad range of substrates (i.e. phenols and arylamines)by catalyzing the monoelectronic oxidation of the compounds to highly chemically reactive radicals. These radicals usually undergo further chemical or enzymatic reactions and sometimes they are able to act at the distance as redox mediators, owing laccases to be used in a wide variety of environmental or industrial processes. Nevertheless, most fungal laccases work better under acidic pH, thus limiting its prominent biotechnological potential.In this work we show results on the selection and characterization of a newly isolated litter-degrading basidiomycete as producer of atypical laccases showing significant activity at near-neutral pH. **Methods:**Basidiomycetes were screened on PDA plates for their ability to oxidize chromogenic ABTS (non-phenolic) and 2,6-DMP (phenolic) substratesunder different pH (2, 4, 6, and 8). Selected strain was cultivated under submerged conditions on a medium containing laccase inducers (350μM $CuSO_4$ + 2% ethanol). After 12 days, mycelium was removed from cultures and a concentrate was obtained by ultrafiltration(10 K) of the extracellular medium. Profiles of pH-activity were studied with ABTS and 2,6-DMP in the pH range from 2.2 to 8.2. Biochemical characterization of crude enzyme was carried out by SDS-PAGE, Zymography and 2D-PAGE. **Results:**Color plate assays allowed the selection of a newly isolated basidiomycete (*Psathyrella* sp CU 24), as a potential producer of atypical laccases, as it showed oxidizing activity on non-phenolic ABTS at pH 8. Crude laccase from this strain exhibited wider than usual pH-activity profiles, on both non-phenolic (ABTS) and phenolic (2,6-DMP) substrates. An apparent double pH optimum behavior was also observed with phenolic 2,6-DMP, suggesting the presence of laccaseisoforms with optimal activity at near neutral pH. Zymography analysis on Native and Semi-Denaturing SDS-PAGE developed at pH 8 with ABTS and 2,6-DMP confirmed the presence of “neutral laccases”. 2D-PAGE indicates that the native laccase is produced as a consortium of at least four isoenzymes of around 40 K*Da* and pI´s of 3.8, 5.2, 5.4, and 5.6, respectively. **Conclusions:** Color plate screening and crude enzyme assays used in this study allowed the selection of the basidiomycete *Psathyrella* sp CU 24 as a new source of “neutral” laccases. This newly isolated native basidiomycete produces a consortium of laccases that exhibit unusual pH-activity profiles, displaying noteworthy activity at neutral pH on both phenolic and non-phenolic substrates.

Measurement of the enzymatic activity of laccase in the wild strain 4287 and R*ho1::hyg* strains of *Fusarium oxysporum* f. sp. *lycopersici* under induction conditions and presence of a chelating agent.

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Mexico is the second leading exporter country of tomato; however, in the last ten years, the areas cultivated with this vegetable have been affected by environmental and pathological factors, causing a decrease in production. In this case, one of the most important disease because of its impact and distribution is the fusariosis, caused by *Fusarium oxysporum* f. sp. *lycopersic*i. This fungus has a complex mechanism of enzymes involved in the infection process; among these, the laccase enzyme, a phenoloxidase whose function is to protect the microorganism against phenolic compounds released by the plant. Therefore, in this study the enzymatic activity of laccase was analyzed in wild type 4287 (pathogenic strain) and mutant *rho1::hyg* strains (nonpathogenic strain) to observe its behavior in presence of metals and metal chelators, since this enzyme could participate together with other enzyme activities in the pathogenicity of the fungus.

Fresh spores were inoculated in PDB medium for 17 hours to obtain germlings which were transferred to induction medium under the following conditions: basal medium for laccase or BL medium (control) and BL medium with addition of the chelating agent BPS (Bathophenanthrolinedisulfonic acid disodium); this for an incubation period of 6 days to obtain mycelia. The mycelia were broken by sonication in Tris-HCl buffer, in the presence of protease inhibitors and, subsequently, the cell fraction was obtained by centrifugation at 5000 rpm. Measuring the enzymatic activity of laccase in the cell fractions was performed using the chromogenic ABTS agent. With the results obtained in the control condition, a higher specific activity was observed in the mutant strain compared to the wild type (mean significant difference). This difference could be due to lack of the protein Rho1 in the mutant strain produces an upregulate. In the BPS condition, we observed a major activity in the wild type strain and a decrease in the mutant strain.

The results so far obtained require further experimentation for their corroboration, regarding the analysis of more lots under the same conditions and with the addition of another chelating agents BCS (bathocuproinedisulphonic acid) and metal compounds $FeSO_4$ and $CuSO_4$.

Studying of the phenotype from the mutants with deletion ape2 gene and function of leucine aminopeptidase yspII from Schizosaccharomyces pombe

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The leucine aminopeptidase yspII (**LAPyspII**) from Schizosaccharomyces pombe is a metallopeptidase of M17 family, depends of Mn^{+2} and hydrolyses leucine from the N-terminal protein and peptides. This enzyme is homohexameric (6 x 54 kDa) with 320 kDa. The Lys292 is fundamental for the catalysis. The function of LAPyspII is unknown. In recent works, we obtained mutants with deletion of *ape2* gene: one diploid mutant (4XΔape2) and one haploid mutant (826 ape2/Δape2) with one mutant allele. The haploid strain not expressed the mRNA of *ape2* gene and there is not enzymatic activity of LAPyspII. Therefore for the purpose of approximating the possible role of the LAPyspII, we analyze the phenotype of mutant about of the velocity in the different phases of the cellular growth.
We worked with the haploid mutant (4XΔape2) and the original strain like positive control (4X). With nitrogen starvation was obtained synchronized cells in G1 phase of cycle cellular, with the purpose to start a homogeneous population. After that a growth cellular curve was made in function of time where we observed that the mutant 4XΔape2 was growing more slowly in the start of logarithmic phase (20 hours) with a duplication time of 3.46 hours compared with 2.56 hours of the strain control (4X). With phase contrast microscopy we observed that in the end of the logarithmic phase (30 hours) appear dead cells in the mutant 4XΔape2, with a significant decrease, almost 50% of lysed cells when they enter at the stationary phase (34 hours). In the DAPI staining for the nucleus we observed important changes in the morphology cell, finding enucleated cells, like an uneven distribution of chromosomal material, which indicates that there are problems in cell division. On the other hand, in the control strain (4X), it had an increase in the medium logarithmic phase with a little increase in the mRNA (20%) of the ape2 gene, being the major increase in the enzymatic activity of the LAPyspII (40%). Finally, these events decrease at the end of the logarithmic phase (stage in which the mutant starts the lysis). We can propose that LAPyspII has an important role in cellular division process (mitosis and/o meiosis) along the cell growth and is important for prolonged cellular growth as in the final log and stationary phase where the mutant begins to abnormally divide and die.

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Obtaining mutants haploids and diploids by deletion of *ape2* gene of leucine aminopeptidase yspII from *Schizosaccharomyces pombe*

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Leucine aminopeptidases (LAPs) are metallopeptidases from M1 and M17 families with a diversity functions: in mammals processed antigenic and bioactive peptides. In microorganisms, LAPs bind to DNA allowing the transcriptional repression, meiosis and events of recombination specific-site. *Schizosaccharomyces pombe* is a unicellular eukaryotic yeast used for the expression of recombinant proteins with human interest and/o viral, vaccine production and new drugs. Therefore, is important to know the proteolytic system to detail for your utilization. The leucine aminopeptidase yspII (**LAPyspII**) of *S.pombe* is a metallopeptidase (M17) depends of Mn^{+2}. The LAPyspII is encoded by *ape2* gene on the chromosome I. The enzyme hydrolyses leucine from the N-terminal protein and peptides and is inhibited by bestatin. LAPyspII of 320 kDa is homohexameric (6 x 54 kDa). Lys292 in the active site is critical for catalysis. The function of LAPyspII is unknow. The use of mutants has been help to understand of the peptidases function. Therefore, the main purpose of this work was obtain mutants with deletion of *ape2* gene.
It was obtained a disrupted construction (ABuraCD), having as selection gene, the ura4 marker flanked for homology regions of *ape2* gene: AB (329 pb corresponding to 5´ from start promoter) and CD (305 pb corresponding to 3´of ORF ending). These sequences allow the integration to chromosome by homology recombination and gene replacement. When we incorporated the disruptive construction to haploid strains of *S.pombe* 201399 (4X) and diploids (826), were obtained probable mutants that grow in absence of uracil: 32 haploids clones and 18 diploids clones. With chromosomal DNA of probable mutants was amplified for PCR the *ape2* gene and one fragment for corroborate the correct insertion of ura4 in the desired locus. We confirmed 3 mutants for deletion: 2 haploid strains (4XΔape2) and 1 diploid strain (826 ape2/Δape2), in which only one allele is mutated. The haploid strain not expressed the mRNA of *ape2* gene and there is not enzymatic activity of LAPyspII. The diploid strain expressed 50% of the mRNA of *ape2* gene and there is 50% of enzymatic activity of LAPyspII.

Herrera-Camacho I, Rosas-Murrieta NH, Rojo-Domínguez A, Millán L, Reyes-Leyva J, Santos-López G, Suárez-Rendueles P (2007). Biochemical characterization and structural prediction of a novel cytosolic leucyl aminopeptidase of the M17 family from *Schizosaccharomyces pombe. FEBS. J.* 274**,** 6228-6240.

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cDNA OBTAINING AND THREE-DIMENSIONAL STRUCTURE OF MARADOL PAPAYA (*Carica papaya* L.) ENDOXYLANASE

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Abstract

The endo-1, 4-β-xylanase (EC 3.2.1.8) is one of the major enzymes responsible for the degradation of the cell wall in many fruits during maturation because it hydrolyzes to xylans of hemicellulose which are attached to the cellulose, decreasing its firmness; however there is no report in the Maradol papaya, although it is the most important in Mexico for their high levels of production, consumption and export to the United States. This wort reports the sequence of cDNA, as well as the structure of the pre-endoxylanase and endoxylanase of Maradol papaya.

A cDNA was obtained using degenerate oligos, which encodes for a theoretical 584 amino acids protein, with a similarity in a 99% with the papaya Sunset endoxylanase. The three-dimensional structure deduced of the pre-endoxylanase, from its amino acids, obtained with the RaptorX Server (Kallberg *et al*., 2012), presents twenty nine regions, of which the mature endoxylanase has seventeen, eight α helices and nine folds in β sheets of the catalytic domain, however in the central part there is a space going from one end to the other of the protein, being a site of interaction specific between the substrate and the protein. In the center are the glutamic acids which act as donors of protons in the reaction of acid/base that takes place during the interaction of the xylanases with its substrate (Manikandan *et al*., 2006). The proposed three-dimensional structure presented three modules, one corresponding to the module that binds to carbohydrates, other to the active site and a last that their function is unknown. In previous studies (Iniestra-González *et al*., 2013), was observed that the second module has endoxylanase activity showing a theoretical molecular weight of 32 kDa.

EXPRESSION AND BIOCHEMISTRY CHARACTERIZATION OF A HYALURONIDASE FROM THE *Scolopendra viridis* Say VENOM

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Introduction: The hyaluronidases are enzymes that cleave hyaluronan and are considered as a "spreading factor" [(1)]; therefore they have been currently used in the pharmaceutical area. This enzyme is present on animal venoms (scorpions, snakes, spiders, centipedes, etc.), facilitating the penetration of venom into the prey. Previous to this study, we have observed hyaluronidase activity on the venom of centipede *Scolopendra viridis* Say [(2)].
Objective: Perform the heterologous expression of a hyaluronidase from the venom of *Scolopendra viridis* Say.
Experimental strategy: The centipedes were collected in the State of Morelos. Hyaluronidase activity was identified in the venom of this specie through zymograms (hyaluronic acid 2mg/ml of gel) [(3)]. Otherwise, the components of crude venom were separated by High-performance liquid chromatography (HPLC) to identify the component with such activity. Peptide sequencing was carried out by mass spectrometry in the IRCM. For heterologous expression, specific primers were designed based on the amino acid sequence of 10 kDa peptide obtained by mass spectrometry. Total RNA was extracted from 10 pairs of venomous glands of *Scolopendra viridis* Say seven days after milking the centipedes. First-strand cDNA was synthesized of total RNA using the 3'RACE. The primers were designed to amplify the sequence of hyaluronidase from Scolopendra cDNA by PCR.
Results: We have observed five proteins with hyaluronidase activity (Fig. 1) in Scolopendra venom. A partial sequencing of the 10 kDa peptide was obtained and it was possible to identify the gene for a hyaluronidase in the cDNA of Scolopendra viridis Say from that sequence.
Conclusions: A peptide of 10 kDa was detected with hyaluronidase activity in the venom of *Scolopendra viridis* Say and also we designed specific primers to identify the gene for this.

Prot.	PM (kDa)
1	83
2	10
3	28
4	18
5	13

Fig. 1. Two-dimensional gel in the presence of hyaluronic acid. The presence of white spot indicates enzymatic activity.

Acknowledgements. We thanks M. B. Herlinda Clement for helpful for the loan of the HPLC. This work was supported by the Consejo Nacional de Ciencia y Tecnología (CNACYT).

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"Transcriptional Analysis of the*Arabidopsis thaliana*TFIIB*2*mutant"

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Plants are continuously affected by diverse environmental factors and therefore they have developed mechanisms to respond to these stress conditions. One of the major mechanisms to adaptation to stress is the regulation of gene expressionthat together define the response to the adverse conditions; which is carried out by various transcription factors (TF's). The TF's have the function of acting as regulators of the expression of various effector molecules involved in the perception and signal transduction of stress. In our research group, we isolated 15 T-DNA mutants from *Arabidopsis thaliana*that shown a phenotype of insensitivity to the disaccharide trehalose*(tin = treahaloseinsensitive)*;in one of the mutants *(tin14),* the T-DNA is inserted close to the gene that coding for the basal transcription factor *TFIIB*. Through analysis *"in silico"*in the genome from *A. thaliana* we found that there were two gene sequences which correspond to factor TFIIB and six sequences related to the TFIIB factor (BRP).By RT-PCR experiments on wild plants from *A. thaliana*, we have determined that the *TFIIB2* gene, is induced dehydration and salinity (NaCl) conditions and also an alternative version (generated by alternative splicing) of the original transcript, is synthesized by retention of third intron. Wepropose that alternative TFIIB2 form, acts as a negative regulator of gene expression from "housekeeping" genes to lead to the expression of genes involved in tolerance to abiotic stress. Using microarrays technology, we compared the transcriptome of the wild plant with a mutant by T-DNA insertion in the TFIIB2 factor, both normal growth conditions and under stress conditions (dehydration and salinity).The results obtained to date make us propose that the basal transcription factor TFIIB2 is an early response factor in the signaling cascade during abiotic stress.

Antimicrobial peptides and commercial antibiotics synergistic effect evaluation in bacteria from diabetic foot ulcer

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Diabetes is a chronic degenerative disease that ranks in sixth place in prevalence in Mexico. There are different types of diabetes, the most common in Morelos is diabetes type 2, 60% of Morelos state population had been diagnosed with this disease. Diabetes mellitus type 2 is a metabolic disorder characterized by hyperglycemia because of insulin. Obesity is thought to be the primary cause of type 2 diabetes, it is initially managed by increasing exercise and dietary changes. If blood sugar levels are not adequately lowered medications such as metformin or insulin may be needed. Long-term complications from high blood sugar can include heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure which may require dialysis, and poor blood flow in the limbs leading to amputations. One of the acute complications is diabetic foot infection, which generates large costs in the health sector, a complication that could not be controlled efficiently. Result of not having diagnosis of gravity, conduct to amputations of the lower extremities of patients; because bacteria have generated resistance to various types of commercial antibiotics used for treatment. Therefore it is urgent to find an alternative treatment to combat diabetic foot infections. In this work we propose to use a mixture of commercial antibiotics with antimicrobial peptides (AMP), using two variants of Pandinin 2, such as: Pin2 (G) and Pin [14], in order to enhance its in vitro effect. Both variants are derived from Pin2 obtained from the venom of the scorpion *Pandinus imperator*, the derivative Pin2 [14] WGLKGLKKFSKKL is a smaller sequence of Pin2, with reduced hemolytic activity than parent peptide Pin2 and without altering its antimicrobial activity greatly and with higher therapeutic indices. PIN2 (G) has a modification in the Proline residue 14 (P14), it was changed to a glycine (G14), which is structurally more flexible than Pin2, it also has reduced haemolytic activity. To perform these experiments two strains of *Pseudomona* were used ATCC y DFU3. The last one was isolated from a patient with diabetic foot ulcer, and identified by morphofology at microscope and in selective and differential media, API Pseudomona and Chromoagar Pseudomona, this strain was kept under -20 ^{o}C. These strains are going to be used in Antibiogram assays with the commercial antibiotics.

Protein engineering with immunoglobulin-like fold

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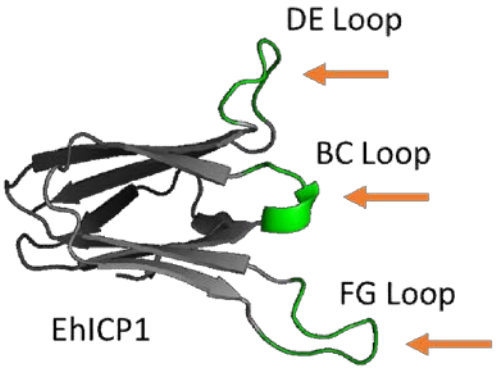
Protein engineering is a science that tries to customize the function of proteins by mutating their genes. In the last years, with the increase in the amount of data in protein structural information databases, the major work in the field of protein engineering have been focused in one single question, how structure determines function? Our understanding of the mechanisms of molecular recognition have allowed us to create new predicted recognition sites on some proteins, such proteins are defined as scaffolds and they come with a certain structural features. In recent decades significant efforts have attempted to establish multiple platforms for the generation of specific binding proteins, all of them obtained by protein engineering, this strategies can be used as alternatives to traditional monoclonal antibodies in both medical and industrial applications (Gebauer and Skerra, 2009; Löfblom, and. Frejd, 2011).



In this work,the ability of accept new peptide sequences (directed to a target protein) in each loop of the inhibitor of cysteine protease 1 of *Entamoeba histolytica* (EhICP1) was evaluated, as shown in the figure. In our group we have found that the EhIPC1 has a stable Immunoglobulin-like fold. Based on a structural analysis that we performed, mutants designed to bind to a specific protein (which is also a cancer biomarker)were generated.

Our results show that the EhICP1 may be a viable option for the design of new proteins with predicted specificities compared to others protein scaffolds, according to the information obtained in this work, the three loops of EhICP1 are likely to be mutated by paratopes against target proteins and also keep its fold. Furthermore the analysis of protein-protein interaction confirmed the complex formation between some mutants and the target protein.

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Generation of recombinant strains of *Bacillus thuringiensis* that synthesize the endochitinase ChiA74 and mosquitocidal Cry proteins

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Bacillus thuringiensis is an insecticidal bacterium used for the control of lepidopteran, coleopteran and dipteran larvae of importance in agriculture and public health. Recently, chitinases have been used as sinergystic elements of the insecticidal activity of the Cry proteins, but most of the studies have been focused to control lepidopteran and there are little reports about the dipteran larvae control (Ramírez-Suero et al., 2011). In particular, the sinergystic effect of chitinase with Cry proteins have been tested using extracellular enzymes and there is not report about the development of mosquitocidal strains of *B. thuringiensis* expressing chitinases as inclusion bodies (Barboza-Corona et al., 2014). In this work, we developed two mosquitocidal recombinant strains able to synthesize the endochitinase ChiA74 as inclusion bodies. The first strain synthesizes both the Cry19A protein (60 kDa) and the endochitinase ChiA74Δsp (*B. thuringiensis* 4Q7-*chiA74Δsp-cry19A-orf2-pSTAB*), whose expression was controlled by the pcytA-STAB system. The second strain is a recombinant *B. thuringiensis israelensis* transformed with the *chiA74Δsp* under the control of its wild promoter and transcriptional terminator (*B. thuringiensis israelensis-chiA74*Δsp-pHT3101). In both recombinant strains it was observed an increment in the endochitinase activity and sporulation but there was not effect on the crystal sizes compared with the wild and nontransformed bacteria. With zimograms, it was confirmed that both strains produce the endochitinase ChiA74 with molecular mass of ~70 kDa, In particular, *B. thuringiensis* 4Q7-*chiA74Δsp-cry19A-orf2-pSTAB* showed an endochitinase activity of ~ 119 mU/mL, ~ 11 x10^7 spores/mL and crystal area of ~ 2.45 μm^2, whereas the nontransformed strain (*B. thuringiensis* 4Q7-*cry19A-orf2-pSTAB*) showed ~ 3.7 mU/mL, 7.5 x10^7 spores/mL and ~ 2 μm^2, respectively. Alternatively, *B. thuringiensis israelensis-chiA74*Δsp-pHT3101 had an increment in the endochitinase activity of 4 times compared with the wild strain, and an augment in the sporulation of 2 times. Data show here indicated that recombinant strains have the potential to increase the mosquitocidal activity of *B. thuringiensis,* but it requires confirmation through bioassays.

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Identification and cDNA cloning of a hyaluronidase from theskin secretion of *Trachycephalustyphonius*

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Key words: amphibians, skin secretion, biological activities,cloning,hyaluronidase

The skin of amphibians plays an important role in their protection against predators and harmful abiotic factors. Consequently, the skin of amphibians contains bioactive components such biogenic amines, steroids, alkaloids, peptides and proteins with interesting biological activities to safeguard them [1].Some of these peptides and proteins contain antimicrobial, analgesic, cytotoxic and lytic functions, among others [2].In this work, we describe the identificationof a hyaluronidasefrom the skin secretion of the frog *Trachycephalustyphonius,* which were captured in the state of Tamaulipas, México. The skin secretions were extract by manual stimulation. The identification of the hyaluronidase was performed using zymograms in non-denaturing SDS-PAGE conditions in the presence of hyaluronic acid.The enzyme activity was measured by TRU and it was similar to that found in the venomof tarantulas. The skin secretions were also liofilized, resupended and separated by reverse phase chromatography to purify the hyaluronidase for N-terminal analysis and further biochemicalcharacterization.To obtain the full primary structure of the enzyme, the total RNA from the skin of *T.typhonius*was extracted from a clean-cut of a secretory gland,and the mRNA were used to construct a cDNA library. To identify the hyalurodinasetranscript a PCR amplification using the 3´RACE protocol was performed. For that, two oligonucleotideswere designed from the alignmentof hyalurodinases from embryonic amphibians, arachnid venoms,insecthemolymphand humans. The most conserved regions wereconsidered. The transcript wasamplified and ligated in TOPO 2.1.*Trachycephalustyphonius* skin secretion contains a highly active hyaluronidase, and it can be an interesting candidate for medicine.

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Kinetics of fatty acids accumulation in *Chlorella vulgaris* jointly immobilized with *Azospirillum brasilense* at three temperatures.

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Abstract

The *Chlorella* and *Azosprillum* jointly immobilized system has been proposed as model to study the prokaryote-eukaryote interactions. A lot of studies have been carried out with this system in several research subjects (de Bashan and Bashan, 2008). This work had as the main objective quantified and identified the main fatty acids during the interaction at three temperatures.

Methods

Fatty acids were quantified and identified using GC-MS (Sato and Murata, 1988).

Results

Main fatty acids found were 16:0, 16:1, 16:3, 18:1, 18:2 and 18:3. The behavior of the kinetics is variable, however is possible see that the temperature and the presence of the bacteria exert a positive effect. Previous works in our group show that the fatty acids accumulation is directly related to de AcetylCoA Carboxylase activity.

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Proteomic approach to the early stages of pozol fermentation

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Key words: Proteomics, fermentation, pozol

Pozol is a mexican traditional beverage elaborated and consumed by various ethnic groups in the southeastern of Mexico. Pozol is obtained from the natural fermentation of nixtamal (heat- and alkali-treated maize) dough. The main compound in maize dough is starch (72-73%). Other carbohydrates such as sucrose, glucose, fructose and maltose are in smaller proportion and are mostly lost during nixtamalization, so starch remains as the carbohydrate available for fermentation (1). A wide variety of microorganisms have already been isolated from this spontaneous fermentation; these microorganisms include fungi, yeasts, lactic acid bacteria, and non-lactic acid bacteria (2). However, only few bacteria are amylolytic and all of them weakly amylolytic, when compared with other bacteria (1). In an attempt to explain how a very low content of sugars can support a diverse and abundant microbiota, a proteomic approach was designed by our research group to understand this fermentation (3). It was observed that the highest variation of pH in pozol fermentation occurs in the first 24 hours. After this time the pH of the fermentation is constant, for this reason we believe that the early stages of pozol fermentation have an important and interesting metabolic activity for study. On the basis of the aforementioned reasons, our principal objective is to use proteomic technology to identify proteins involved in the consumption of different carbon sources, in early stages of pozol fermentation. To develop a comprehensive proteomic approach to the early stages of pozol fermentation, we obtained samples of pozol from Villahermosa local market. The samples were taken every 3 hours and immediately cooled to stop the fermentation process. Protein extraction was done by a method developed specially for this starchy fermentation (3). After that, the samples corresponding to 0, 9, 24 and 48 hours were selected for the proteomic study. This analysis is currently underway.

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Relationship between the increase in the oxidative state and the improvement of infectivity in the *Isariafumosorosea*conidia

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Key words: entompathogenic fungi, infectivity, superoxide anion, general oxidative stress.

Introduction. Approximately1000 species of insects are considered pests[1]. Natural enemies of this insects are used in biological control, among whichare the entomopathogenic fungi (EF) as*Isariafumosorosea*. The infectivity of the EF towards target organisms, is one of the most important features for their selection and application in open fields.

Metodology. Two strains of *Isariafumosorosea* (CNRCB1 and ARSEF3302) were exposed to two different concentrations of O_2: normalwith 21% (**NA**), and the other one, enriched with 26% (**26**)[2].The infectivity of the conidia produced in both treatments was determined, as well as the degree of general oxidative stress[3,4]and the superoxide concentration[5].

Results. Treatment **26,** cause an increase in infectivity of the conidia of both strains of *I. fumosorosea*as the final survival value (S) of the *G. mellonella* larvae was significantly lower than the value obtained with the conidia exposed to the **NA** (Table 1).

Table. 1. Infectivity test of the two strains of *I. fumosorosea*. Final survival value (S, %) of *G. mellonella*larvae is shown. Asterisks denote statistically significant differences between treatments in each strain studied (t Student, α= 0.05).

Strain	Treatment	S (%)
I. fumosorosea **CNRCB1**	**NA**	35 ± 10*
	26	0 ± 0
I. fumosorosea **ARSEF3302**	**NA**	80 ± 0*
	26	55 ± 10

Similarly, the treatment **26** increased both the degree of general oxidative stress[4] and in the superoxide concentration in the conidia,in the twostudied strains (Fig. 1).This is in agreement to the fact that an increase in ROS, in the entomopathogenic fungus *I. fumosorosea*, cause arise in the antioxidant enzyme catalase activity[6]. Similarly, this enzymatic activity has been associated with an infectivity increase in othersentomopathogenicfungi, including *Metarhizium anisopliae*[7] and *Beauveria bassiana*[8].

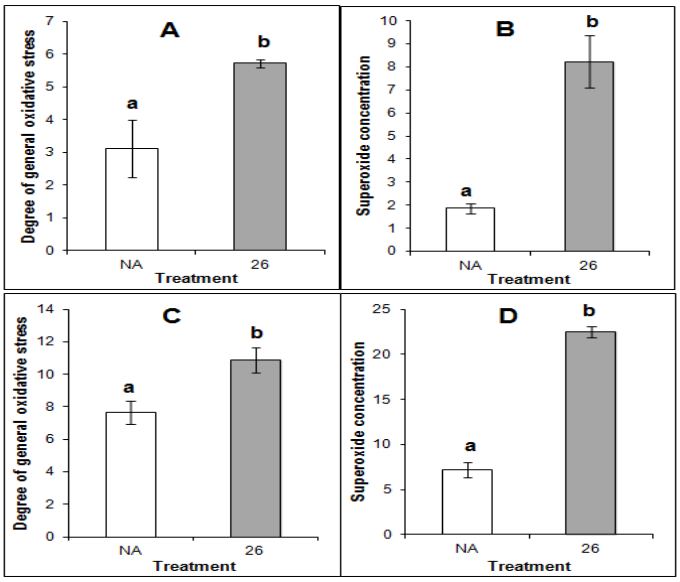

Figure1. Quantifying of the degree of general oxidative stress and concentration of the superoxide anion in the conidia of two strains of *I. fumosorosea:* CNRCB1 (A and B) and ARSEF 3302 (C and D). Different letters indicate significant differences between treatments (t Student, α= 0.05).

Conclusions. The treatment with an oxygen-rich atmosphere improved the infectivity of two strains conidia of *I. fumosorosea* to *G. mellonella* larvae. This is associated with the increase of the oxidative state of conidia.

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Identification of a peroxidase related to post-harvest insect resistance of maize (*Zea mays* L) by activity-directed proteomics

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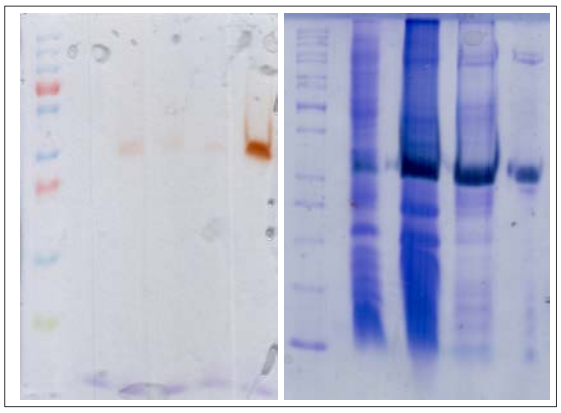
Maize (*Zea mays* L). is one of the most important crops for human nutrition and animal feedstock. Mexico has been considered as the origin and diversification center of this cereal. This is reflected by many varieties, which are still cultivated by so-called smallholders, i.e. farmers with limited access to land and production resources.

Since maize is also used now as raw material for 'bio'-fuel generation, the production of maize cannot keep pace with the increasing demand. This leads to raising prices, which affects net-importers of this basic food, such as Mexico. Reviewing the product chain reveals that almost 40% of the worldwide maize production is lost due to biotic and abiotic factors, before and after the crop growing. One of the main causes of post-harvest losses in tropical and sub-tropical areas is the attack by insects, mainly of *Prostephanus truncatus, Sitophilus zeamais* and *Sitotroga cerealella*. Thus, reducing these post-harvest losses, could significantly improve the availability of maize in disadvantaged regions.

It was found that the phenolic compounds and peroxidase activity contribute significantly to the resistance of maize grains against insect pests. Therefore, we developed an activity-directed proteomics approach for identifying peroxidases, which are correlating with insect resistance.



Studying the highly insect-resistant *Z. mays* variety p84 c3, we found that more than 90% of the total peroxidase activity was provided by a single isoenzyme. Combining classic protein purification with peroxidase-activity directed proteomics, we could identify a protein with similarity to B6T173_MAIZE (or ZmPrx35), a class III peroxidase. Isolating the cDNA of this protein from *Z. mays* p84 c3 kernels and recombinant production of this protein in *Escherichia coli* confirmed the POD activity of this novel enzyme involved in insect resistance of maize. Our findings could contribute to the screening for insect resistant maize varieties and support marker-assisted breeding.

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Hydrolytic enzymes of membrane and cell wall assay secreted by endophytic bacteria as an antifungal factor against *Mycosphaerella fijiensis*

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Mycosphaerella fijiensis is a fungus that produces Black sigatoka in banana plants making economic losses in a 100% of commercial banana (*Musa acuminata* Cavendish) production around the world, besides the fungus also have an impact in other banana varieties that have economic importance. Black sigatoka is a strictly foliar disease, which inhibits the photosynthetic capacity of the plant producing a premature ripening and a loss of weight of the fruit. Nowadays, chemical control (fungicides) is the alternative to combat this plague, but it is not the most effective treatment, causing an annual cost of over 500 millions of pesos, besides this method increases the resistant strains and causes an ecologic damage. New control alternatives are emerging, and biological control seems to be the best option.

In this research we analyzed antifungal factors (membrane and cell wall hydrolityc enzymes) produced by *Pseudomas aeruginosa, Enterobacter cloacae, Enteorcoccus faecalis* and *Bacillus cereus* -banana endophytic bacteria- they were selected by its highly antifungal effects in previous works, using an inactive mycelium of *M. fijiensis as a* culture medium. The results showed an 80% of fungal inhibition when the supernatant from bacteria grown in a fungal cell wall medium was used, also we observed enzyme activity of 119.7 U/min of glucanase, 8.7 U/min chitinase, 37.87 U/min protease and 61.85 U/min lipase in the supernatant of these bacterias.

A microscopic hyphae assay was performed using commercial chitinase, the supernatant obtain from *P. aeruginosa* and the active bacteria (*P. aeruginosa)* employing three dying treatments. The results showed mechanical damage in the hyphae when used 1U of commercial chitinase after 24 hours incubation. Also when *Pseudomas* was cultured with active mycelium of *M. fijiensis*, we observed a biofilm formation after 24 hours of incubation and a severe mechanical damage after 48 hours incubation.

This research may contribute in the development of a possible biofungicide formulation in the combat of Black sigatoka.

Influence of Rho1 in the expression of *lcc2*, *lcc3* and *lcc5* in strains of *F. oxysporum* f. sp. *lycopersici*

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Fusarium oxysporum f. sp. *lycopersici* is a fungus fitopathogen that causes large economic losses in the tomato crops. This fungus possesses a complex enzymatic mechanism that allows it to infect to tomato plants. Rho1p is a protein involved in the biogenesis of the cellular walls through the regulation of the activity of the β-1, 3-glucan synthase (GS) as well as the organization of the actin cytoskeleton and polarized secretion.

In this work, we analyzed the expression of the genes *lccl, lcc2, lcc3, lcc4, lcc5* and *lcc9* in the wild-type 4287 and mutant strains *rhol::hyg* (non-pathogenic strain) and *rho1::hyg+rho1*^{G14V} (pathogenic strain) of *F. oxysporum* f. sp. *lycopersici*. For the analysis of the genes, were inoculated fresh spores on PDB medium for 21 hours at 27°C and 220 rpm. After of that period, the germlings obtained were transferred to the induction medium with sucrose at 0.25% as carbon source. The strains were incubated for 3 days at 27°C and 220 rpm. The breaking of the micelium was obtained by sonication, afterwards, the samples were centrifuged at 2000 rpm for 5 minutes at 4°C. The tablet was discarded and the supernatant (enzymatic extract) was used to measure the enzymatic activity also the quantification of proteins. In order to measure the enzymatic activity of laccases, we used as substrate the chromogenic agent 2, 2' – azino bis (3-ethylbenzthiazoline-6 sulfonic acid, ABTS Sigma).

Under the conditions applied in this work, we observed an expression similar to the genes *lccl* and *lcc9* in the strains of *F. oxysporum*, whereas the gene *lcc4* did not show any expression. The genes *lcc2, lcc3, lcc5* and *xyl3* showed an expression different in the three strains of *F. oxysporum*. Additionally, we analyzed the specific activity of laccases, observing a similar activity in the mutant strains *rhol::hyg* and *rho1::hyg+rho1*^{G14V}, these activities being larger in comparison with the wild-type strain 4287. According to the obtained results, it is suggested that the state of the protein Rho1 could have an influence in the regulation of *lcc2, lcc3* and *lcc5* at a translational and/or transcript level.

Application of marine yeast for biological control of the phytopathogenic fungus *Aspergillus flavus*on post-harvested *P. vulgaris* L.

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Key words:*Phaseolus vulgaris L.,Debaryomyces hansenii*, biological control

Introduction

During the last years, the most important problems facing the snap bean (*P. vulgaris* L.) agroindustryare spoiling problems associated with postharvest aspergillosis caused by *Aspergillus flavus*(1)responsible for important economic losses on the legume industry worldwide. Biological control using microbial antagonists has become a good alternative.

Methodology

Biological control of postharvest spoilage of snap beans with *A. flavus*using *D. hanseniiw*as evaluated at different post-treatment times. *In vitro* fungal growth inhibitionon PDA agar plates was done at 29°C, 85% humidity for 7 days.Fungal growth was addressed with standard mycological methods;grown diameter was measured every day and confirmed with specific molecular probes. *In vivo*fungal spoilage of snap beans and fungal growth inhibition assays with *D. hanseniiw*ereevaluatedin a biosecured climate chamber at 29°C, 85% humidity for 7-15 days.Fungal growth inhibition and marine yeast colonization of snap beans was also monitored by scanning electron microscopy.

Resultsand discussion

Symptoms of Aspergillosis on snap beans occurred seven days after they had shown spoilage at 29^{o}C, 85% humidity.*A. flavus* isolated from infected snap beans had macroscopic characteristics similar to the first inoculated fungus, and it reinfected fresh snap beans.*In vitro* antagonism of *D. hansenii* showed 80% inhibition on PDA agar plates (P <0.05) whereas spore germination was inhibited since the first 6-h yeast-fungi antagonism assay (P <0.05).Aspergillosis symptoms were not detected on snap beans previously treated with *D. hansenii*; however,snap beans treated with dextrose and yeast showed a 20%spoilage incidence (P <0.05) whereas those treated with dextrose plus surfactant and yeast had 60% disease incidence (P <0.05). *D. hansenii* colonization on snap beans is achieved with a yeast suspension of 1x10^{6}cfuas revealed by scanning electron microscopy. Biofilm formation was observed in snap beans where the disease had not progressed, which suggeststhe possibility to use *D. hansenii* as possible biocontrol agent to prevent fungus spoilage ofpost-harvested vegetables.

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Synthetic production of a Teparybean lectin: generation of the genetic construction and insertion into*Agrobacterium tumefaciens*

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Several studies report that some plant lectins have differential cytotoxic activity against cancer cells. Particularly, a Tepary bean(*Phaseolusacutifolius*) lectin fractionhas showed cytotoxic effects on human cancer cell lines. However, these molecules are not currently used as therapeutic agents because, in part,the high costs, time consuming and low yields of the conventional isolation and purificationprocesses. The heterologous production oflectins from simpler organisms has not been a viable alternative because glycosylation patterns are subjected to the enzymatic machinery of each cell type. Therefore, this work aims to express and purify a syntheticTepary bean lectinby genetic modification of Tepary bean plants with a new strategy that will preserve the native glycosylation pattern and thus, their differential cytotoxic activity. For this, a gene construct composed byTepary bean lectinthat engages a rizo-secretion signal peptide and a hexa-histidinetale in the coding region was produced. This genetic material was inserted into bean plants via *Agrobacterium tumefaciens*. Currently,*in vitro* reconstitution of genetic modified plants is in progress. Upon reaching their development, plants will be grown in hydroponics and the product obtained in root exudates will be separated by affinity chromatography in order to purify the lectin, perform the biochemical characterization and assess its cytotoxic effect on cancer cell lines.

Key words: Genetic construction, lectins, Tepary bean, *Phaseolus acutifolius.*

Characterization of biologically active cyclic dipeptides from bacterial isolates originating in pools of CuatroCiénegas, Coahuila.

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The CuatroCiénegas basin in México has been described as an important biodiversity reservoir within the Chihuahua desert. The area is a small (<840 km^2) intermountain valley where different water systems are located. Most of the aquatic habitats are not permanent. Between locations, environmental conditions can vary in water chemistry, flow rate and the volume of spring discharge. Moreover, most aquatic habitats in this area are extremely oligotrophic due to the almost negligible phosphorous levels. While some studies have sought to describe and characterize the diversity of prokaryotes in the basin, little has been to understand and interpret how the populations are related, if exist communication between them, through which molecular mechanisms performed
competition for nutrients, or mechanisms used to control microbial populations. Thus, the study of the mechanisms that bacteria use to compete by resources in this environment has ecological and evolutionary interest and could lead to relevant biotechnological discoveries. On the other hand, cyclic dipeptides (CDPs) also known as 2,5-diketopiperazines (DKPs) are relatively simple compounds. The ability of microorganisms to produce diketopiperazines is widespread and published data have shown that about 90% of Gram-negative bacteria produce them. Diketopiperazines have also isolated from Gram-positive bacteria, fungi and higher marine organisms. Biosynthesis of CDPs can be achieved by dedicated nonribosomal peptide synthetases (NRPS). Recently was discovered a new biosynthetic route involving a named cyclodipepdide synthases. Important biological activities of diketopiperazines are antibacterial, antifungal, antitumour, antiviral. In addition, DKPsare able to activate or antagonizeLuxR-mediated quorum-sensing systems of bacteria, and considered to influence cell-cell signaling. In our work we evaluated the production and biological activity of CDPs of bacterial isolates originating in pools of CuatroCiénegas, Coahuila with the purpose of know if thesemolecules have influence in the control of bacterial populations.We evaluated bacterial isolates using competition assays and selecting those isolates with ability to inhibit the growth of other bacterial isolates.For the selected isolates was carried out extractions with ethyl acetate solvent and then analyzed by HPLC and GC-MS for compounds identification.The ability of the extracts to inhibit the growth of bacterial isolates was tested, and results were compared with data obtained of the HPLC and GC-MS analyses. The presence of CDPsand also the
2,4-diacetylphloroglucinol (2,4-DAPG) was confirmed in the culture mediums. We propose that CDPsand 2,4-DAPG areinvolved in microbial population controloccurring in CuatroCiénegas pools.

Alkaloid synthesis during seedling development in *Argemone mexicana* L

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Argemone mexicana L (Papaveraceae) is known aschicalote or Mexican prickly poppy. It has been used in the traditional Mexican medicine for different purposes. This plant can produce benzylisoquinoline alkaloids (BIA) from the benzophenanthridine (sanguinarine and chelerythrine) and protoberberine (berberine) groups. These alkaloids have been identified in plants from the superorders Magnoliflorae and Ranunculiflorae, but in few instances occur in the same species, such as in *A. mexicana*.In adult plants, sanguinarine is found exclusively in the roots and seeds, whereas berberine is distributed throughout the entire plant. During seedling development,sanguinarine could be detected in radicles, even prior to cotyledon emergence. Berberine accumulation requires the formation of cotyledons, even when it is not detected in them and remains restricted to radicles. These data suggest the participation of aerial tissues in the synthesis, even though they are not involved in the accumulation in these early phases of development. Upon appearance of the first pair of true leaves, berberine distribution reaches the aerial parts. The expression profiles of eight genes involved in the early, common biosynthetic reactions for these alkaloids, as well as in the specific ones leading to sanguinarine and berberine, were analyzed in aerial and underground tissues of developing seedlings of *A. mexicana*. Results are discussed considering the possible participation of such tissues in the biosynthetic process, considering the occurrence of transport mechanisms.

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Analysis of *Trichoderma atroviride* –plant interaction with fungal strains that overexpress the *SwoI* gene.

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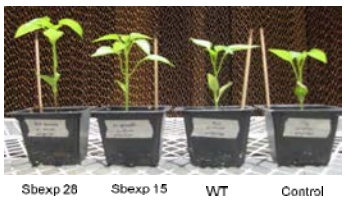
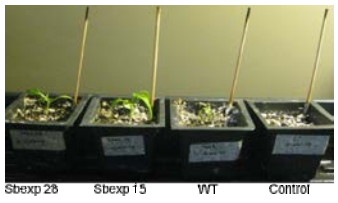
Trichoderma spp. are free-living fungi that are common in soil and root ecosystems. They are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonization of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. Colonization of plant roots by *Trichoderma* can protect plants against diseases and environmental stresses such as salinity and drought, and an improve plant growth and development (Brotman 2013). Swollenin, a protein first characterized in the saprophytic fungus *T. reesei*, among other characteristic, the swollenin contains expansin-like domain (Saloheimo, et al 2002). *SwoI gene* in *T. atroviride* has been also reported and is similar to the swollenin of *T. reesei.* These proteins have been involved in cell wall remodeling, which is a process that *Trichoderma* has to face in order to colonize the root system of plants. Brotman, et. al 2008 observed overexpression of swollenin showed a remarkably increased ability to colonize cucumber roots and capable of stimulating local defense responses.

To better understand the mechanism underlining the plant-*Trichoderma* interaction we followed changes in plant growth and colonization of *Capsicum* roots and measured abiotic stress tolerance mediated by *Trichoderma* strains that overexpress the *SwoI* gene. Our results suggest that swollenin plays a major role in root colonization since plants inoculated with the overexpressor strains produce more biomass than those inoculated with the wild type strain. These plants also endured better cold-stress. This study represents an important step forward in understanding the nature of the non-pathogenic plant *Trichoderma* interaction, and may contribute to the efforts to improve *Trichoderma* biocontrol abilities.



Plant growth with overexpresion and wild type strain



cold stress tolerance of plant with overexpresion and wild type

Key words: Root colonization, Swollenin, Defence mechanism of plant, *T.atroviride*

Generation and characterization of truncated versions of MelAtyrosinase from *Rhizobium etli*expressed in *Escherichia coli*

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Tyrosinases are copper-containing enzymes involved in melanin synthesisfound and described for many phylogenetic groups [1] including *R. etli*, although little information has been generated for the latter. The *melA* gene from *R. etli* CFN42 was cloned in *E. coli*resulting in a 609 amino acid MelA protein which was found to have tyrosinase activity using L-tyrosine as substrate[2, 3].A variant (*melA*c) was generated from the *melA*gene being 106 amino acids shorter from the amino terminus which retained melanin production capability[4].Computational analysis of *melA*c rendered a possible signal peptide (31 amino acids) at the start of this truncated MelA version which has not been confirmed for functionality. This arises the possibility to generate, identify and characterize new truncated mutants capable of retaining activity and thus revealing certain important structure-function relationships for this enzyme.In this report, a mutagenic approach was employed to construct a mutant library and to identify the minimal MelA sequence encoding for a protein with tyrosinase activity.The methodused for the library construction was a PCR primer codon deletion with designed oligonucleotides,developed at our institute (unpublished method). This technique enabled us to generate a library of sequential amino-and carboxy-terminus MelA truncated mutants for16amino acids from the amino terminusand 16 amino acids from the carboxyl terminus (Figure 1).The first step taken in the construction of the library was deleting the 31 aminoacids for the proposed signal peptide.Afterwards, 1000 colonies of the library of MelAtruncated sequences wasscreened for activity in *E. coli* MC1061, growing in solid medium supplemented with tyrosine for melanin production and 26 mutants were randomly selected and sequenced. Five colonies were found to retained tyrosinase activity. Tyrosinase activity of the screened mutants was also measured from cell extracts of *E. coli* clones expressing truncated versions of MelA.

The molecular, biochemical and functional characterization performing in this work will be useful to increase the knowledge of this protein.

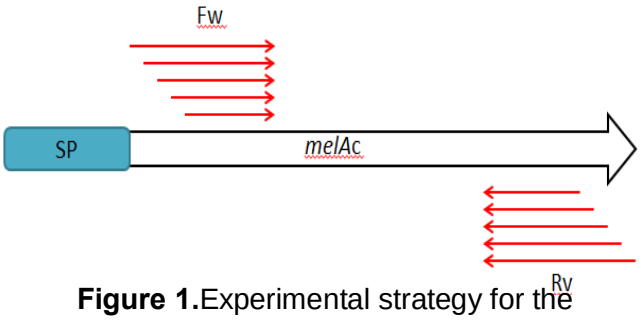

Figure 1. Experimental strategy for the construction of the library of truncated versions of *melA* gene.

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The physicochemical characterization and antioxidant potential of*Bromeliakaratas* fruits

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Bromeliakaratas L. or cocuixtle (Xocuixtle: xocolt=sweet and sour, ixtle=fiber), “piñuela” and “guamara”, a monocotiledonea plant has little purple fruits like banana with sweetand sour taste, high in fiber and a diabetic sugar quality (Casillas, 2010). From some *Bromelia*species cysteine protease was recently characterized (Valles*et al.,* 2007) and endopeptidases (Pardo*et al.,* 2000). The high concentration of antioxidant metabolites in the*Bromelia* genus fruits could be compared with BTH and ascorbic acidantioxidant effects (Moyano*et al.,* 2012). The main of this work was the physicochemical characterization and antioxidant potential of *B. karatas* fruits. The collected fruits were used to determine fruit size, seed numbers, pulp and peel weigh.

For chemical characterization water and ethanol extraction of pulp and peel were used. The total phenolics content was determined by Folin-Ciocalteu method using ethanolic extracts (Gutiérrez*et al.,* 2008).The potential antioxidant activity was determined using lyophilized ethanolic extracts verified through two spectrophotometric models in vitro: The reduction in DPPH (2,2-difenyl-1-picrylhydrazyl) (Brand-Williams *et al.,* 1995) and ABTScation(2,2-azinobis-3-etilbenzotioazolin-6-sulfonic) (Re *et al.,*1999). Additionally, using TLC carbohydrates were characterized. Therefore, using 50 fruits, the average seed and total fruit weigh were 2.7 g and 19.04 g respectively. The fruit size average was 5 cm long and 2.5 cm width. Carbohydrates TLC represented important sugars in pulp and peel, these corresponded with the sweet pulp taste, and quality carbohydrates composition. Phenols and flavonolsconcentration was higher in peel than pulp; this probably corresponding to peel color. However, after 30 days the pulps of ripening fruits had more phenols and flavonols. High antioxidant activity has been observed in fruit pulp extract.The cocuixtle fruits are a high source of antioxidants and they could be a potential use in biomedicine.

Metabolic characterization of tomato plants using low-temperature plasma (LTP) as a new ambient ionization technique

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For the metabolic characterization of plants, mass spectrometry (MS) is one of the most powerful techniques due to selectivity, sensitivity and identification capabilities. Additionally, it is a valuable analytical tool for untargeted metabolic fingerprinting [1].

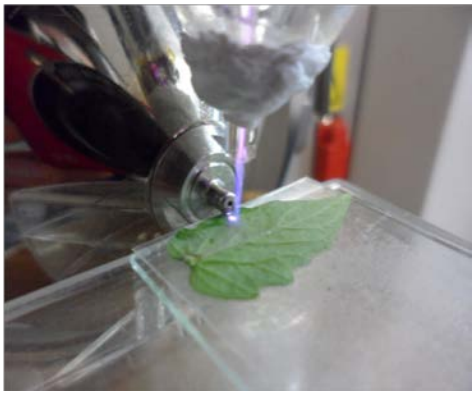
In the last decade, ambient mass spectrometry has attracted increasing interest. Various ionization sources can operate under ambient pressure and temperature, which allows the direct analysis of metabolites from plant tissues [2].

In our Laboratory of Biochemical and Instrumental Analysis we constructed a novel ambient ionization source based on low-temperature plasma (LTP). Using the LTP source, we detected different classes of pure compounds (alkaloids, volatiles, amino acids, terpenes and others). Recently, we demonstrated the generation of molecular images from plant leaves and fruits. Interestingly, LTP ionization permits the detection of volatile and semi-volatile compounds, which are difficult to analyze by conventional methods, such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI).

However, an efficient evaluation of potential of LTP in the study of plant metabolism is still lacking. Therefore, we decided to study the metabolic fingerprints and 2D images generation of different genotypes of tomato (*Solanum lycopersicum*) plants by LTP-MS.

The above genotypes are characterized by changes in the metabolic pathways related to the systemin wound response pathway associated to jasmonic acid (JA) [3,4]: 1) suppressor of prosystemin-mediated response (*spr2*) mutant plant, deficient in the jasmonic acid (JA) synthesis and emission of volatile organic compounds; 2) a prosystemin over-expressing plant (*35S::PS*). The mutants will be compared with their cv. Castlemart genetic background.

Preliminary results demonstrate that the different genotypes can be distinguished by their metabolic fingerprint. Statistical analysis indicate that the mutant plants generate changes in particular metabolic pathways.



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ELABORATION OF FERMENTED FRUIT BEVERAGES

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INTRODUCTION. Fermented foods have been produced since remote times through the transformation of substrates (edible or not). A great variety of fermented foods are produced in Mexico since prehispanic times, Mexico has used its natural resources to the development of fermented foods and beverages, which vary according to geographic area, cultural preferences, and the particular characteristics that distinguish them, resulting in a wide biodiversity of these fermented beverages (Steinkraus, 1996). Among the various sources that have great potential for industrial applications for the production of fermented foods are sugarcane, malt, grape and agave, which can get beer, mezcal, tequila, white and red wine.During fermentation several microorganism transform sugars such as sucrose, glucose and fructose in ethyl alcohol.
In this work, we take two fruits for making a fermented beverage: the passion fruit and mango, (*Passiflora edulis y Mangifera indica)*, due to its nutritional characteristics and high sugar content.

METHODOLOGY. The juice was prepared with fruit (passion fruit or mango) and water at boiling temperature for 30 min, pH (3.6-4) was adjusted with sodium bicarbonate and sucrose were added to 200 g/L. Subsequently, *Saccharomyces cerevisiae* was activated and added to the juice (1 g/L), for the fermentation starting with a temperature of 30 °C for 5 days under aseptic conditions. After the % ethanol was determined by fractional distillation

RESULTS. Fermented beverages mango and passion fruit with a light orange appearance, smooth consistency, alcoholic odor was obtained. The passion fruit fermented beverage provided 18.24% ethanol and mango drink 18.4% ethanol.

CONCLUSIONS. According to the NOM-142-SSA1-1995 of alcoholic beverages, the % alcohol wines and fortified wines is 10-15% and 10-20%, respectively. The data obtained according to the fractional distillation are within the limits set by that rule us. In this work, we prepared a novel fermented beverage from passion fruit and mango, using their nutritional properties.

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Expression and preliminary characterization of amarantin acidic subunit and amarantin acidic subunit modified with bioactive peptides

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Abstract

In this research was carried out overexpression of acidic amaranth subunit (ACM3) and acidic amaranth subunit modified in its third variable region by bioactive peptide VYVYVYVY and C-terminal region by bioactive peptide IPP (ACM3.3.4). Expression of recombinant proteins were carried out by *E. coli* BL21-CodonPlus(DE3)-RIL at fermenter level using lactose like inducer. The best productivity of the recombinant proteins was attained at 6 h after induction. The yields achieved for ACM3 and ACM3.3.4 were 1.88 g/L and 1.16 g/L, respectively. Analysis by SDS-PAGE gels shows that recombinant proteins were expressed as inclusion bodies. Therefore, both proteins were solubilized with 6 M urea and purified byInmobilyzed Metal Affinity Chromatography (IMAC) and through refolding protocol the proteins were refolded in refolding buffer (0.02 M phosphates pH 7.5, 0.2 M NaCl) by dialysis process to carry out characterization of their structure by Circular Dichroism (CD) and fluorescence. Also, isoelectricpoint (pI) was determined in both proteins by isoelectric focusing (IEF). Characterization of these proteins is with the goal of establishing criteria to make way for its use in the formulation of food and/or its application as additives

Isolation of lactic acid bacteria which produce bacteriocins from ranchero cheese.

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Bacteriocins are antimicrobial peptides of ribosomal synthesis, which are produced by wide kind of microorganisms, mainly lactic acid bacteria. The main mechanisms vary and may include pore formation, degradation of cellular DNA and inhibition of peptidoglycan synthesis. Bacteriocin producing bacteria have been isolated from vegetables, fish, meat, and dairy food.

Ranchero cheese is consumed fresh and is elaborated from raw milk of cow. With the aim to curdle the milk, chunks of veal curd or liquid curd are employed (mix of enzymes).

Due to this process, ranchero cheese has been considered as a genuine Mexican cheese. Nowadays there is a great interest in the study of the biota associated with regional products in order to produce the same products to the industry and to explode the biotechnological potential of the biota. The aim of this work was isolate and identify lactic acid bacteria which produce bacteriocins that inhibit the growth of food-associated pathogens.

128 strains were isolated from ranchero cheese from Boca del Río, Veracruz. The isolates were tested forinhibiting the growth of 4 pathogens: *Listeria monocytogenes*, *Staphylococcus aureus, Salmonella enterica* subsp. entericaserovarTyphimurium and*Brucellaabortus.* Only61 isolates presented inhibitory activity in the spot on lawn assay against at least 1 pathogen strain. Agar well diffusion assay was used as confirmatory assay, we observed that the supernatant of only 1 isolate showed inhibitory activity. This isolate was identified by biochemical and molecular tests as *Enterococcus faecalis*QR10, and it inhibited the 4 pathogens mentioned before. The supernatant was treated with proteolytic enzymes and it lost inhibitory activity, in this way we demonstrate that the inhibitor factor has protein origin.

Isolation and characterization of fungi for the biocontrol of *Phytophthora capsiciL*. and their protective effect in *Capsicum annuum L*. plants.

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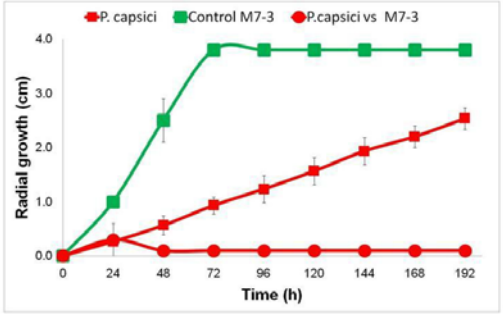
The cultivation of pepper (*Capsicum annuum L*.) in the state of Zacatecas is one of its most important socio-economical activities. In the period of 2010-2012, 309 612 tons of pepper were produced, with a value of production of around 2.16 billion of Mexican pesos, on average (SAGARPA, SIAP). Also, Zacatecas is the main producer of dried pepper in Mexico. However, pepper cultivation is affected by different phytopathogen fungi, such as *Phytophthora capsiciL.* This oomycete is the causal agent of pepper plant blight, one of the most destructive diseases that attacksthe roots, stems, leaves, and fruitsof the pepper plant, causinglosses of 60 to 90% the cultivated area. In order to control this phytopathogen, high amounts unspecific and toxic fungicides are applied to the field, increasing the costs of cultivation and resulting in a risingwidespread fungicide-resistance. An economically and environmental friendly alternative to fungicides is the use of some bacteria and fungi as biocontrol agents. Biocontrol agents can antagonize the growth of the phytopathogen by restricting the availability of space or nutrients, also they can activate the plant defense mechanisms against the phytopathogen. This work was aimed to isolate fungi with biocontrol activity against *P. capsici L*. and evaluate their protective effect in *C. annuum L.* plants. Pure culture of fungi strains were obtained from samples of farming soils, mining-derived soils, and their growing plants (rhizosphere, roots and leaves), all collected in the state of Zacatecas. 75 fungal strains were confronted with *P. capsici L.* on PDA medium to obtain the kinetics of growth-inhibition. 20 fungal strains caused inhibition from 75 to 100% of *P. capsici* growth. Some of these fungal strains inhibited the phytopathogen growth as early as from the 3rd day of cultivation (Fig. 1 and Graph 1). *In-vitro*cultivation of seedling of *C. annuum*inoculated with *P. capsici*caused blight symptoms at the 5th day, showing rotting of roots, wilting of leaves, brown to black stem lesions, and defoliation (Fig. 2a). Co-inoculation of *C. annuum* seedlings with *P. capsici* and with the selected biocontrol fungal strains, resulted in a reduction of the phytophthorablight symptoms, *i.e.* co-inoculation with fungal strain M5R-3 resulted in a 70% reduction of blight symptoms, showing healthier plants even at 15 days of confrontation (Fig.
2b). These results show that fungal isolates with biocontrol activity against *P. capsici* are potential agents to protect pepper plants from phytophthora blight disease.

Figure 1. Confrontation of *Phytophthora capsici* (**P.c.**) and fungal strain **M7-3**. Third day of cultivation in PDA medium



Graph 1. Growth kinetics of *Phytophthora capsici* (■), fungal strain **M7-3** (■), and the growth of *P. capsici* in confrontation with M7-3 (●).

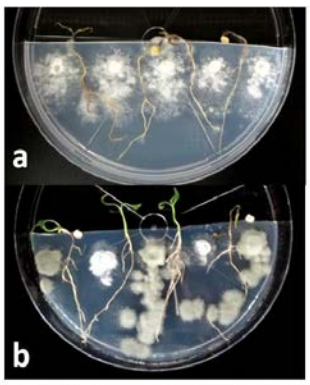


Figure 2. *C. annuum* seedlings inoculated with **(a)** *P. capsici* and **(b)** co-inoculated with *P. capsici* and fungal strain M5R-3. 15 days of cultivation in M9 medium

Peptides of different protein fractions from Chia seeds (*Salvia hispanica*) produced inhibitory activity against Angiotensin I-converting enzyme

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Chia seed is an annual plant of the Lamminae family that grows in semiarid climates and native from Mesoamerican[1]. Chia seed popularity has been increased due to its different biochemical compounds that include high content of phenolic compounds, unsaturated fatty acids, dietetic fiber and high amount of proteins[2], these features may provide effective benefits for the human health[3]. Total protein content in Chia seed is higher than most of traditionally utilized grains. Recently the main protein fraction in Chia seeds correspond to globulins, albumins, and prolamins and glutelins are present in low rate[2]. Many studies have focused on the isolation, purification and digesting of protein fractions to obtain peptides with a biological function against important diseases in human health. Angiotensin I-converting enzyme (ACE) is a peptidylpeptidase hydrolase that plays an important physiological role in the regulation of the blood pressure, to controlade the hypertension, treatments are based on the inhibition of the ACE enzyme. Proteins of Chia seeds are well-known precursors of a range of biologically active peptides that were used to block activity of ACE[4], however to date none research has probed which proteins fraction from Chia seed can inhibit the activity of ACE. The aim of this research was to fractionate the different protein fractions, including the Chia seed flour by different solvents and the fractions were separated by poliacrylamide gel electrophoresis (SDS-PAGE) and subsequent two dimensional gel electrophoresis (2-DE), by the way, protein fractions and seed flour were hydrolyzed by commercial peptidases to release the Chia seeds peptides and evaluate the inhibitory activity of these peptides against ACE *in vitro*. Chia protein fractions revealed that are different in a SDS-PAGE protein patterns and these same patterns were totally different in 2-DE maps. With respect of the peptides from the protein fractions digestion, the peptide profiles showed low molecular sizes in SDS-PAGE patterns. Peptides confronted against ACE enzyme showed that globulin and albumin peptides inhibit by similar manner the ACE activity, but glutamine and seed flour inhibited less, and change prolamine peptides were the last fraction that inhibited in less proportion the ACE activity. These results show that peptides encrypted in Chia protein fractions and seed flour have different inhibitory activity against ACE and these biomolecules could be alternatives for the hypertension treatment.

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Analyzingthe function the*arg2* gene encoding the small subunit of carbamoyl-phophatesynthetaseas a transformation marker in *Trichoderma atroviride*

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Trichoderma atroviride is a filamentous fungus of great economic and biotechnological interest due to itmycoparasite pathogenic fungi, which attack many crops impinging in the food production on the world. The genome of five species of the genus *Trichoderma*are available now in public database, of which about the function of40% of the genes is hypothetical. That highlightsthe necessity of tools that allow us to perform functional genomics to understand the role of the genes in *T. atroviride*.

Gene selection markersto carry out functional genomics are scarce in *T. atroviride*, most of them are gene resistant to antibiotics, and these chemicals are expensive. Auxotrophic strains to use the own genes for complementation have been implemented by massive mutagenesis (physical or chemical), which is not specific causingrandomly changes into cell genome. Therefore, the implementation of new techniques for the generation of selection marker and consequently the study of genesare necessary to generate selection markers without the backgroundcreated by massive mutagenesis strategies.

The *arg*2 gene encodes thesmall subunit of carbamoyl-phophatesynthetase,which is essential for arginine synthesis.Mutants lacking *arg*2 gene (Δarg2), were obtained by double-join PCR. A first PCR amplifies the 5 '- and 3'-endflanks of the *arg*2 gene, excludingin this way the full open reading frame (ORF). A second PCR joins these fragments with the *hph*gene conferring resistance to the antibiotic hygromycin B. A thirdPCR amplifiesthe wholecassette using nested primers. With this fragment we carried out protoplast transformation to obtain auxotrophic mutants unable to growth on minimal medium.Addition of 20mM .arginine restore growth of the Δarg2 mutants to a similar rate as the wild type strain. Characterization of auxotrophic strains and delimitation of minimum length of the *arg*2 gene for its use as a transformation markerarebeing evaluated.

Cloning and recombinant expression of Elongation Factor 1-α of *Leishmania infantum*

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BACKGROUND: *Leishmania infantum* is an intracellular parasite that causes the potentially mortal visceral leishmaniasis (VL) in America. This parasite is transmitted to human by bite of sandfly of genus *Lutzomyia*. Elongation Factor-1α(EF-1α) of *L. donovani*, specie that causes VL in old world, has been described as anantigenic protein with dual function: a canonical function in eukaryotic cells, involved in protein synthesis; and unconventional function, as activator of SHP-1 phosphatase and inhibitor of macrophages microbicidal activity against *Leishmania*, but in *L. infantum*infection we unknown which is the key role of EF-1αin host-parasite relationship.
OBJECTIVE: Theobjective of this investigation was the cloning and recombinant expression of EF-1α of *L. infantum* to study that is the key role of this molecule in host-parasite interaction in VL.
METHODOLOGY: The gene *Elongation Factor 1-α*of *L. infantum*(ATCC 5013) was cloned by PCR using specific primers designed *in silico* with Oligo software 7.0, and the sequence of *L. infantum*registered in GenBankXM_003392352.1. The PCR product was inserted in a vector of pETSUMO expression system using *Escherichia coli* BL21 as host. Recombinant protein was expressed as six-Histidinefusion protein, and then purified using a chromatography column HiTrap charged with nickel.
RESULTS: A PCR product of approximately 1350 bp was obtained with primers CEF1aR (TTACTTCTTCGCAGCCTTCGCGG) and CEF1aF (ATGGGCAAGGATAAGGTGCACATGA) and afterward this product was sequenced. The partial sequence obtained (1298 bp)was 100% of similarity with*EF-1α*gene of *L. infantum*strain JPCM5in BLAST. The expression and purification of the product of expression of gene*EF-1α*of*L. infantum*inserted in pETSUMO shown a protein of 60 kDa evidenced with Coomassie stain on SDS-PAGE, corresponding to the expected product.
CONCLUSIONS: We cloned the gene *EF-1α*of*L. infantum* as suggest the partial sequence (96.1% of the full length gene) and BLAST analysis. The gene expression as recombinant product is consistent with molecular weight expected suggesting that is the rEF-1α of *L. infantum*. Opening the scenario to continue studying the key role of this molecule in host-parasite relationship in VL.

Insecticidal activity of δ-PaluIT1 in *Heliothis virescens* and *Apis mellifera*.

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Insects are the most diverse group of animals over the earth, with more than a million different species; this group displays great importance in biology, medicine and agriculture. Lepidoptera larvae, affect several crops of importance such as cotton, corn, wheat, sugar cane, generating economic losses. Traditional control of pest insects had been done by the use of chemical pesticides. These chemicals have several disadvantages, for example they have effect in many insect killing them even the beneficial ones, allow insects to development resistance and are environmental contaminants. Among the new strategies to develop bioinsecticidal products spider venom offers several toxins which target voltage gate ion channels, for example: Calcium (Ca_V), Sodium (Na_V) and Potasium (K_V). δ-PaluIT1 is one of four insecticidal toxins isolated from the venom of *Paracoelotes luctuosus* spider, this toxin displays insect selective activity over *Spodoptera frugiperda, Diatraea magnifactella* and *Galleria mellonella* larvae with LD_{50} from 6.2 to 9.5 µg/g of larvae. In a electrophysiological study over the cordon nodal of *Periplaneta americana* it was observed that the δ-PaluIT1 interacts with site 4 of the Na_v channel and was able to modulates site 3. To understand specific differences between sites 3 and 4, that allow us to predict, if δ-PaluIT1 can interact with other Lepidoptera species, we performed a global bioinformatic analysis of *para type* Na_V and α subunit and local analysis of sites 3 and 4. Using the available protein sequences from Gene bank of insect *para type* Na_V, and BLAST suite program results showed that *Heliothis virescens* has a low similarity, that is it's α-subunit only has a 9.1% similarity in loop1 of Site 4 and non in the loop2 of the same site among other Lepidoptera. But for *B. mori, B. mandarina, P. xylostella, H. amirguera* and *A. mellifera*. Results indicated a similarity around 72% for the loop1 and 100%for loop2 in the same Site. When loop 2 of Site 3 was analyzed it seems to be more conserved, since a similarity of 81 to 100% in all Lepidoptera analyzed. It can be predicted that δ-PaluIT1 will not have insecticidal activity against *H. virescens* because the percentage of similarity in loop1 Site 4 is low. However the question about δ-PaluIT1 insecticidal activity in *A. mellifera* its more difficult to predict since a 73% in loop1 site 4 was founded, further work will be performed to bioassays δ-PaluIT1 in *Heliothis virescens* and *A. mellifera* to probe these results.

Peptides with antihypertensive functions of protein fractions from the canary seeds (*Phalaris canariensis* L.)

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The canary seed belongs to the Poaceae family and is native of the Mediterranean region and traditionally the main use is as food for birds[1]. Canary seed has increased interest due to its good nutritional value and could serve as an alternative source of gluten-free cereal grain[1]. Canary seed contains high levels of crude proteins and is rich in cysteine, tryptophan and phenylalanine, crude fat and phenolic compounds[2]. Due its high protein concentration, canary seeds could have potential as a food crop because of its high protein content, which is higher than oat, wheat and rice[2]. Canary seeds were used as folk hypertension treatment[3]. Recently protein fractions of this seed were characterized as prolamin and glutelin proteins as the main fraction, followed by albumin and globulin, by addition, the seed flour was hydrolyzed and the respective peptides were confronted against ACE (angiotensin convertin-I enzyme involved that plays an important physiological role in the regulation of blood pressure) and inhibited its activity[3]. However to date has not been tested which fraction is involved in the ACE inhibition. The objective of this research was obtain the protein fraction by different solvents and subjected to protein separation by poliacrylamide gel electrophoresis (SDS-PAGE) and two dimensional gel electrophoresis (2-DE), and also, subjected to *in vitro* gastrointestinal digestion conditions to obtain peptides that were confronted against ACE. The protein fractions showed differences in SDS-PAGE and these differences were corroborate in 2-DE. With respect of protein fractions digestion, peptide profiles showed low molecular sizes in SDS-PAGE patterns. Peptides confronted against ACE enzyme showed that globulin, prolamin and albumin peptides inhibit by similar magnitude ACE activity, and glutamine and seed flour inhibited less the ACE activity. The results point out the possibility of obtaining bioactive peptides from protein fractions that have a high potential as a nutraceutical food in prevention of cardiovascular diseases.

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Stability evaluation of bioemulsifier by*Acinetobacterbouvetii* in polluted water with diesel

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Keywords: Bioemulsifier, *Acinetobacterbouvetii*, bioremediation, diesel

Introduction: Currentlyremovingxenobiotic hydrophobiccompounds such as dieselfromwaterby bioemulsifiers (BE)have beenconsidered a technological alternative over traditional remediation techniques.BE areamphiphilic moleculesproduced by microorganisms. BE have characteristicssuch as:biodegradability,compatibility with the environment, low toxicity, high selectivityand their activity even in extreme temperature, pH and salinityconditions(1).The aim of thisstudy was to evaluatethe stability ofBEproduced by*Acinetobacterbouvetii*in the emulsificationofdieseldispersedin water atdifferent pH, salt concentration andtemperature.

Methodsand materials: BEwas produced in a 1.0 LAirlift bioreactor(superficial gas velocity, 0.6 cm s^{-1}; 72 h; 30°C).Recycled cooking oilwas usedas the sole sourceof carbon and energy(initial concentration 1.11 g L^{-1}),mineral medium(2) and*A. bouvetii* was inoculated ($1x10^6$ CFU mL^{-1}).Emulsifying activity (EA)was determined according to the standardmethod (3) at different pH (2, 4, 7, 9 and 10), NaClconcentration (0, 10, 20 30 and 50 g L^{-1}) and temperature (25, 40, 60, 80 and 121 °C).As controln-hexadecane (pH 7.0, 0.0 g NaCl L^{-1} and 25 °C) was used. Analyses of variance andHolm-Sidaktests (α=0.05)were performedfor statistical analysisof experimental data.

Results:BE produced by*A. bouvetii*was stable atthe differenttestedpH;significant differences were not observed inEAneither n-hexadecane (HXD) nor diesel, as an average we measured5.94±0.37 EU mL^{-1}whilstEA control was 5.24±0.56 EU mL^{-1}.NaClconcentrationsignificantly affected theEA,the higherAEvalues were observedwithHXD (an averageof 8.53±0.65EU mL^{-1}) and diesel(8.70±1.30EU mL^{-1}) with 30 and 50 g L^{-1}, respectively.Temperaturesignificantly affected theAE, the higher assayed temperature (121°C)produced the higherEA(for HXD16.48±0.87 EU mL^{-1} and16.99±0.79 EU $mL^{-1}$for diesel).Our resultswere similarto those reportedforBEproduced by*A. calcoaceticus*subsp.*Anitratus*SM7 (4) and BE by*A. radioresistens* KA53 (5).

Conclusion:BEproduced by *A. bouvetii*wasstableemulsifyingdieseldispersedinwater at differentpH,NaCl concentration andtemperatures which is afavourable featurefor the application ofBEinremediationof water contaminated withdiesel.

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AtGRDP2, a novel glycine-richdomainprotein, involves in plantgrowth and development

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Glycine rich proteins (GRP) have been reported in a wide variety of organisms including plants, mammalians, fungi, and bacteria. Plant glycine-rich protein genes exhibit developmentally regulated and tissue-specific expression patterns. Herein, we present the characterization of the *AtGRDP2* gene using Arabidopsis null and knockdown mutants and, Arabidopsis and lettuce over- expression lines. *AtGRDP2* encodes a short glycine-rich domain protein containing a DUF1399 domain and a putative RNA recognition motif. qRT-PCR analysis showed that *AtGRDP2* transcript is mainly expressed in Arabidopsis floral organs. Arabidopsis *Atgrdp2* mutants and *35S::AtGRDP2* overexpression lines exhibit alterations in development. The *AtGRDP2* over-expression lines display a fast-growing phenotype, observed from early stages of development towards flowering; while the *Atgrdp2* mutants show an opposite phenotype. Likewise, transgenic lettuce plants over-expressing the *AtGRDP2* gene manifest increased growth rate and early flowering time. Our data reveal an important role of *AtGRDP2* gene in plant development, positioning it as a good candidate for biotechnological applications.

Quantification of genetically modified MON810 maize (Zea mays L.) leaves by qPCR and dPCR

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Abstract

Nowadays the polymerase chain reaction (PCR) is the most common technique used to analyze modern biotechnology-derived traits. In particular, real-time PCR (qPCR) is a sensitive, quantitative and fast technique, however it has some limitations such as the need forreference calibrators,whichare not easy to get in Mexico. These limitations have been overcome by the new technique digital PCR (dPCR) which allows the amplification of one molecule of DNA based on the concept of limit dilutions used to enrich minor sequences through a partition process, enabling the quantification without a reference calibrator.Crop leaves arethe main kind of samples analyzed in Mexico, so in thepresent work,absolute quantification of mixed leaves, from genetically modified MON810 maize, were measured by qPCR and dPCR. The dPCR technique was validated first with two certified reference materials(CRM) with the event MON810, ERM®-AD413 and DMR436IIa, plasmid and powder, respectively, these were quantified to verify the OpenArray® Real-Time PCR System. The results obtained were comparable with that reported in the certificate. Subsequently, mixtures from modified leaves with the event MON810 and conventional maize leaves were made (10%, 1% and 0.1%) and quantified by qPCR and dPCR. The qPCR analysis fulfilled the minimum performance requirements for analytical methods of GMO testing such as limit of quantification, amplification efficiency, R^2 coefficient, dynamic range and the estimation of uncertainty, however we conclude that the values obtained by dPCR were more accurate, since a proportional ratio between the GM percentage for the leave 100% GM and mixtures made from itwere 41.87, 3.69, 0.3 and 0.031% with dilution factor close to 1:10, corresponding to that used to prepare the mixtures measured gravimetrically and we expected to obtain a number close to 50% taking into account the parental origin of the transgenic allele, that in these case commercial lines are inherited paternally. These results indicate that both methods could be applied to determine the copy number ratio in GM leaves.

Identification of microRNAs responsive to chronic drought, during three growth stages of *Brachypodium distachyon* Bd21.

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ANTIMICROBIAL PEPTIDE EXPRESION IN *E. COLI*

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Antimicrobial molecules have been widely studied in the last few years due to its high abundance in living organisms and its great potential use as new antibiotics. Because of its protein nature,these small molecules between 15-50residues make it difficult for bacteria and other microorganisms to develop resistance. However, its expression as recombinant proteins has been quite difficult.Currently, most of these peptides are expressed using affinity tags such as GST to minimize the damage of the peptide to *E. coli* mainly.Recently, it has been demonstrated the use of a SBD (Starch binding domain) in the immobilization of recombinant proteins on starch granules without interfering in the activity or properties of both the SBD and the fused protein with good expression levels.This SBD has been used as a purification tag with successful results.Therefore,the objective of this work is to express a recombinant active antimicrobial peptide (LL37) using this SDB as an affinity tag to minimize the damage caused on *E. coli* and its purification with starch. The antimicrobial peptide LL37 is regularly produced by humans as part of the innate immune system; it has a wide antimicrobial spectrum with potential applications in pharmacy and food industries.

A dual affinity taggedLL37 was constructed, rendering a fusion protein SBDtag- LL37-6xHis (figure 1) with protease cleavage sites between the N and C terminal sides of the peptide to free it after purification. In order to avoid adding extra amino acids to the C terminal of the peptide this was modified to match the thrombin recognition site. These modifications will not reduce the activity but may increase it. Normally LL-37 has a 6+ charge with this change a 7+ chargeexpected. Its expression has been confirmed in *E. coli* BL21 DE3 pLysS (figure 2) and is currently under optimization.Binding capacity at the moment of the writing is being evaluated onto raw corn starch granules.Finally, to verify the antimicrobial activity of the modified LL-37, the peptide will be initially tested against *E. coli* and *M. luteus*.

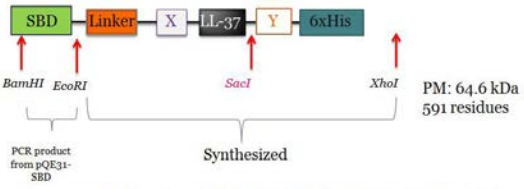

Figure 1. SBD-LL37 protein design. X enterokinase recognition site, Y thrombin recognition site

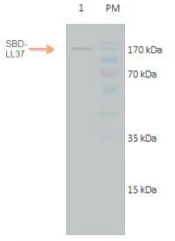

Figure 2. Western Blot anti His. Showing expresion of the protein SBD-LL37

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The overexpression of the *Amaranthus hypochondriacus NFY-C* gene modifies the growth rate and confers abiotic stress resistence in Arabidopsis

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The physiological flexibility of plants to adapt to adverse environmental conditions has generated the interest to identify the genes responsible of different kinds of biotic and abiotic stress tolerance. Now days, has increased the study of grain amaranth due the high protein content in seed and the agronomic characteristics that allow it to grow in poor soil unsuitable for growing grain (Omami *et al*., 2006). According to this, amaranth shows one of the principal alternatives to the production of good quality aliments in saline soils with long periods of drought and erratic rains (Espitia-Rangel *et al*., 2010). Taking as a tool the *Amaranthus hypochondriacus de novo* transcriptome, addressed to the identification of induced genes in stress conditions, has been possible identify that *AhNFY-C* increases it expression in leaves of six weeks old *Amaranthus hypochondriacus* plants, when they was subjected to more than one type of stress. The *AhNFY-C* gene encoding one of three subunits that make de *nuclear factor-Y*, which is constituted by *NFY-A, NFY-B* and *NFY-C.* It has been observed that the overexpression of the *NF-YB* subunit from Arabidopsis and Poplar confers drought tolerance in Maize and Arabidopsis plants respectively (Nelson *et al*., 2007; Han*, et al*., 2013) unmodified crop yield. However, it is not known the role of *NFY-C* subunit from Amaranth when it is overexpressed in a model plant as Arabidopsis and if this modification could confer stress tolerance. The results obtained showed that the constitutive expression of *AhNFY-C* in Arabidopsis cause a modification in grow rate, involve a delayed flowering, an increased inflorescence length, and a higher performance. Besides, the overexpression occasioned an increment tolerance in osmotic stress and drought in Arabidopsis plants.

AtNTC, A NOVEL ARABIDOPSIS GENE INVOLVED IN DEVELOPMENT AND STRESS RESPONSES

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Plants are sessile organisms that cope with different environmental factors (drought, salinity and extreme temperatures) during their life time. The understanding of the molecular mechanisms underlying stress responses is still a big challenge. Information on plant genome sequences, including the discovery of novel genes and molecular markers, has increased steeply with the usage of high-throughput DNA sequencing technologies. It has been estimated that ca. 38% of the *Arabidopsis thaliana* genes encode proteins of unknown function, many of them might be involved in development and stress responses.

Recently, we identified a non-characterized Arabidopsis gene (*AtNTC*) containing a Nucleos-tra2-C domain. This domain has been described in mammals and related with a Na^+ dependent nucleoside transport function. *In silico* analysis revealed that the Nucleos-tra2-C domain is widely distributed in the plant kingdom, finding orthologues of the Arabidopsis *AtNTC* gene in different plant species. Characterization of a T-DNA null mutant line of the *AtNTC* gene revealed that it is involved in Arabidopsis development. In particular, *Atntc* mutant lines have longer primary roots than WT plants, conversely silique size is reduced and the number of seed produced by each silique is also diminished. Furthermore, salt stress experiments using both WT and *Atntc* mutant lines revealed that the mutants are more tolerant, and have higher survival rates than WT plants after salt stress recovery. These phenotype might be related with a increased root size in the mutants. Changes in the expression pattern of the *AtNTC* gene under control and stress conditions are also presented.

Isolation and characterization of microorganisms with tolerance to toxic concentrations of lead and zinc, and their effect on the growth of *Arabidopsis thaliana* under conditions of lead toxicity.

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The state of Zacatecas is the major producer of Agin Mexico, in 2012; 2.3 million of tons of Agwere produced. Zacatecas is also an important producer of Au, Cu, Pb and Zn. However, the long history of mining activities, the predominant open pit mining processes and the scarce remediation actions; have caused the accumulation and dispersion of heavy metals in soils, water and air, across the state. Hg, As and Pb are the main heavy metals found in the environmental passives derived from the mining activities in Zacatecas and can be found at concentrations that triple or quadruple the permissive values of reference (SEMARNAT), imposing a high risk for the environment and the ecosystem by their inclusion into the trophic chain. A remediation strategy is the use fungi and bacteria for the removal of heavy metals from soil and water. Identification of tolerant strains to toxic concentrations of heavy metal is a key factor in order to stablish suitable bioremediation strategies. In addition, the utilization of these microbes in conjunction with plants can improve the ability to fixate/translocate the heavy metal resulting in an efficient strategy for bioremediation. The aim of this work was the isolation and identification of bacteria and fungal strains with tolerance to toxic concentrations ofZn and Pb, and the possible promoter effect in the absorption of Pb by *Arabidopsis thaliana*. Pure cultures of bacteria and fungi were obtained from samples of mining-derived soils,water bodies, abandoned open pit mines, and their growing plants (rhizosphere, roots and leaves), all collected in the state of Zacatecas. From the fungal strains collection, 73 isolates were cultured in PDA medium supplemented with 100 to 500 ppm of Pb or Zn. 19 fungal strains showed tolerance to 500ppm of Pb, and only 6 fungal isolates were tolerant to 500ppm of Zn, indicating the higher toxicity of Zn. A set of 6 fungal strains were tolerant to both Pband Zn at 500 ppm; *i.e.*, fungus strain M10- MP7 showed a similar growth rate when cultured in 500 ppm of Pb or Zn, compared with control culture (Fig. 1). Interesting changes in the micro- and macromorphology, in the synthesis of pigments, and the growth rates were observed for distinctive fungi at the evaluated concentrations of heavy metals. Concerning to the bacterial isolates, 384 bacterial strains were cultivated in NA medium supplemented with 500 to 1500 µg/mL of Pb, and Zn was added at concentrations of 500 to 1000 µg/mL.74 bacterial strains were tolerant to 1500 µg/mL of Pb, and 23 bacterial isolates were tolerant to Zn at 1000µg/mL; 13 isolates were tolerant to both, Pb and Zn, at their highest concentrations evaluated. Most of the bacterial isolates with tolerance to Pb showed a brown color (Fig. 2b) compared with the control culture.

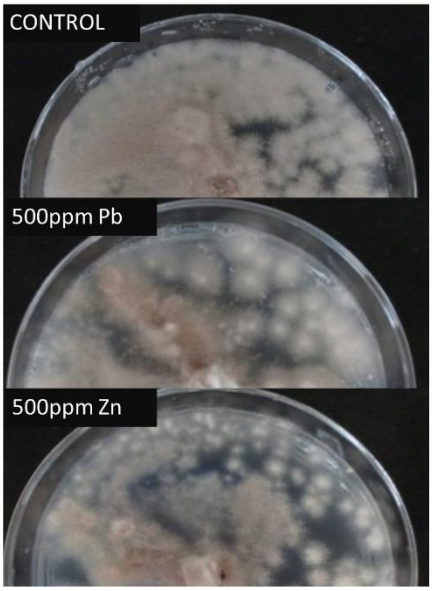

Figure 1. Fungal strain M10-MP7 grown in PDA medium supplemented with Pb or Zn.

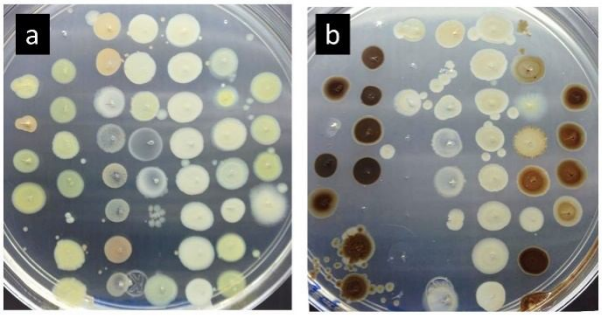

Figure 2. Bacterial strains cultured in **(a)** NA medium and **(b)** NA medium supplemented with 1500 µg/mL of Pb

(Fig. 2a). Bacterial strains MH5-3 and M2-MP4caused a promoter effect in the growth of *A. thaliana*grown in presence of 100 µM of Pb. These results suggest that tolerant bacterial and fungal strains have the potential as promoter agents for bioremediation.

"A new transformation vector to increase the accumulation of trehalose in *Nicotiana tabacum* transgenic plants"

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Plants are continuously affected by a variety of environmental factors. As they arebiotic environmental factorsthat arecausedbyotherorganismssuch as symbionts, parasites, pathogens, herbivores, and competitors, on the other hand the abiotic factors include, for example, extreme temperatures (heat, cold, and freezing), too high or too low irradiation, water logging, drought, lack of nutrients in the soil, and excessive soil salinity. As especially drought and salt stress are becoming more serious threats to agriculture and the natural status of the environment. The impact of abiotic stress on plant growth and development has been and still is a major research topic. Have developed various strategies to contend with these adversities, as identification and characterization of genes associated with plant responses to stress are crucial to the development of new cultivars with improved tolerance. An important pathway that has been linked to abiotic stress tolerance is the trehalose biosynthetic pathway. In plants only one pathway for trehalose biosynthesis exists; a two-step process involving TPS (trehalose-6-phosphate synthase) and TPP (trehalose-6-phosphate phosphatase) thatsubsequently dephosphorylates trehalose 6-phosphate to produce trehalose. Trehalose production seemed to be exclusively reserved for stress resistant plants, living inextreme habitats. Based on its excellent characteristics, several attempts to engineer plants that produce more trehalose with the aim to improve stress tolerance and yield under stress conditions have been undertaken in a variety of plant species. Heterologous expression of yeast trehalose biosynthesis genes in plants showed an increased stress tolerance. In the present workwe construct a plasmid to overexpress a gene fusion of yeasttrehalose-6-phosphate synthase and trehalose-6-phosphate phosphataseunder the control of two tandem copies of theCaMV35S promoter and transferred into*Nicotiana tabacum* by *Agrobacterium tumefaciens* LBA4404.Transgene insertion into the genome was analized by PCR andexpression by RT-PCR in tobacco transgenic plants. Several independent homozygous lines were selected in the presence of kanamycin and analyzed.Trehalose content and abiotic stress tolerance (drought and salinity) was determinedand compared with tobacco transgenic plants transformed with a single CaMV35S promoter directing the same gene fusion for trehalose accumulation.

CHARACTERIZATION OF PROTEIN EXTRACTS FROM TWO STRAINS ABLE TO HYDROLYZE METHYL PARATHION

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Keywords: methyl parathion, *Burkholderia*, hydrolysis.

Organophosphate pesticides such as methyl parathion are extremely toxic. However, is still commonly used in some countries, including Mexico. Their indiscriminate and irresponsible use has led to contamination and accumulation in soil, air and water as well as agricultural and aquatic products. Organophosphate pesticides are strong inhibitors of acetylcholinesterase in insects and non-target organisms too, including humans. The ability of microorganisms to degrade xenobiotics has aroused great biotechnological interest to remediate contaminated sites with these compounds and to degrade wastes and obsolete pesticides. We have isolated two bacterial strains from agricultural soils with a history of pesticide application, which have the ability to hydrolyze methyl parathion (MP). Later, these strains were identified by genomic DNA isolation and sequencing of 16S rRNA gene, belonging to the genus *Burkholderia* sp. In order to optimize the hydrolysis process, the enzymatic activity for both protein extracts and whole cells was measured by varying conditions of pH (4 – 9) and temperature (20 – 70^{o} C). The effect of different divalent cations (Cu^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}) in the enzymatic activity, was evaluated too. In addition, we analyze whether the PM hydrolyzing enzyme is constitutive or inducible by studying intracellular protein extracts from cells grown in mineral medium supplemented with PM as sole carbon source and cultured cells in mineral medium with glucose. We identified and characterized the enzyme of interest by SDS-PAGE.

Role of potassium uptake systems in *Azospirillum brasilense* Sp245 osmoadaptation.

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Salt stress is an adverse factor that restrict the production of economically important crops. Actually, about 40% of the world area has potential salinity problems, this affect not only the plants but also has a negative impact on the fitness of beneficial microorganisms. Among these we can found *Azospirillum brasilense* Sp245, which is a nitrogen-fixing bacteria and produces plant growth regulating substances. In order to adapt to osmotic fluctuations, soil bacteria of the genus *Azospirillum* accumulate compatible solutes such as glutamate, proline, glycine betaine and trehalose. However, recent studies have shown that there is a more immediate response to saline stress: accumulation of potassium. This cation accumulates by a number of different transport systems which are widely distributed in different bacteria species: Trk, Kdp, Kup1 and kup2 and allows to bacteria acquire a tolerance to high salt concentrations. By a search in the genome of *A. brasilense* Sp245, we have found that the four systems mentioned above are present and by an analysis of this genes (*trk, kdpA, kup1 and kup2*) we will assess the importance that each system has when the cells are exposed to different salt concentrations (NaCl). This will be performed by the mutant's generation of *A. brasilense* Sp245 on each potassium uptake systems.

Antimicrobial and Biochemical Characterization of Actinomycetes Antagonists of Phytopathogenic Microorganisms Isolated from Soils of Michoacán

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Pepper (*Capsicum annuum* L.) is an important crop in Mexico and pepper wilt caused by *Phytophthora capsici* (PC) *Fusarium oxysporum* (FO) and *Rhizoctonia solani* (RS) is one of the diseases that affect it. The disease control is performed mainly by applying large amounts of agrochemical compounds and the search for alternatives with less environmental impact is required, so the finding and use of biological control agents could contribute to reducing the environmental impact that the application of pesticides cause and meet the demand for organic products. Actinomycetes may be employed as biological control agents due to their ability to produce bioactive compounds as antibiotics. These microorganisms are present in soil and interact with phytopathogenic microorganisms. Therefore the aim of this study was to characterize soil actinomycetes isolated from Michoacán with antimicrobial activity against PC, FO and RS, to further determine the biochemical mechanism by which the inhibition of microbial pathogens occurs. To evaluate the *in vitro* antagonism between actinomycetes and plant pathogens, three experiments were established, each test was placed in a completely randomized experimental design with 87 treatments (85 Michoacán actinomycetes, *Streptomyces lydicus* of Actinovate^{AG} and PC, FO, RS as controls). The response variable evaluated was the area of inhibition (%) of plant pathogenic microorganism growth (AIMF-PC, FO, RS) due to actinomycetes. Subsequently, the production of cellulase, pectinase, chitinase and phosphatases for the 10 strains of actinomycetes with the highest antimicrobial activity was determined. For RS, there were significant differences between strains (Tukey, $p \leq 0.05$) for AIMF-RS, being the best strains MABV07, MABV45, MABV37 and MABV47 AIMF-RS with greater than 50%. in the case of PC, there were statistical differences (Tukey, $p \leq 0.05$) among the 85 strains of actinomycetes for AIMF-PC, 32 strains inhibited the growth of PC over 60% and 14 inhibited the growth of PC completely (MABV01, MABV09, MABV24, MABV30, MABV37, MABV38, MABV39, MABV40, MABV42, MABV45, MABV47, MABV48, MABV49, MABV65 and MABV74). Finally, to FO, the results also indicate statistical differences between treatments (Tukey, $p \leq 0.05$) and of the 85 strains tested, 12 showed a AIMF-FO than 60%. To confirm the inhibitory activity of the strains tested, a second experiment was established in dual confrontation culture. The results showed that 12 strains AIMF-FO showed a greater than 40%, the strain MABV63, it presented an AIMF-FO greater than 50%. Finally a positive correlation between the production of cellulase, pectinase, chitinase and phosphatases with the inhibitory capacity of 10 strains of actinomycetes to RS, PC, FO, found result suggesting that the mechanism by which the actinomycetes are acting is by producing of enzymes involved in the formation of the cell walls of the phytopathogenic microorganisms.

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Different speract gradients attract *Strongylocentrotus purpuratus* spermatozoa

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Abstract

Sperm must find the oocyte to fertilize and chemotaxis is essential for marine organisms. It has been shown that spermatozoa of some sea urchin species (*Arbacia punctulata* and *Lytechinus pictus*) exhibit chemotactic responses. Speract is a chemoattractant belonging to the SAP (Sperm Activating Peptide) family, and isolated from *Strongylocentrotus purpuratus* egg jelly layer. Speract stimulates *S. purpuratus* and *L. pictus* spermatozoa metabolism (respiration, increases in cGMP levels, intracellular pH, Ca^{2+}, etc). In spite of decades since the isolation of speract from *S. purpuratus*, sperm chemotaxis has not been demonstrated in this species. In the present work we investigated whether *S. purpuratus* spermatozoa can exhibit chemotactic responses. For this purpose, we exposed *S. purpuratus* sperm to defined speract gradients and found they accumulated in response to that chemoattractant. We determined that shallow speract gradients are unable to generate the accumulation response but steep ones do. In conclusion we have shown that *S. purpuratus* spermatozoa do accumulate in response to speract.

In Vivo assessment of probiotic characteristics indigenous lactic acid bacteria

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Background. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO, WHO, 2006). Since 2012 (Alegría-Mundo) in ITTG was performed isolation and characterization of lactic acid bacteria with probiotic potential. Particularly the BAL-03 strain was reported by González-Escobar 2013 by assays *in vitro*, with high survival rate of gastrointestinal conditions, deconjugation of bile salts, exopolysaccharide production, etc.. However, for an microorganism to be considered as a probiotic, FAO/OMSS (2006) recommend the strain identification and evaluation in animal models before developing clinical trials in humans. The aim of this study was to evaluate three probiotic characteristics of lactic acid bacteria isolated from a fermented beverage, by *in vivo* tests.

Materials and methods. BAL-03 strain was provided by the microbial ITTG collection. The molecular identification of the DNA of the BAL-03 strain was made by using the Kit 'ZR Fungal / Bacterial DNA MiniPrep',PCR and sequencing by MacroGene. The toxicity test was carried out for 21 days with female mice using a control group and 3 study groups which were given a bacterial dose 10^8,10^9, 10^{10} CFU/ml/day. It was observed body weight, water intake, food, and finally macroscopic observation and weight of the liver and spleen was monitored (Yakabe *et al*., 2009). The hemolytic activity test was performed by plating blood agar (Santini *et al*., 2010).

Results. The DNA sequence was compared with sequences in the alignment tool BLAST" of the NCBI database, and it is 95% similarity to *Lactobacillus plantarum*. As for the toxicological evaluation no symptoms of urination and/or diarrhea, or adverse effects in the study groups were presented. There was no statistically significant difference in the consumption of water, food and body weight between the experimental groups. The weight of the liver and spleen of the study groups showed no statistically significant difference from the control group. Macroscopic observation did not reveal effects associated with consumption of *Lactobacillus plantarum* on these organs. No hemolytic activity was observed on blood agar plates.

Conclusions. According to the assessments made, *Lactobacillus plantarum* is safe; however, testing such as bacterial translocation, intestinal adhesion is necessary to confirm that the strain is a probiotic effective and safe.

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Electrophoretics patterns of pregerminatedmaize and field performance

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Introduction

In nature, seeds may undergo repeated cycles of imbibition-drying and germinate only when conditions are optimal; eventually, they can die. The pregerminative seed treatment, or osmopriming, has been proposed as a mean for better emergency underfield conditions even seedlings resistance to stress. The treatment involves seedsoakingfor a few hours in gibberellic acid solution, potassium nitrate, polyethylene glycol, polyamines, and a combination of these and other compounds. After imbibition, seeds were dried at room temperature andwereconveniently stored.After the seeds were planted the percentage of germination was improved, among other attributes.

Materials and methods

One corn seeds bulk (1 Kg) of two selected locations on the IT Roque underwent imbibition for 6 hours in a solution of potassium nitrate, 6g/200 ml water on March 2014 were dried at 27 ° in a convection oven and stored to sowing on 21 April. Four plots were planted in three rows each, 5 m long furrows and days to emergency were registered at two leaves stage, plant height, ear height and yield. Embryos treated seeds and untreated, proteins were extracted with 100 mM phosphate buffer pH 7, 2 mM PMSF and run on denaturing polyacrylamide gels.

Results

The germination percentage of the April 7, 2014 was 88% for untreated red maize; 86% for red treaty maize. For yellow untreated maize was 82% and for yellow treated maize was 46%. By 26 June, the germination percentage was maintained for untreated red and was 44% for red treaty. Yellow treaty seed had a percentage of 48% and untreated yellow seed 64%. Red corn seeds pretreated emerge to 8.7 days; the untreated emerge to 9.97 days. Yellow corn seeds treated emerge to 8.8 days; the untreated to 10.6 days. Red with treatment took 10.8 days to develop two leaves, untreated 12.5 days. The yellow treated took 10.8 days to have two leaves and untreated yellow 12.6 days. Some changes are observed in protein banding seeds treated with respect to untreated, there seems disappearance of some bands in the yellow treated. There is a clear effect of pretreatment on seeds and seedlings of maize.

The ISC Genetic System Involved in the Virulence of Pathogenic Bacteria of Plant

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The iron-sulfur [Fe-S] clusters are cofactors of a variety of proteins that play a wide variety of important functions in life, such as respiration, central metabolism, gene regulation, DNA replication and repair, etc. In recent years the mechanisms involved in the biogenesis of [Fe- S] clusters have been the subject of intense research in model organisms (*Escherichia coli* and *Saccharomyces cerevisiae*). In bacteria, the biogenesis of [Fe-S] clusters is encoded by different groups of genes organized in operons, which are able to regulate their gene expression in response to the concentration of the substrates with which they interact. To this day three systems have been described for the biogenesis of [Fe-S] clusters: NIF, ISC and SUF. The ISC and SUF systems have in common the genetic regulation by the regulator transcipcional IscR. *Dickeya dadantii* (synonym *Erwinia chrysanthemi*) is a phytopathogenic bacterium that causes soft rot in many plants of economic importance, the three systems has biogenesis of [Fe-S] clusters, which appears to confer high capacity virulent. It has been shown that IscR protein regulates genes involved in oxidative stress, some anaerobic respiration and two genes related in the biogenesis of [Fe-S] clusters (*erpA* and *nfuA*); IscR also negatively regulated the ISC operon and positively SUF system under oxidative stress and iron deficiency respectively. This genetic control is attributed to center [2Fe-2S] of IscR, it acts like a "sensor" of the number of clusters available in the cell. In another sense *Pseudomonas syringae* is a phytopathogenic bacterium that affects a large number of plants of economic importance in agriculture and industry. For example, the pathovar *phaseolicola* causes halo blight of bean (*Phaseolus vulgaris*). This plant pathogen to interact with their host faces the generation of oxidative stress and nutrient limitation, such as iron, that allow the bacteria reproduce. The [Fe-S] cluster biogenesis system have been proposed as a mechanism for the adaptation of microorganisms to hostile conditions such stress generated by superoxide agent and iron starvation. Therefore the aim of this study was to determine the effect of regulator IscR of *D. dadantii* (Dd) and *P. syringae* pv. *phaseolicola* (Psph) in its virulence. To this end we constructed mutants by molecular biology techniques in the genetic system ISC of Dd and Psph. The mutants were subsequently tested in oxidative stress, iron starvation and virulence in arabidopsis plants, african violet and beans.The advances that
have so far are the genetic construct (*iscR*::*aphA*-3) to the null mutant *iscR*$^-$ by homologous recombination in a wild type strain of *D. dadantii*. The null hypothesis of this null mutant *iscR*$^-$
is that regulation of the biogenesis [Fe-S] clusters in the cell is altered and this alteration will result in the loss of virulence of Dd.In the case of Psph was demonstrated by *in silico* analysis
that putatively only the [Fe-S] cluster assembly system ISC is present in its genome, so we developed a methodology to study its biological function in this plant pathogen, whereby the
hypothesis has so far in this pathosystem is that the ISC system is important in virulence

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Biochemical and immunological studies of mannitol dehydrogenase in strain YR-1 of *Mucor circinelloides* isolated from petroleum contaminated soil

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Summary

Key words: alcohol dehydrogenase, environmental pollution, hydrocarbons, metabolism. Alcohol dehydrogenase (ADH) is an enzyme that catalyzes the conversion of alcohols to aldehydes and ketones. The reaction is reversible, reducing carboniles compounds to alcohols using NAD or NADP as coenzyme. Some of the substrates that this enzyme may use are aromatic and aliphatic alcohols with lineal or branched chain (Reid and Fewson, 1994).
One of the important aspects in fungal metabolism is its capacity of using compounds considered as harmful, such as, hydrocarbons that contaminate soil (Arrambarri y Cabello, 1994). Therefore, some enzymes like alcohol dehydrogenases, particularly, mannitol dehydrogenase, have demonstrated its importance as catalyzers in the metabolism of these compounds.

Mannitol is a polyalcohol of 6 carbons and it is one of the most abundant biochemical compounds in the biosphere. A number of functions have been addressed to mannitol in filamentous fungi; storage of carbon hydrates, stress tolerance, dispersion and/or release of spores. The development of techniques of genetic manipulation in filamentous fungal has accelerated the understanding of its functions and mannitol metabolism (Solomon et col. 2007).

The subject microorganism of this work is strain YR-1 of *Mucor circinelloides*. In general, it is a mesophilic specie that can grow in presence or absence of oxygen. It has some outstanding characteristics at a basic and practical level; it is easy to induce different morphological growths, either filamentous or yeast in response to a change in environmental conditions (Van Heeswik and Roncero, 1984). It has been suggested that the yeast phase is the result of a fermentative metabolism while the mycelial phase is oxidative (Orlowski, 1991).

In *M. circinelloides* YR-1, isolated from hydrocarbon contaminated soils, the studies have focused in researching the influence of different kinds of compounds (alcohols and aromatic and aliphatic hydrocarbons), on the production of the ADH enzyme(s) that use short and long chain alcohols and dihydrodiols as sustrate and that are involved in the pathways of degradation of aliphatic and aromatic hydrocarbon, respectively (Zazueta et col. 2008). Previous studies have demonstrated that strain YR-1 of *M. circinelloides* has a great variety of ADHs, which perhaps, are involved in the metabolic machinery able to adjust to degradation and use of aromatic and aliphatic hydrocarbons, and alcohols too.

In our study group, we have explored the use of zymogram stains of electrophoretic gels as first approach for the identification of ADH type carbonile reductases (ADH's) present in strain YR-1 of *M. circinelloides*. In this particular work, we were able to determine the pH and cofactor with which mannitol dehydrogenase has best activity. In another study, using immunodetection and silver staining we found that the enzyme mannitol dehydrogenase (MDH) is monomeric.

Purification and characterization of antimicrobial peptides derived from the venom of *Scolopendra polymorpha*.

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Antimicrobial peptides (AMPs) comprise the main defense mechanism against bacterial infections in insects and amphibians. AMPs are cationic molecules of low molecular weight. This class of peptides has been isolated in bacteria, plants, in the skin of amphibians, in the venom of bees, spiders, scorpions and centipedes. In the venom of S. subspinipes mutilans has been reported Scolopendrín 1 (Wenhua et al., 2006) and Scolopin 1 and 2 (Peng, 2009). These peptides have antimicrobial and antifungal activity. González in 2010 reported antimicrobial activity against *S. aureus* in the venom of *Scolopendra viridis* Say (now identified as *S. polymorpha*). The aim of this study was to characterize an antimicrobial peptide in *S. polymorpha* venom. Centipedes were milked by mechanical stimulation, resuspending the whole venom (VT) in bidistilled H_2O and quantitated by the Lowry method. Venom was separated by electrophoresis on Tricine-SDS-PAGE at 16%. Bands of protein were electroeluted, dialyzed, quantitated by absorbance (λ 280 nm) and taken to dryness. The antimicrobial activity was determined by the agar diffusion method against *S. aureus* (ATCC
29213) at a concentration of 1 to 2 x 10^8 CFU / ml of culture and an O.D. of from 0.070 to 0.13
(λ 600 nm). Each of the bands electroeluted was tested for antimicrobial activity. Five bands of various sizes (67, 37, 20, 17 and 13 kDa) showed activity. From them, the 13kDa band showed the highest antimicrobial activity, this band was named *SPC13*. Minimum inhibitory concentration (MIC) of *SPC13* was determined by monitoring the kinetics of inhibition and measuring the change in absorbance (630 nm) versus time of incubation in the presence and absence of the peptide. Five different concentrations of *SPC13* (9, 4.5, 2, 1 and 0.5 µg / µl) were used; ampicillin (5µg/5µl) was used as a positive control, and medium inoculated with the bacterium, as negative control. MIC values obtained from the active peptide were analyzed using Graph Pad Prism statistical package on a statistical ANOVA and Tukey a single queue (α
<0.05). The hemolytic activity of *SPC13* was assayed in human erythrocytes; after half an hour
at 37 ° C of incubation, the absorbance at 415 nm of supernatant was measured. The absorbance value of the positive control (Triton X-100) was taken as 100% hemolysis. Finally, sequencing by mass spectrometry of *SPC13*, revealed two sequences with 98% identity with histone H3 reported in 2010 in *S. viridis* (GenkBank: DQ222181.1). *So far, SPC13* is the antimicrobial peptide with the highest molecular weight reported within the genus Scolopendromorpha; according to their minoacid sequence could be considered the first report of an histone with antimicrobial activity within the specie *S. polymorpha* and the class Chilopoda.

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Cloning and bioinformatic and biochemical characterization of a novel glycosyl hydrolase family 10 of Bjerkandera adusta.

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Functional genomics enables the study of new sequences coding for enzymes and structural proteins that contribute to better understand microbial physiology. Moreover, the description of new biocatalysts with functionality in industrial conditions is a novelty for modern biotechnology. Limitations as *in vitro* microbial cultures, the expression of functional proteins in specific culture conditions, and methods for the detection of enzymatic activities, are disadvantages to study cultivable microorganisms. Massive sequencing stands out among new search methods for coding sequences and allows the annotation and characterization of new transcriptional units. *Bjerkandera* is a fungal genus that groups several Bacidiomycetes causing white rot of wood, and its enzymes have great potential for biotechnology applications. A genomic library was constructed from *Bjerkandera adusta* and screening through PCR using degenerate primers for xylanases allowed us to detect a clone with a partial sequence that showed homology to family 10 glycosyl hydrolases. The characterization demonstrated that the gene sequence was incomplete at the 5’ and 3' ends. Genome Walker and 5'RACE methodologies were used to complete the sequence towards the 3’ and 5' ends respectively. The genomic DNA sequence of the gene is comprised of 1500 bp. Blastx showed the existence of domains of glycosyl hydrolases and homology with fungal glycosyl hydrolases. The best hits of the Blast analysis suggest this gene codes for a xylanase. Furthermore it was demonstrated that the gene was complete as we detected an open reading frame including initiation and stops codons for translation. Currently, we are working on the cloning and expression of the gene in *Pichia pastoris*, for the subsequent functional characterization of the enzyme. This study demonstrates that the application of genomic tools for annotation, description and characterization of new genes is a novel and useful approach to study the physiology of poorly characterized organisms.

Viability and germination of seeds of *Swietenia macrophylla King* mediated by tetrazolium staining.

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Introduction. Mahogany (*Swietenia macrophylla*, Meliaceae) is the most valuable and intensively exploited Neotropical tree, also known as big-leaf mahogany, is a tropical tree species native to Central and South America. The viability of fresh mahogany seeds is around 80–90%, although the viability of stored seeds can vary. In general mahogany seeds will not retain an acceptable level of viability if stored at room temperature and in humid conditions for more than about 3 months [1]. Therefore, is important to know the factors affecting the viability and germination of their seeds because their only means of natural propagation is by seeds. Being the feasibility the property which defines the ability of a seed to generate a new plant. Mahogany is considered a recalcitrant species whose viability can be reduced by up to 60% [2]. The tetrazolium test is the fastest way to determine the viability of a seed and is the most used worldwide. The aim of this work was characterize the mahogany seed viability using tetrazolium as indicator.

Materials and methods. The seeds were collected from tree elite. To determine the ability of the seed to absorb water, imbibition kinetics was performed with three replicates with 60 seeds. The seeds were placed in Petri dishes with cotton saturated with distilled water. To estimate the percentage of viable seeds (VS), a staining protocol by tetrazolium (0.5 and 1%) was standardized at different times (3, 8, 12 and 24 hours) using 10 seeds with 2 replications. The percentage of viable germinating seed (VGS) was estimated by germination tests in Petri dishes with cotton saturated with distilled water. 180 seeds were used in three replicates. The percentage of not germinating viable seeds (NGVS) was estimated as NGVS= VS- VGS.

Results. The maximum absorption (30-35%) of water was reached after 24 hours of imbibition. The image analysis showed a correlation with the second stage (phase II) of kinetics of imbibition. The tissues of embryo showed more stained areas in comparison with control. After 24 hours the impregnation with tetrazolium, the percentage of seed viability was 87% (VS), our results are similar to those reported for other authors [3]. Our results demonstrate that the seeds exhibited 87% viability (VS), with 72% of germinated seeds (VGS) and 15% of NGVS (dormant seeds). In conclusion, these results confirm that mahogany is recalcitrant tropical forest specie.

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Design and biological comparison between alpha helical and beta-defensinantimicrobial peptides

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The constant emergence of drug resistant bacterial strains, as a consequence of the unmeasured use of commercial antibiotics, has redirected the pharmacological research towards the development of new antimicrobial agents (AMPs). They offer a promising solution to this drawback because their mechanisms of action are not limited to a single receptor. However, these molecules have some disadvantages such cost of chemical synthesis, peptide stability and cytotoxicity to eukaryotic cells when compared to conventional antibiotics. Here, two types of antimicrobial peptides are designedand their biological activities are compared.For the design of an alpha helical antimicrobial peptide, the model structure of the 24 merPin2was used. This peptide has a Proline residue in its middle region, which gives high antimicrobial and hemolytic activity. Here, the Proline was substituted for a more flexible structural motif with the intention to reduce its hemolytic activity. Therefore, Pin2 variants, with substitutions of the P14 for G, V, GV, VG, GVG or GPG, werechemically synthesized. The hemolytic activities of the Pin2 variants GV, GVG and GPG were between 50-75% lower than that of the parental peptide and the antimicrobial activity in the dilution assay were 6.25-12.5 µM for all variants. Based in these results, twoshort peptides were designed havingonly 14 and 17 residues. Both peptides Pin2[14] and Pin2[17] showed antibiotic selectivity against *E. coli* in both the diffusion and dilution assays. In addition, Pin2[14] showed 25% hemolysis at 100 µM while Pin2[17] did not show any hemolytic effect at the same concentrationincreasing their therapeutic indexes were up to 32times higher than that of the parental peptide.On the other hand, human beta-defensins (hBDs) have a structure composed of antiparallel beta strands stabilized by three disulphide bridges.These molecules are expressed soluble in different tissues and exert antimicrobial and antiviral activities on them. Here, two peptides, hBD11 and hBDconc, were synthesized chemically andshowed notoxicicityonhuman erythrocytes. Theyhad MIC values between 12 and 25 µM in the diffusion assay method. However in the dilution assay, hBD11 showed a bacteriostatic effect against *E. coli* and its MIC value towards *S. aureus* was 100 µM.Finally, the hBDcon showed poor selectivity towards *S. aureus*,but the MIC value for*E. coli* inhibition was 12.5 µM. The results showed that the alpha-helical variants represent a better alternative than that of beta-Defensins for designing new antibiotic peptides to be used as therapeutic agents.

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Exposure Effects of toxic dinoflagellates on gene expressions related to cell cycle regulation in *Crassostreagigas*

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Introduction*Crassostreagigas* accumulates marine toxins asdiarrheic shellfish poisoning (DSP) and paralytic shellfish toxins (PST) associated to dinoflagellates*Prorocentrum lima*and *Gymnodiniumcatenatum*. DSP are acidic polyether toxins includingokadaic acid (OA). The main OA effect is the specific inhibition of serine and threonine phosphatases 1 (PP1) and 2A (PP2A) resulting in hyperphosphorylation of many cell proteins. On other hand,PST are a group potent neurotoxic alkaloids whose mode of action involves a reversible and highly specific blocking of ion transport by the sodium channel and thus of the action potential in excitable membranes. Althoughtoxic effects have been extensively reported in bivalve mollusks at cellular and physiological levels, genomic approaches have been scarcely studied.Therefore, the objective of our study was to determine the effects of *P. lima*and *G. catenatum*on farmed *C. gigas* juveniles (3–5 mm) during a sub-chronic (7 and 14 days) exposure.
MethodologyFor the bioassays, groups of 20 oysters (by triplicate) were exposed to three diets (1)mixed diet *P. lima* (3×10^3 cell. mL^{-1}) combined with a fixed amount of *Isochrysisgalbana* (0.75×10^6 cell. mL^{-1}); (2) mixed diet with *G. catenatum* in same condition as the previous one; and (3) diet with only *I. galbana*as nontoxic control.The effects of both dinoflagellates on *C. gigas*analyzing the expression levels of three genes involved in cell cycle regulation: (a) p21 protein (*Cg-p21*); (b) chromatin assembly factor 1 p55 subunit (*Cg-cafp55*); (c) elongation factor 2 (Cg-*ef2*)by polymerase chain reaction and real time quantitative PCR werechanges in time and dinoflagellate species.
Results and conclusions The results in our work showed that the analyzed genesexhibited a constitutive expression under normal conditions but inducible under sub-chronic exposure.The highest expression levels were found at 7 days with the two mixed diets, but the oysters fed with *P. lima*were most affected at the levels of*p21 protein* (9 fold) andCg-*cafp55* (8 fold) above the nontoxic control. The oysters fed with *G. catenatum*showed the highest expression level of *Cg-ef2* 4 foldabove the nontoxic control.The whole cell ingestion of *P lima* and *G. catenatum*caused a clear mRNA modulation expression of the genesinvolved in cell cycle regulation. The presence of DSP and PST through *P. lima*and *G. catenatum*cells, probably resulted in eliminating some cell cycle control points compromising the integrity of the genome and other critical cellular processes in oysters; the consequences could be an abnormal replication control or coordination loss between DNA-replication and cell cycle progression.

Changes in polyamine profile of *Arabidopsis* in the interaction with *Trichoderma atroviride*.

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Arabidopsis thaliana represents a functional system to study beneficial plant-microbe interactions like *Trichodermaspp*. The establishment and interaction development between Trichoderma and plants are accompanied by an exchange of signaling molecules between both organisms. These signaling molecules may act as hormones that stimulate plant defense, plant growth and development. It is known that plant polyamines metabolism undergoes remarkably changes during plant – microbe interactions. Polyamines (PAs) are small aliphatic polycationic compounds that have been recognized as plant growth and development regulators since are involved in many essential physiological processes in a wide variety of organisms.

To evaluate if the growth promotion observed in Arabidopsis seedlings during Trichoderma interaction is dependent of physical contact, we analyzed Arabidopsis seedlings of 2-weeks old growth in a divided system interaction with *T. atroviride*. Two conditions were used, in the first one both organisms (plant and fungus) growth in different sides of the plate in MS medium (MS- MS), in the second both organism growth together in one side of the plate in MS medium (MS-TA). It was observed that seedlings that are growing in MS – MS conditions are bigger than seedlings growing in MS–TA at three and five days post interaction (dpi).

In order to assess if the polyamine metabolism is involved in the improvement of Arabidopsis seedlings growth by Trichoderma, PAs levels were detected in their free and soluble forms in total plants and were quantified by HPLC. At 3 dpi *T. atroviride* provoked a diminished in Spd and Spm levels between 20 and 30%, and for Put levels a 40% decreased was observed in both conditions in comparison with control seedlings. In contrast, at 5 dpi the PAs profile was different between both conditions, while in MS-MS Put levels increased 50%, during MS-TA the increased was about 200%. Interestingly, Spd levels decreased in 10% in MS-MS contrary with the increment of 170% observed in MS-TA. There were no differences in Spm levels during MS-MS, while in MS-TA an increase of 25% was detected in comparison with control samples. Because is evident a change in PA profile during Arabidopsis – Trichoderma interaction we suggest that PAs metabolism could be involved during this beneficial association.

Evaluation of antibiotics in milk content of the main producing areas of Chiapas, Mexico.

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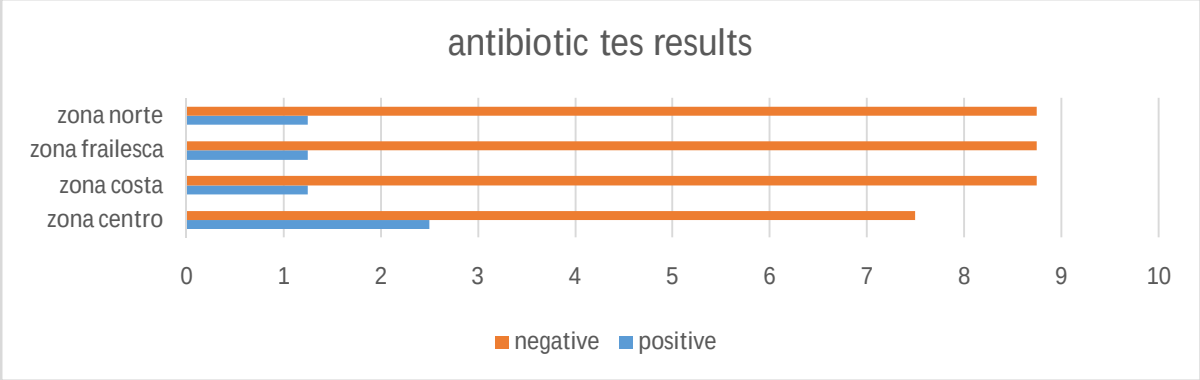
Milk is a complex nutritional product that has more than 100 substances found either in solution, suspension or emulsion in water. According to the FAO, pesticide residues drugs, antibiotics, or other chemicals not own the same food are considered as a risk factor in public health and is a limiting factor in the economic development of any country. Different residues in food of animal origin generate low quality products and pose a risk to the health of consumers, producing acute or chronic toxicity, mutagenic and carcinogenic effects, body development disorders, allergic reactions and bacterial resistance phenomena, among others. These side effects have led to waste in general and hazardous drugs veterinary health are regulated. The use of antibiotics in livestock production is a reality and a need, however, to apply such drugs must have a dose, route of administration, appropriate withdrawal period and proper identification of cows in treatment to prevent accidental contamination of the milk from healthy cows. Excessive and inappropriate use of antibiotics has fostered the rise of resistant organisms and allergic reactions, including (Varnam and Sutherland, 1994). Despite the age of the existing international regulations, only until recently in our countries are paying attention to this health problem and have begun to adopt new measures to recognize the residual effect of drugs and other substances in food of animal origin, which aims to achieve greater competitiveness of livestock products in international markets. No data reported related to the content of antibiotics in milk in the state of Chiapas, it is important to know the possible presence of these concentrations and to offer alternatives to reduce or eliminate their consumption. In this work the presence and concentration of antibiotics and some quality parameters of milk in the 4 major milk producing areas of Chiapas was evaluated. The presence of antibiotics in milk was determined using the test Charm MRL Beta-lactam / Tetracycline. Other quality determinations were performed according to NOM-155-SCFI-2012 and NOM-243-SSA1-2010.

Results.-There is presence of antibiotics in milk, other tests gave different results, some of them show mainly that remain inadequate hygiene practices and also no information and content regulation of antibiotics in milk chart below shows the relationship samples that were positive and were allowed by the SSA values.



In the downtown area, are some of the few industries in the state of Chiapas milk and this represents a risk to the consumption of milk.

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Novel thioesterase for degradation of toxic aromatic compounds. Functional and structural implications.

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Thioesterases (TEs) are classified into EC 3.1.2.1 through EC 3.1.2.27 based on their activities on different substrates, with many remaining unclassified (EC 3.1.2.-). Many of them are involved in the degradation pathway of aromatic compounds. Several genes encoding thioesterases have been identified in metagenomic libraries prepared from environmental samples. Thiosterases could be detected from activated sludge metagenomes and expressed in heterologous systems. The aim of this study was the cloning and heterologous expression of a putative thioesterase (ThyesT_ar) in *Escherichia coli* BL21 using the pET expression system. Screening of an amplified metagenomic library with a tributyrin-containing medium led to the isolation of a clone exhibiting lipolytic activity. This clone carried a 2,096 -pb DNA fragment encoding for five genes, one of which (*thyest_ar)* was implicated in the phenylacetic acid degradation in bacteria. The *thyest_ar* gene encodes a protein of 135 aa with a calculated molecular mass of 14.2 kDa, displaying
24-64% amino acid sequence identity to a few characterized bacterial *PaaI*_thioesterase, a tetrameric acyl-CoA thioesterase with a hot dog fold described in the 4-hydroxybenzoyl-CoA thioesterase from *Pseudomonas* for 4-chlorobenzoate degradation. ThyesT_ar was expressed as a recombinant protein and overexpression metabolic advantages in presence of models substrates analyzed. Phylogenies analysis and structural modeling of protein sequence infer evolutionary relationships with thioesterases family 13. Accessing the metagenomic pool esterases can be an immediate source of novel biocatalysts, or yield enzymes that can be further specialized by directed evolution. This ascribed ThyesT_ar as a novel thiosterase with a strong potential in industrial applications. This project is supported by the National Council for Science and Technology (CONACyT) through grant
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Evaluation of antiviral and toxic effect of secondary metabolites present in fractions and methanol extracts of *Rhoeo discolor* (L'HerHance) and *Callisia fragrans* (L. Woodson) on influenza virus (H1N1)

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INTRODUCTION. Medicinal plants produce secondary metabolites with pharmacological action on other living organisms[1]. Plants *Rhoeo discolor* and *Callisia fragrans* Commelinaceae family members, are investigated by presenting important medicinal applications such as anti-inflammatory and gastrointestinal infections, antiradical, antioxidant, antimicrobial, antiviral[2]. This study objective to evaluate the effect of secondary metabolites present in methanol extracts and fractions obtained from leaves of *R. discolor* and *C. fragrans* on viral activity pandemic influenza A (H1N1).

MATERIALS AND METHODS. Methanolic extracts were obtained from leaves *R. discolor* and *C.fragrans, then* secondary metabolites were identified by thin layer chromatography(TLC), and quantified for spectrophotometric techniques followed by obtaining fractions through preparative chromatography. These fractions and extracts were used for antiviral testing in Madin Darby Canine Kidney (MDCK) cells lines, with Influenza virus AA/Yucatán/2370/2009 (H1N1).The cytotoxicity of fractions was determined in relation to cell viability of 50% (CC_{50}), and then the reduction of cytopathic effect (CPE) was evaluated., this proof allow to know the concentration inhibiting 50% (IC50) of the viral activity. For the above tests the cells were inoculate in 96-well plates at a density of $1X10^5$ cells/well and incubated at 37 °C under 5% CO_2. The virus suspension employed was a multiplicity infective (MOI) of 0.01. Thus, a binding inhibition assay in MDCK cells input is also performed by plaque assays as a positive control using 6'SLN (6-sialyl-N-acetilactosamina). Finally the RNA was extracted and the expression NP gene was analyzed by qRT-PCR.

RESULTS

Phytochemical analysis. The composition of the leaves of the plant *R. discolor* in relation to the contents of total phenols, flavonoids and saponins were 2.25mg.g^{-1}, 0.67 mg.g^{-1}, 10.32 mg.g^{-1}dry matter, respectively, while the concentrations of tannins and coumarins were 0.66 mg.g^{-1}, 0.47 mg.g^{-1}of dry matter respectively and total phenol content, flavonoids and saponins were 3.04 mg.g^{-1}, 0.71 mg.g^{-1}, 8.31mg.g^{-1}dry matter, respectively for *C.fragrans*, while the concentration of tannin and coumarins were 0.89 mg.g^{-1}, 0.51mg.g^{-1}dry matter respectively.

Cytotoxic evaluation. This was performed on MDCK cell lines. The CC_{50} values of the *R. discolor* and *C.fragrans* fractions were identified at a concentration of 0.5 to 1 mg.mL^{-1} and for the crude extracts were 4 to 7 mg.mL^{-1}.We found that any fractions or crude extract were not toxic. **Reductionof the cytopathic effect.** We assessed whether extracts and fractions possess activity to reduce or prevent the cytopathic effect, of which the selectivity index ($IS=CC_{50}/IC_{50}$) was obtained and the co-treatment in which one of the fractions *R. discolor* provided significant antiviral activity, the fraction MF1 had an IS of 32.06 with an IC_{50} value of 0.027$\pm$ 0.07 µg.mL^{-1}. MF1 probably act at the level of the hemagglutinin preventing entry of the virus into the cell.

Expression of viral RNA by qRT-PCR. To level of co-treatment was used as positive control to sugar (6'SLN). The plaque assay determined if there plaque inhibition or decrease over time. Assay the expression of NP gene of virus co- treated with 6'SLN and the fraction MF1 was analyzed by qRT-PCR method. The fraction MF1 inhibits viral replication from the 15 minutes of treatment was not observed NP gene expression, thus the positive control (6'SLN) inhibits viral replication from the 45 minutes. However, the positive control treatment expresses a number of copies of the NP gene of 74.541 representing 96.34% of inhibition viral compared to control viral expresses a number of copies of 2035.83 after 15 minutes. Therefore, there is not statistically significant difference between the fraction MF1 and 6'SLN with a confidence interval of 95%. **CONCLUSION.** The fraction MF1 of *R.discolor* plant blocks virus entry into the host cell, as an inhibitor of the fusion of the viral haemagglutinin with sialic acid from the cell, as evidenced by the NP gene copy number detected by qRT–PCR analysis.

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Zn^{2+} preconditioning triggers mechanisms that increase the cadmium accumulation capacity in photosynthetic *Euglena gracilis*.

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Zn^{2+} is and essential heavy metal that reacts with glutathione (GSH) to form the bis-glutathionate of zinc (Zn-GS_2), which one is the best co-substrate for phytochelatin synthase from the photosynthetic cadmium hyper-accumulator *Euglena gracilis* (*Eg*PCS). The *Eg*PCS is an important enzyme because phytocheatins (PCs) are necessary to carry out the inactivation and compartmentalization mechanisms against Cd^{2+}. In this way, *E. gracilis* is able to accumulate more than 0.1 $mg/g_{dry\ weight}$ of this non-essential heavy metal.

Heavy metals hyper-accumulator plants have been achieved by over-expression of PCS or other enzymes related with the mechanisms of cadmium inactivation; and these plants have been developed keeping in mind the heavy metal bioremediation of soils. Unfortunately, molecular strategies are not available for many aquatic organisms with biotechnological potential (like *E. gracilis*).

Our research group has demonstrated that a 38% increase in the Cd^{2+} accumulation capacity of *E. gracilis* is obtained by a preconditioning with 1.5 µM Hg^{2+}; however, the Hg^{2+} toxicity impede propose this protocol as a biotechnological approach to develop hyper-accumulator aquatic organisms. Nevertheless, this background shows that it is possible to increase the cadmium accumulation capacity of *E. gracilis* cells through a preconditioning with other heavy metal.

In this regard, the goal of the present work is to understand the metabolism of GSH and PCs when *Eg*PCS activity is promoted with Zn^{2+}, in order to propose a method to achieve heavy metals hyper-accumulators aquatic organisms.

The preconditioning of *E. gracilis* to Zn^{2+} was carried out with 200 - 1,500 µM during at least 10 cellular generations (2 weeks). These concentrations were selected because there are around the IC_{50} for Zn^{2+}. Results shown that the susceptibility to Cd^{2+} of cells preconditioned to Zn^{2+} is the same than control cells (grown under 20 µM Zn^{2+}); however, those cells were able to accumulate more Cd^{2+}. The highest accumulation level (108% up to control cells) was observed in *E. gracilis* preconditioned with 400 µM Zn^{2+} ($Eg_{Zn\ 400}$). In regard to PCs content, $Eg_{Zn\ 400}$ non exposed to Cd^{2+} synthesize the same levels of PCs in reference to control cells, but it shows 32 and 85% more capacity to synthesis GSH and PCs, respectively, in presence of 200 µM Cd^{2+}. This result suggests that GSH and PCs biosynthesis pathway is stimulated by the overload of Zn^{2+}, which is revealed when Cd^{2+} generates stress conditions.

In our work we discuses about the transcriptional and kinetic mechanisms involved in this phenomena; nonetheless, conclusions at this point are 1) *E. gracilis* preconditioned to Zn^{2+} triggers mechanisms that increase its cadmium accumulation capacity; and 2) There are a correlation between this phenotype and the capacity to synthesize GSH and PCs.

Micropropagation and induction of organogenesis *in vitro* of *Heliocarpus appendiculatus* Turcz and *Trema micrantha* L. Blume.

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Introduction. The "papel amate" is made from the bark of trees jonote that naturally found in rain forests and cloud forests. Considered originating of Mexico and Central America comprising different genres: *Heliocarpus appendiculatus, Trema micrantha L. Blume.* (Hatchondo,1987; Rios Morales,1999). At this time both species have the same problem that *Ficus* some decades ago, that is, they are at risk due to overexploitation. In view of this and taking into account that the rescue of some commercial species is one of the main primary lines of biotechnology as a basic tool for the improvement and spread of forest species. This work aimed to evaluate different growth regulators for induction of organogenesis.

Methodology. For shoot induction used MS medium was (Murashigue and Skoog, 1962) with a factorial design with 2 x 3 x 3 (Explants: hypocotyls and cotyledons, 3 growth regulators (BAP, Kinetin and Spermidine) at three concentrations (0.5, 1.0 and 3 mg L^{-1}).

Results and discussions. In the next table shows that higher percentage of induction of shoots in *Heliocarpus* was presented with Kinetin and BAP. *Trema* presents difference in the three growth regulators however had results with spermidine.

Results confirm that each species responds differently to the way growth regulators, which is essential for the development of individual.

% shoots by explant					
Growth regulators	**mg L^{-1}**	***Heliocarpus appendiculatus***		***Trema micrantha***	
		Hypocotyls	**Cotyledons**	**Hypocotyls**	**Cotyledons**
BAP	0.5	8.33±3.73	0.0 ± 0.0	7.5±2.5	0.0 ± 0.0
	1	8.89±4.44	0.0 ± 0.0	15±5	0.0 ± 0.0
	3	6.11±3.89	0.0 ± 0.0	7.5±2.5	0.0 ± 0.0
Kinetin	0.5	5.56±4.30	0.0 ± 0.0	7.5±2.5	0.0 ± 0.0
	1	6.11±2.99	0.0 ± 0.0	10±5	0.0 ± 0.0
	3	6.67±4.16	0.0 ± 0.0	7.5±2.5	0.0 ± 0.0
Spermidine	0.5	7.22±4.34	0.0 ± 0.0	15±5	0.0 ± 0.0
	1	7.78±3.69	0.0 ± 0.0	15±5	0.0 ± 0.0
	3	4.44±2.22	0.0 ± 0.0	7.5±2.5	0.0 ± 0.0

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A novel modelfor the study of heavy metal stress in plants.

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Key words: *Lupinus campestris,* arsenic, transformation.

In Zacatecas State,mining activities are well known for their deleterious effects on the environment. Those effects are mainly due to the deposition of large volumes of mining wastes on the soil, generating huge areas contaminated with heavy metals (HMs).Plants growingon HM-rich soils display unique adaptation mechanisms for surviving in such habitats.Previously we found that ***Lupinus campestris*** is widely distributed those HM-rich soils. The aim of this investigation is to establish the bases to work at molecular and physiological level with *L. campestris* to propose it as model to study the plant responses to HMs stress. First we performed vegetation analysis and we evaluated physical and chemical soil properties on HM-rich area where *L. campestris*is present, in order to know their ecological importance and distribution. Then we established the protocols for germination, *in vitro* stress analysis and micropropagationto obtain information about the physiological responses to HMs. Finally we generated root transformation protocols to start the molecular dissection of HMs responses. We found that *L.campestris*is the dominant specie (IVI 32.6) on contaminated area (“Francisco I. Madero” Zacatecas, México) and grows mainly in zones where Pb concentrations are high (224 ppm).To start the work on laboratory, we generated a efficient germination protocol. We evaluatedscarification treatments and we found that together thermal and mechanical treatments were necessary to obtain 100% of synchronized germination (onMurashigue and Skoog medium at 10%). We were able to micropropagate*L. campestris*, whit a multiplication rate of 3. The regenerated plants were separated in two groups, the first one was used for acclimation in greenhouse and the second one was grown on MS media supplemented whit HMs (AS, Pb and Hg) and the concentration of each HMs was measured with a X-ray spectrometer.We found that *L. campestris*is an As and Hghyperaccumulator plant. We evaluated also root development of seedlings growing on media (MS 0.1x) supplemented with HMs, we found thatthe root length decreases as the concentration of As increases. Finally we were able to generate transformed roots in*L. campestris*(70% of efficiency), using *Agrobacterium rhizogenes* (strains K599 and ARqua1). *L. campestris*is auseful tool to obtain information about the physiological and molecular responses of plant to HMs.**Acknowledgements**. Ciencia Básica CB-2010-01 00000000015685 “Búsqueda de los componentes moleculares que participan en la respuesta de *Arabidopsis thaliana* a la presencia de metales pesados”.We are thankful to Fraire-Velazquez S.,forkindlyprovidingus*Agrobacterium rhizogenes* (strains K599 and ARqua1).

Isolation and characterization of a thermostable lipase from a cDNA library of the white rot fungus *Bjerkandera adusta*

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Enzyme technology has developed new approaches for the development of strategies in productive processes. Lipases from prokaryotes and eukaryotes are enzymes used in a wide range of biotechnology applications involving synthesis and hydrolysis reactions for the production of paper, cosmetics and detergents among others. The objective of this work was to characterize a novel lipase gene isolated from a cDNA library of the Basidiomycete *Bjerkandera adusta* grown on crude oil. The gene (lipase: LipBj) consists of 1100 bp and encodes for a protein of 322 amino acids with a predicted molecular mass of 34.4 kDa. Sequence alignment revealed that LipBj has a maximum amino acid identity of 47 % and 43% hypothethical proteins from *Phanerochaete carnosa* and *Fibroporia radiculosa* respectively. The catalytic triad or LipBj was predicted to be Ser164-Asp252-His285 with Ser164 in the conserved pentapeptide GXSXG. Glycosylation analysis suggested that LipBj has potential to be N-glycosilated but not O-glycosilated. There is no predicted signal peptide according with the sequence analysis. A tridimensional modeling of LipBj shows that it has an α/β hydrolase-like folding. Preliminary results show that the recombinant LipBj, expressed from *Pichia pastoris* is able to hydrolize β-naphthyl acetate, an inespecific substrate to detect esterase/lipase activity. Moreover, the purified fraction of LipBj has activity in the presence of ρ-nitrophenyl laurate and lypolytic activity using olive oil as substrate. Biochemical characterization of LipBj revealed that it is a thermophilic esterase that exhibits optimum activity at the pH range of 6 to 7 and temperature ranges of 70-90ºC. All these properties shown by LipBj and its high thermostability may render it a potential candidate for industrial applications since thermostable lipolytic enzymes have been used in biopolymer synthesis, as well as pharmaceutical, cosmetic and biodiesel production.

qPCR to assess the robustness of a manufacturing process to remove adventitious viruses from biopharmaceuticals.

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How the intensity of an electric field can modify the physiology of *Aspergillusniger*growing in solid state culture?

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Introduction: Electric field(EF)have been used for different aims on microbial physiology, one of those can bemetabolic modificationse.*g.*enhancing protein production [1] or pollutant degradation [2]. In our group, it was demonstrated that alow intensity EF promoted an increase in hexadecane (HXD)mineralisationand low biomass production [3]. Nowadays, details of the physiologic microbial responses regarding low intensity EF are not well understood. EF can promote different microbial responses, depending on: exposure time, EF intensity and type of microorganisms involved. The aim of this work is to shed light onphysiological responses of *A. niger*exposed todifferent low intensities EF. **Methods:***Aspergillusniger* was produced in a 0.5 L horizontal cylindrical solid state culture bioreactor provided with electrochemical devices. Perlite was used as inert support; it was impregnated with mineral medium and HXD (180 mg g^{-1}) as sole carbon and energy source. A controlled EF was applied during 24 h after spores germination (4.5 d of culture), the independent variable was electric current intensity (3, 4.5 and 6 mA). A bioreactor control without EF was used to compare with. The response variables were: HXD degradation (measured by FID-GC), biomass (estimated by protein content) and spores viability (germination spores percentage). All determinations were carried out after 12 d, in duplicatedand data were then evaluated by ANOVA($\alpha < 0.05$) and Tuckeyproof. **Results:** HXD degradation was significantly affected by the highest controlledEFintensity assayed (6mA), increasing from 78% to 90% with 0 and 6 mA, respectively.Biomass production was significantly affected in all assayed EF, decreasing from 160 to 40 mg (g of dry matter)$^{-1}$with 0 and 6 mA. Our results are positive responses because the efficiency of HXD degradation (high degradation with low biomass) was enhanced with EF.Finally, *A. niger* spores viability after EFexposure decreased from 80 to 35 % with 0 and 6 mA, respectively. **Conclusion:** *Aspergillusniger* enhanced HXD degradation efficiency when electric field was applied within the assayed EF intensities. However, spores viability after EF application decreased when EF intensity increased.

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Timely detection of Mycobacterium using DNA microarrays

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Infection with the TB bacteria (*Mycobacterium avium subsp. paratuberculosis*) in animals is found worldwide. The infection occurs in different species of domestic and wild ruminants as well as various species of non-ruminant herbivores and carnivores. These organisms are aerobic bacillary form and do not form spores. Tubercle bacilli can be found in urine, colostrum, milk, semen and feces. Transmission is performed by the fecal-oral route; can also be excreted in colostrum and milk of subclinically and clinically affected females, not to mention the spread by fomites. You may also be performed in utero transmission. The disease may be be asymptomatic per years, so that the bacillus is detected only see displayed characteristic symptoms that indicate infection. The precos diagnosis of infections with Mycobacterium is of great importance, because in the asymptomatic period there is a high risk of the disease spreading without some control of it. Generally the detection of tuberculosis is made by culture and immunological methods that can last from 2-3 weeks, although in some cases it requires up to 8 weeks. In order to increase the sensitivity and specificity for infection with the tubercle bacillus, now have been testing different molecular protocols can give a precos diagnosis of the disease. In this field the PCR reaction of DNA, has provided the opportunity to obtain a rapid diagnosis with high sensitivity and specificity even in cases where the permanence disease asymptomatic, so that today the method considered vital to prevent the spread of TB in the population at large. In this regard, our group has developed a methodology for detection of Mycobacteria from samples from different sources suspected to be infected with these organisms. Our proposal consists of a DNA microarray containing specific molecular probes, distributed on-chip in the form of numerical codes, and the probes are directed against the most common mycobacteria in cattle (*avium paratuberculosis, bovis, intracellulare, smegmatis and tuberculosis*). The test is, to amplify and fluorescently mark by multiplex PCR test samples, followed by hybridization on the microarray. The method has shown robust specificity and sensitivity, giving results in a maximum of 24 hours compared with conventional distribution methods, and probes as numeric codes simplifies the interpretation of results.

Establishment of *in vitro* shoot and root cultures of *Argemone mexicana L.*

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Medicinal properties of Argemone mexicana (Papaveraceae) due to the presence of benzylisoquinoline alkaloids (BIAs) from the benzophenanthridine (sanguinarine) and protoberberine (berberine) families. The BIAs biosynthesis occurs through the entire life cycle, although following certain developmental patterns and associated to specific tissues. This suggests that the biosynthetic cell specialization could be progressively arisen. It is not clear the specific participation of the aerial and underground tissues in the biosynthesis of the different Argemone alkaloids. In order to establish the biosynthetic capacity of these parts, *in vitro* cultures of rootless shoots and of isolated roots were obtained and characterized. The phytochemical profile of the tissue cultures was evaluated by HPTLC analysis, also molecular parameters of the biosynthetic pathway were studied using RT-PCR. All data was compared withentire plants.

Enzymatic synthesis of polyphenylene disulfide-like compounds from aromatic dithiols

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Aromatic polymers containing sulfur have shown interesting characteristics, such as a high melting temperature, heat stability and semiconducting properties when compared to polymers containing other heteroatoms such as oxygen or nitrogen[1]. Polyphenylene disulfide is a polymer made up by aromatic rings connected with disulfide bonds; however, the synthesis of these polymers has not been broadly studied. These polymers can be produced by direct oxidation of an aromatic dithiolic monomer in order to generate thiyl radicals which couple to each other without the occurrence of radicals attack at any of the ring carbon positions due to the radical stabilization on the sulfur atom[2]. The monomer oxidation can be catalyzed by oxidoreductases, enzymes that constitute a clean and green option due to the use of milder reaction conditions than chemical methods[3].

In the present work the synthesis of polyphenylene disulfide-like compounds was studied using *Coriolopsis gallica* laccase (LCg) as the catalyst and 1,4-benzenedithiol as the substrate. The reaction was carried out in aqueous media (10% acetonitrile) and an insoluble product was recovered. The obtained powder was analyzed by infrared (FTIR) and Raman spectroscopy, evidencing the disappearance of thiol groups and the formation of disulfide bonds. Electronic Ionization Mass Spectrometry (EI-MS) showed the presence of the cycle tri(1,4-phenylen disulfide). This result is interesting since reactions with laccases using thiols as substrates have not been extensively reported. On the other hand, the kinetic constants of this reaction were determined with the aim of getting a complete kinetics study of the reaction mentioned above. The obtained values for these constants K_M=0.045 mM, k_{cat}=252 min^{-1} and k_{cat}/K_M=5600$min^{-1}mM^{-1}$ were in good agreement with the reported values for LCg and other fungal laccases with different substrates[4].

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Avocado snakin (*PaSn*)cDNA cloning into pCAMBIA-*PaSn* vector for enhancing fungal resistance in strawberry plants using *Agrobacterium* mediated transformation

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ABSTRACT

Antimicrobial peptides are small proteins that have been described in plants and animals, which are related to the innate defense. Snakina-1 (SN1) and snakina-2 (SN2) are the active peptides reported in *Solanum tuberosum* cv. Desiree with antifungal activity againstpathogenic fungi such as *Fusarium solani* and *Botrytis cinerea*.Homologous genes to these peptides have been identified and characterized in different plants. Recently, the gene snakin *PaSn* has been reported for Mexican native avocado (*Persea americana* var. *drymifolia*) (Gene Bank accession KC012806) and in order to determine the effect of avocado snakin (PaSn) on pathogenic fungi, there will be studies of heterologous expression of the gene coding for PaSn in transgenic plants of strawberry (*Fragaria* x *ananassa* Cv. Aromas) treated with *Botrytis cinerea*. Here, we report *PaSn* cDNAcloning into pCAMBIA2301 to obtainpCAMBIA-*PaSn* construction (Figure 1), the resultant binary vectors were introduced into *Agrobacterium tumefaciens* strain GV2260 via electroporation.Transformation of strawberry was performed by a co-culture method using crown explants and transgenic plants were selected on Murashige and Skoog agar plates containing kanamycin (150 µg/mL). The resulting lines are maintained under cultivation for their molecular characterization and evaluation of the expression of the gene *PaSn*.

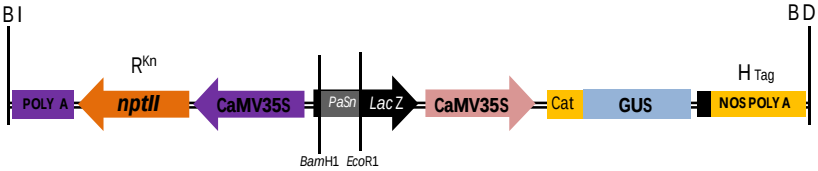

Figure 1. Construction pCAMBIA2301-*PaSn* cloned into*Agrobacterium tumefaciens*.

Assessment of cross-reactivity and neutralizing capacity of single chain antibodies directed against Mexican scorpion venoms.

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The scorpionism is a health problem that occurs worldwide. In Mexico around 300,000 cases are reported annually. From 254 species of Mexican scorpions only eight belonging to Centruroides genus are dangerous to humans. Envenoming is caused by several peptides which modify the gating mechanism of voltage-dependent Na^+ channels resulting in most of the toxicity symptoms.

Currently, scorpion poisoning is treated with anti-venom of equine origin, corresponding to immunoglobulin (Fab ')$_2$ fragments obtained from immunized horses with telson extracts of 4 species of dangerous scorpions. Alternatively, it has been proposed a more rational strategy that seeks to neutralize the venoms by means of human antibody fragments corresponding to the variable domains of the heavy and light chains known as single chain variable fragments (scFv). The strategy followed to obtain specific scFvs against toxic component of the venoms has been the combination of phage display and directed evolution. The scFvs have several advantages: small size, rapid diffusion and tissue permeability, easy expression in bacteria as well as high specificity and affinity for the antigen.

Initially in the group of Dr. Becerrril, two scFvs against the more abundant toxin of *Centruroides noxius* scorpion venom (Cn2) were identified. From these two scFvs, several matured variants capable of neutralizing other toxins and venoms of Mexican scorpions were obtained by means of directed evolution processes. In this work, the recognition of some scFvs to toxins or toxic fractions from the venoms of *C. sculpturatus* (distributed in Arizona and Sonora causing 24,000 sting cases) and *C. tecomanus* (from Colima responsible for 7834 accidents) was assessed.

The results show that the scFvs recognize with high affinity several of the toxic components of the venoms of both species. This result is explained by the high sequence identity of the toxins. In conclusion, some scFvs are promising antibody fragments which can be matured by directed evolution in order to obtain scFvs capable of neutralizing the venoms of these two species and gathered with other similar scFvs to constitute a recombinant anti-venom of human origin.

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Bifunctional gene fortrehalosesynthesis induces changes in photosynthesis in wheatplantsundersalinity

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Salinity is a major environmental factor that limits crops development and productivity, because ithas a strong effect in photosynthesis, protein synthesis energy productionand lipid metabolism.Wheat is one of the most important crops in the world;it is used in several industries and is an important staple for millions of people worldwide. However, wheat is adversely affected by environmental factors like hydric stress.The aim of the present study was to analyze the photosynthetic parametersin wheat plants transformed with *ScTPS1-TPS2* gene for a bifunctional enzyme that catalyzes trehalose synthesis under salt stress.Three lines of plants transformed with *ScTPS1-TPS2* gene under stress inducible promoter RD29A were subjected to salinity with 200 mM and 400 mMNaCl. Physiology and morphology of the transformed and untransformed plants, as well as salt stress treatment effects on gas exchange variables, chlorophyll fluorescence, and dry biomass of plants were evaluated.A decrease in gas exchange and quantum efficiencyof photosystem II in untransformed plants subjected to 200 and 400 mMNaCl was observed, and a reduction in the total dry biomass for plants treated with 400 mMNaCl.Transformed plants showed higher quantum yield, water use efficiency andcarbon assimilation efficiency. In conclusion, plants overexpressingthe bifunctional enzyme showed tolerance to salinity.

Purification and identification of peptides with antimicrobial activity present in the venom of *Scolopendra viridis*

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Antimicrobial peptides (PAMs) identified to date have been classified according to their charge, anionic or cationic. Close attention to the PAMS has been focused since they can be excellent candidates for the development of new antimicrobial agents. In fact, some PAMs have already entered to clinical trials, for example: Magainina, an Antimicrobial Peptide identified from Xenopus laevis has been used to treat diabetic foot ulcers (Rossi et al., 2008). Many antimicrobial peptides from arthropods, such as insects and scorpions, have been intensely studied (Müller et al., 2008). Three peptides with antimicrobial and antifungal activity in *Scolopendra subspinipes mutilans* have been reported (Wenhua et al., 2006; Peng et al. 2009). The objective of this work is to identify and characterize antimicrobial peptides contained in the venom of Scolopendra viridis. Venom from S. viridis was collected by mechanical stimulation and quantified by the Lowry method. The separation of proteins from venom was carried out by two different methods: anionic-exchange chromatography using as eluant a buffer in gradient (20mM to 1 M CH_3COONH_4 pH 4.7) and HPLC using a gradient (A: 0% H_2O 0.1%TFA to B: 60% CH_3CN 0.1%TFA) in 60 min. Fractions obtained from both methods were tested for antimicrobial activity. The test was conducted by the agar diffusion method against S. aureus (ATCC 29213) at a concentration of an OD of 0.070 to 0.13 (λ 600 nm) by placing 3 to 5µg of each fraction to be analyzed; ampicillin was used at a concentration of 5 mg/ml as positive control. To identify the molecular weight of proteins present in fractions with activity a 12 % SDS-PAGE was conducted. Individual proteins for each fraction with activity were tested too. Four proteins of various sizes (26, 41, 61 and 118 kDa) showed antimicrobial activity. Finally, hemolytic activity of these proteins was assayed in human eritrocytes.

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Heterologous expression of intrepicalcin, isolated from the venom of scorpion *Vaejovis intrepidus*

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Introduction: Scorpion venom is a complex mixture of biologically active peptides which exhibit a variety of biochemical activities and pharmacological functions. The toxin family which affects calcium ion channels is called calcins, characterized structurally by having an inhibitory cysteine knot motif. These toxins can penetrate cellular membranes and alter calcium release from ryanodine receptors. From a cDNA library of the Mexican scorpion *Vaejovis intrepidus* a calcin like sequence was identified. The peptide was named intrepicalcin. This communication describes its heterologous expression and function. **Materials and methods:** The nucleotide sequence of the gene coding for intrepicalcin was obtained by means of the PCR technique. The PCR product was ligated to the expression vector Pet22b. The *E. coli* BL21DE3 strain was used for transformation, expression and purification of the peptide by affinity columns, followed by high performance liquid chromatography. **Results and discussion**: The correct construction of the gene coding this peptide was confirmed by sequence analysis. The recombinant intrepicalcin was also confirmed by direct amino acid sequencing and mass spectrometry analysis. The effect of the recombinant intrepicalcin was assayed electrophisologically using artificial membranes, but also by means of binding of [^{3}H]ryanodine to skeletal muscle vesicles containing ryanodine receptors. Intrepicalcin (10 nM to 1µM) enhances the binding of [^{3}H]ryanodine to the channels, induces the appearance of a long-lasting sub-conducting state of the channel and stimulates the release of calcium from sarcoplasmic reticulum vesicles. **Conclusions:** The electrophysiological effects recorded from the intrepicalcin clearly shows that it is working in a manner analogous to other known calcins. It is the first calcin isolated from this species of scorpion, and it is the first calcin ever expressed heterologously.

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Study of genes involved in the stress response in *Saccharomyces cerevisiae* and their effect during Agave juice fermentation.

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According to the Mexican norm:NOM-006-SCFI-2005, the Tequila is defined as a “Regional alcoholic beverage distilled from must” that is prepared from the heads of the *Agave Tequila Weber* Blue Variety, hydrolysed or cooked, and then the must could be blended or not with other sugars to a ratio not higher than 49% of total reducing sugars expressed in units of mass and it is subjected to alcoholic fermentation with yeast, cultivated or not.

S. cerevisiae is traditionallyethanol-producer;however, the performance during the fermentation step depends largely on its ethanol tolerance, and it is known that these yeasts are sensitive to higher concentrations of ethanol.Accumulation of ethanol in the medium is a stress factor and it causes inhibition of cell growth and viability, and finallyaffects the ethanol yield. For these reasons, it is desirable that the yeasts used in the production of fermented beverages have a high ethanol tolerance.

The yeasts havea general stress response that involve genes expression related to various functions such as protein synthesis, amino acid metabolism, transport,membrane and cell wall organization.However, there are genes that are expressed under these conditions whose function is unknown. In 2005, Madrigal Pulido analyzed the response of the tequilera strain AR5 of *S. cerevisiae*, at 7% ethanol shock during 30 min and compared with a laboratory strain (S288c) by microarrays assays. He found that 16% overexpressed genes encoded for proteins not classified.Some of these genes arethe open reading frames, *YLR177W* and *YNR034W-A*

In order to study the role of the genes *YLR177W* and *YNR034W-A*, the goals of this project were:

Overexpress the *YLR177W*and *YNR034W-A*genes individually in the *S. cerevisiae*Tequilera strain and laboratory strain. These strainswere characterized by assessment of growth in the presence of ethanol, the ethanol shock response, and performance in high gravity agave juice fermentation.

Subcellular localization of the proteins encoded by the genes*YLR177W*and *YNR034W-A*by GFP tagging.

In general, we found that the overexpression of these genes confers higher ethanol tolerance and the sub cellular localization of the proteins codifies by the open reading frames is cytosolic.

FUNGAL ENDOPHYTES OF *Taxus globosa* SCHLTDL., POTENTIAL PRODUCERS OF SECONDARY METABOLITES FOR BIOLOGICAL CONTROL OF *Phytophthora capsici* AND *Pythium* sp., PLANT PATHOGENS OF MAJOR AGRICULTURAL CROPS.

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The "endophytes" are microorganisms (most of them fungi and bacteria) living within cellular tissues of plants, and apparently they don´t cause any symptoms of disease. In the last decade the endophytes emerge as a new proposal that has been gaining importance in areas such as medicine, agriculture, etc. Endophytes as fungi have the ability to produce bioactive secondary metabolites (Strobel, 2003), which in medicine have been used for drug manufacturing or agriculture for the production of new products used in the control of plant pathogens of economically important crops in Mexico, as Solanaceae. These crops are affected by many kinds of fungi such as *Phytophthora* sp., *Pythium* sp., *Fusarium* sp., *Alternaria* sp., among others, high pathogenicity and aggressiveness that may kill entire fields caused by their spread ease (FAO, 2010). The present work was made to demonstrate the biological activity of endophytic fungi isolated from *Taxus globosa* tree from Oaxaca to control the mycelial growth of plant pathogens *Phytophthora capsici* and *Pythium* sp., through the establishment of double culture, so the extract of these fungal endophytes were evaluated by microdilution tests on a 96-well plate. The results obtained were positive because the endophytes inhibited the growth of phytopathogens. This study showed that *T. globosa* endophytes are able to control the growth of pathogenic fungi and can be proposed as a new alternative to biological control.

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Growth kinetics and effect of pH on the emulsifying activity of the bioemulsifier produced by *Acinetobacterbouvetii*using hexadecane as organic phase

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Background: Some strains of *Acinetobacter*genera are capable to produce an extracellular compound with emulsifying capacity, also has been proved that the chemical nature of the bioemulsifier (BE) depends on the species of the genera which are able toproduce it (1), the BE production by *A. bouvetii* has been reported for first time by our research group (2), and its chemical structure could be different tothosepreviously reported.The aim of this work was to evaluate the emulsifying activity (EA)in the culture brothat different pH values. Such a pH range will allow us to establish the working conditionsto maintain dispersed an organicphase through a continuous aqueous phase.**Methodology:** A pre-inoculum culture was prepared in nutrient broth, then one liter of mineral medium with 7.6 $g \bullet L^{-1}$ hexadecane (HXD) as a carbon source was inoculated with *A. bouvetii* in anAir Lift bioreactor with Ug=0.6 $cm \bullet s^{-1}$. The growth kinetics, pH analysis andEA were performed for a total of 72 h. The kinetic parameters were calculated using the Gompertz model. The EA was used as an indirect measure of production of BE. The EA of the cell-free supernatant at 48 h growth was then analyzed to determinate the pH effect (3.0 to 9.0 range values) by the technique reported by Rosenberg et al. (1979), in brief: different buffer solutions weremixed with a HXD:2-methylnaphthalene (1:1) mixture (1), after vigorous shaking the optical density was measured, an EA unit was defined as the ability of 1 mL of supernatant to raise 0.1 units the absorbance measured at 600 nm**.Results:** The stationary growth phase occurs after 24 culture hours.The growth rate in the exponential phase corresponded to μ=0.546 h^{-1}.The pH varied from 6.5 to 7.2 and growth from 6 to 8.82 Log $UFC \bullet mL^{-1}$ after 48 h. The EA significantly increased after 18 h and reached maximum value (11.5units) during the stationary phase. The EAof the supernatant at the end of 48 h showed significant differences in response between the higher assayed pH (9.0) and the lower pH (α = 0.05, Tukey).**Conclusions:** The EA profile and *A.bouvetii*growth kinetic suggested thatEA was partially associated to biomass. The BE was much moresensitive to acidic pH than those assayed basic values.

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Overproduce of the leucine aminopeptidase yspII of *Schizosaccharomyces pombe* in the *Pichia pastoris* yeast methylotrophic

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The leucine aminopeptidases (LAPs; EC 3.4.11.1) belongs to the M17 family of the metalloenzymes. The LAPs release leucine from the N-terminal of the proteins and peptides. Have recently had an important use in biotechnology, like the recombinant LAP of *A. proteolitica* produced by *E. coli*, this can remove the N-terminal methionine from interferon alpha-2b human. In the laboratory, the Herrera-Camacho group (2007) describe in the *Schizosaccharomyces pombe* fission yeast, the leucine aminopeptidase yspII (**LAPyspII**) like a 320 kDa protein who depends from manganese and can form hexameric complex. However, there is not any work about the recombinant expression of the active enzyme LAPyspII. Therefore, in this study, a heterologous expression system was used in the yeast *P. pastoris* is capable of overproducing recombinant LapyspII active.

From chromosomal DNA of S. pombe strain 972 h- , the LAPyspII open reading frame of *ape2* gene (1542 pb) was amplified from PCR.The PCR product was ligated to the *Pichia* expression vector to obtain the recombinant plasmid pPICZaB- LAP2. The plasmid was incorporated into cells competent of X33 *Pichia pastoris* and the clones were named X33-LAP2. With PCR was determined the obtaining
strains with phenotype Mut$^+$, for overexpress LAPyspII we used BMMY medium
(24 hours) and after for 4 days feeding with methanol. The recombinant protein was detected by western blot in the extracellular fraction and for determine the activity enzyme, we use the specific substrate (leucine p-nitroanilide), who has a good enzimatic activity.

With this project we produced the recombinant LAPyspII from *S. pombe* in the *Pichia pastoris* expression system. The protein was recovered in soluble form and active like a hexamer in the *Pichia pastoris* extracellular fraction. The enzyme recombinant was identified by western blot and activity enzymatic; whose production reaches the highest scores after 4 days of expression with methanol. Finally this work is important because is the first time that produces a hexameric active enzyme in *Pichia pastoris*, with excellent results for biotechnology purpose.

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Neural induction of bone marrow mesenchymal stromal cells and olfactory neuro-epithelial cells.

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The therapy of neural replacement is a promising strategy for the treatment of some neurological diseases; though an optimal source of cells for transplants is still missing. Human bone marrow mesenchymal stromal cells (hBM-MSC) and human olfactory neuro-epithelia precursor cells (hONEPC) have been proposed as such a source because i) they are human adult multipotent cells, ii) they can be obtained by relatively simple procedures, iii) their use do not rise ethical concerns, and iv) if treated appropriately they have the capacity to differentiate into neurons. It is of interest, then to define the best experimental protocols for proliferation and differentiation of these cells.

In the present study, hBM-MSCs and hONEPCS were isolated and characterized as previously described by Castro-Manrreza et al. (Stem Cells Dev 2014, 23:1217) and Benítez-King et al. (J Neurosci Methods 2011, 201:35). The capacity of these cells to generate neural progenitors-like cells was evaluated. Cells were seeded on normal or low adherence plates, and cultivated in specific media to promote growing of neural progenitor cells. N2/B27 or human NeuroCult™ proliferation media containing hEGF and hFGF were used in this study. The morphology, viability, cell number, and changes in the expression of the neural marker nestin were evaluated.

In low-attachment plates, neurosphere-like cellular aggregates were formed in the two culture media. Viability was unaffected but there is no evidence of proliferation monitored by $BrdU^+$ assays. Cells seeded in normal plates do not form floating aggregates. There is an increase in the cell number, assayed by $BrdU^+$. Also, the number of $nestin^+$ cells increases in a time dependent manner, so that after of 30 days almost all cells are $nestin^+$. These results establish an effective protocol to obtain neural stem/progenitor cells from these multipotent somatic cells of human origin. Studies on the expression of other neural progenitor markers and of neuronal differentiation are in progress in our laboratory.

Functional analysis of the *WIP* gene as a developmental regulator in the liverwort *Marchantia polymorpha*

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The non-vascular plant *Marchantia polymorpha* belongs to the Marchantiophyta (liverworts), which is considered a key group for understanding the genetic basis of the innovations that allowed green plants to evolve from their aquatic ancestors. An increasing number of transcriptional regulators has been associated to the emergence of these multicellular organisms that colonized the terrestrial environment, suggesting that the increased complexity of genetic regulatory networks may have promoted this emergence, and it might also have promoted the appearance of vascular plants. To understand how complex gene regulatory networks evolve and trigger different phenotypes that can result in speciation, it is first necessary to elucidate individually the functions of the transcriptional regulators involved.

In this context we are interested in studying the possible role of *WIP* genes in *M. Polymorpha*. The WIP proteins belong to the C2H2 Zinc Finger family, and the WIP subgroup is composed of six members in the model plant *Arabidopsis thaliana.*

In our investigation we found a single gene with sequence similarity to the WIP subgroup in *M. polymorpha*. In *A. thaliana*, WIP1 is important for correct seed development, WIP6 for correct vasculature development and *WIP2* has been proposed to act at the transmitting tract and replum of developing gynoecia and fruits. Its absence affects the correct development of this tissue and has therefore been named *NO TRANSMITTING TRACT (NTT).* However, we have found that *WIP2* is also present at the Shoot Apical Meristem (SAM) and interacts with known SAM regulators. In this work, we are interested in comparing the role of the *WIP* gene in *M. polymorpha* to the role of *WIP2*, its closest orthologue in *A. thaliana.*

Effect of iodine deficiency on the migration and invasion of 3AsubE human trophoblasts cells.

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In early weeks of gestation a low oxygen atmosphere is required for cytotrophoblast differentiation into villous cytotrophoblast cells, in order to invade the uterine spiral arteries, this physiological process is essential for the development of maternal-fetal circulation and nutrients exchange (including iodine) and gases (oxygen). This process is altered under oxidative stress conditions caused by chronic hypoxia, which can alter the differentiation and invasion of cytotrophoblasts, through regulation of hypoxia responsive genes. The HIF and Snail transcription factors can regulate genes involved in trophoblastic differentiation, fusion and invasion causing a shallow invasion and a poor spiral artery remodeling, causing preeclampsia as a consequence. In thyroid gland, iodine deficiency elevated intracellular ROS levels, activating genes involved in angiogenesis as HIF. However, the functional role of iodine in differentiation, migration and invation of trophoblast cells is unknown. Therefore this paper analyzes the effect of iodine deficiency in cell migration and invasion processes using an *in vitro* model of normal human trophoblasts cells (3aSubE). It also analyzes the expression of iodine transporters and HIF and Snail transcription factors participation.
Methodology: The trophoblasts 3aSubE cells were growth at 1x106 for 24 hours, then where treated with 25 mM KClO4 (NIS blocker) and 1.0 mM DIDS (pendrin inhibitor) for 24 hours in presence or absence of CoCl2 (stress inducer) with or without lugol or KI at 5-100µM, subsequently cell viability was analized by the exclusions trypan blue method. Cell migration was measurement with woung healing motility assay and cell migration in the Boyden camara during 0-24 hours with iodine transporters, in presence or absence of lugol or KI. The expression of iodine transporters NIS, pendrin and the HIF and Snail transcription factors were analyzed by endpoint PCR. Results are expressed as mean±standard deviation. Data were analyzed statistically using ANOVA and t-studen. P=<0.05 was considered significant respect to its control. **Results:** The treatments with KClO4, DIDS, CoCl2, lugol and KI did not significantly affect cell viability relative to the negative control. The results show that iodine deficiency affects cell migration, which is significant in trophoblasts treated with 50 and 100 µM lugol at 12 and 24 hours of exposure (p=<0.05) with and without CoCl, compared with treated with KI. When treated with 50 and 100µM lugol increased cell motility, these is observed to close the wound, as well as increased cell migration (p=<0.05). PCR results shown that trophoblast 3aSubE cells express NIS and pendrin iodine transporters and a decrease of HIF and Snail. **CONCLUSION:** Iodine transport in human trophoblast 3aSubE cell line could be mediated by NIS and Pendrine. Results suggest that iodine levels are important in migration and invation trophoblast cells mechanism mediated by HIF and Snail. **Grants:** This study had financial support from Public Health Institute of Universidad Veracruzana grants POA 2013-2014 and CONACyT grant no. CB-2012-01-176513.

Effect of L-carnitine and a phosphorus analog of L-carnitine on *in vitro* insulin-like response

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Diabetes mellitus (DM) affected 382 million people worldwide in 2013 and is estimated to increase to 592 million in 2030. In Mexico it is the leading cause of death in women and the second in men. Antidiabetic drugs in use have a number of adverse events such as gastrointestinal disturbances, hepatotoxicity and weight gain. Recent studies have shown that administration of L-carnitine stimulates production of insulin-like growth factor 1 (IGF-1), which promotes insulin-like response. Our working group synthesized a phosphorus analog® of L-carnitine from amino acids in order to improve their pharmacokinetic properties and decrease toxicity. The aim of this study was to determine the effect of the L-carnitine analog and L-carnitine on adipogenesis and insulin-like response in 3T3-L1 preadipocytes. Nontoxic concentration of each compound resulted in 10 g/mL, rosiglitazone (RGZ) was used as positive control at a concentration of 10 μg/mL. Adipogenesis was induced using DMEM supplemented with fetal bovine serum at 10%, 5 μL/100mL insulin, 0.5 mM IBMX and 0.4 μg/mL dexametoasone, the insulin resistance was induced with 10 ng/mL of TNF-α. The insulin response was assessed by the accumulation of triglycerides determined colorimetrically or by incorporating a glucose analog 2-NBDG by fluorometry. No adipogenic effect on preadipocytes was observed by the L-carnitine or L-carnitine analogue in the absence of insulin; however both compounds increased the insulin-like response in resistant adipocytes. Currently the possible mechanism of action of L-carnitine and the L-carnitine analog is studied.

Palabras clave: Adipogenesis, adipose differentiation, L-carnitine, insulin resistance, insulin-like response.

Characterization of cyclins B1;2 and B2;1 duringmaize germination.

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The succession of events that give rise genetically identical cells in a growing organism is the cell cycle. Itcomprehendsfour sequential phases: G1, S, M and G2. Cyclins are the regulatory partners of a Cyc-Dependent Kinase (CDKs) and together drives the cell across the cell cycle.Catalytic activity is conferred on the CDK at various levels through various protein-protein binding domains and through phosphor-regulation of specific amino acid residues in CDK. Perhaps the most spectacular event is the timed synthesis and destruction of the non-catalytic Cyc that regulates CDK activity.

Cyclins B are mainly synthesized during G2 phase and first retained in the cytoplasm due to a cytoplasmic-retention signal (CRS) region, at G2/M it migrates to the nucleus.As cells enter mitosis there is a burst of protein phosphorylation largely due the activation of a pool of cyclin B/CDK complexes.

In this work we analyzed the abundance of ZmCyclinB1;2 and ZmCyclinB2;1 in maize embryo axes, throughout germination, finding a peak of both proteins at 18 and 24 hrs with a moderate sucrose concentration in the imbibition media. The ZmCyclinB1;2/CDK and ZmCyclinB2;1/CDK related activity showed a peak mirroring protein abundance.

In contrast, embryos in media without sugar or with higher sucrose or glucose concentrationsestablished a differential ZmCyclinB1;2 patron: showing a peak at 24 h in the case of sugar starvation, a peak at 12 h on sucrose and an earlier peak at 6 h on glucose. This result suggests that sugars might alter differentially cell cycle timing on embryo axis during germination.

Preliminary results suggest that ZmCyclinB1;2 and ZmCyclinB2;1 could form complexes withZmCDKA or CDKB. More work is in progress to unravel potential associations with other proteins participating inthe cell cycle such as PCNA and CKS.

IL-6 ROLE DURING PROLIFERATION INDUCTION IN MCF-7 CELLS TREATED WITH ELDERLY SERUM.

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Aging is considered a risk factor to develop chronic diseases and cancer. One feature that relates aging with cancer is chronic inflammation, which has even been called “inflammaging” and is the most important frailty syndrome cause in elderly. A cellular mechanism that can increment pro-inflammatory cytokines is cellular senescence through the senescent associated secretory phenotype (SASP). TNF- α and IL-6 are characteristic SASP components, which along with other cytokines induce phenotypic changes in adjacent cells and are able to promote cell proliferation, migration and even ephitelial-mesenchymal transition. The aim of this work was to incubate MCF-7 cells, a breast human cancer cell line, with serum obtained from elderly people (60-90 years), in order to determine if the SASP components present in elderly serum (ES) induced changes in MCF-7 proliferation rate. MCF-7 were incubated with ES for 24, 48, 72 and 96 h. Cellular viability was determined by tripan blue assay and proliferation with the commercial cell counting kit-8 (CCK-8).Interleukins and growth factors in ES were determined with the commercial kit Bio-Plex Pro™ Human Cytokine, Chemokine, and Growth Factor Assays. IL-6 receptor (IL-6R) mRNA was assessed by semi-quantitative PCR. Our results showed that ES did not affect MCF-7 viability during the different time points measured. ES were analyzed by three different criteria: 1) Age, 2) gender,
3) frailty. Different groups were formed depending on the donor age (<65; 66-70;
71-75 y >76). In that case, all ES groups stopped MCF-7 proliferation after 48 and
72 h incubation, when compared with their controls (proliferation with FBS-5%). While at 96 h, MCF-7 incubated with ES groups 66-70 and 71-75 increased their proliferation against control and ES groups <65 y >76. When ES was analyzed by gender, the same effect was observed, proliferation stopped at 48 and 72 h incubation, and at 96 h, male and female ES increased proliferation against control. The last analysis was perormed in function of frailty. ES from frail, pre-frail and non-frail also stopped cell proliferation at 48 h, but at 98 h the frail ES tend to increase MCF-7 proliferation.
Since these criteria did not represent the differences in proliferation, ES was examined in order of their capability to induce cellular proliferation: low, medium and high. ES that induced higher proliferation presented higher IL-6, IL-10 and GM- CSF, and lower IL-8 concentrations against controls, and did not show differences in IL-2, IL-4 and TNF-α. IL To determine IL-6 role in proliferation, MCF-7 were incubated with ES and IL-6 specific antibody, in that case proliferation decreased to control levels. These data show that age, gender and frailty condition did not alter MCF-7 proliferation, moreover the increase in proliferation is related to higher IL-6 and lower IL-8 concentration in ES.

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Expression of theDystrophin-AssociatedProteinComplex in Human AdiposeTissue

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Background: Thedystrophin-associatedproteincomplex (DAPC) islocated in plasma membrane of myocytes and neuronswhereitprovidessolidmechanicalconnectionbetweencytoskeleton and extracelular matrix [1]. DAPC absence in sarcolema leads to muscular dystrophies and mental retardation [2]. DAPC isalsoexpressed in ratadipocytes [3], and β-sarcoglycannullmice are glucose-intolerant and insulinresistant [4]. Theseresultssuggestthat DAPC isinvolvedadiposetissuephysiology and glucose homeostasis, although DAPC expression in human adiposetissueisyetundocumented. Therefore, weanalyzedthe gene expression of the DAPC components in human subcutaneous and visceral adiposetissue and in differentiatingprimary human adipocytes.
Methods: The gene expression of DAPC componentswasanalyzedby RT-PCR in explants of subcutaneous and visceral adiposetissues of euglycemic normal weight and overweightadultmaledonors. Preadipocytesderivedfromthesetissuesweredifferentiated in culture toanalyzetheexpression of DAPC in thedifferentstages of human adipogenesis.
Results:Subcutaneous and visceral adiposetissuesfrom normal weight and overweightdonorexpressedmRNAsfor Dp427 and Dp71 dystrophin, α and β-dystrobrevins, dystroglycan, αI, βI and βII-syntrophins and α, β, γ, δ, and ε-sarcoglycans. Theexpression of dystropphin Dp71 isdiminishedrelativetohousekeeping gene GAPDH in bothtissues. WealsofoundthatmRNASfor Dp427, Dp71, α and β-dystrobrevins and α-sarcoglycan are differentiallyexpressedduringdifferentstages of subcutaneous and visceral adipogenesis. Other DAPCelements, dystroglycan, αI, βI and βII-syntrophins and β, γ, δ, and ε-sarcoglycansdidnotchange in adipogenesis.
Conclusions:Genes codifyingfor DAPC are expressed in human subcutaneous and visceral adiposetissuesfrom normal weight and overweighteuglycemicmaledonors, and Dp427, Dp71, α and β-dystrobrevins and α-sarcoglycan are diferentiallyexpressed in differentstages of humansubcutaneous and visceral adipogenesis.

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PAPILLOMAVIRUS ONCOGENES E6/E7 AND ESTRADIOL PROMOTE MOUSE EAR REGENERATION BY DIFFERENT MECHANISMS

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The E6 and E7 oncogenes of the human papillomavirus HPV16are highly expressed in the skinof the transgenic mouse line Tg(K6b-E6/E7) (Tg). These Tg mice regenerate their hair follicles continuously skipping the telogen resting phase, and close ear holes more efficiently than wild-type (Wt) animals by promoting re-epithelization and growth (Cell Growth Differ. 11:527-39, 2000; J Invest Dermatol. 128:2894-903, 2008). Notably, the regeneration phenotype is more evidentin females than in males, suggesting that sex hormones play a role. In order to determinehow female hormones, particularly estradiol, influence regeneration, we studiedear hole regeneration under different hormonal conditions. Unexpectedly, ear hole regeneration efficiencywas higherin adultthan in young female mice, and this effect was more evident in the ones carrying the E6/E7 oncogenes. In agreement with the participation of estradiol,reduction of its levels by ovariectomy or the intraperitoneal injection of a selective estrogen receptor modulator (Raloxifene) decreased ear regeneration efficiency in Wt mice, but this effect was not observed in Tg mice, which retain their increased regenerative capacity. These data suggest that estradiol and E6/E7 oncogenes mediate its effect on regeneration by a different mechanism. Interestingly, expression of genes associated with oxidative phosphorylation increased in the tissue in regeneration ofTgmice but not in that of Wt mice, though a downregulation is apparent in ovariectomized animals;this effect might be mediated by Lin28, which also appeared to be up-regulated in Tg but not in Wt mice. The critical role of oxidative phosphorylation in regeneration was supported by the inhibitory effect of metformin which down-regulates this metabolic pathway.We propose that E6/E7 oncogenes promote tissue renewal rather than growth, a phenomenon that is associated withincreased oxidative phosphorylation. In contrast, estradiol might promote regeneration by favoring the recruitment of epidermal progenitors to the injured tissue, as suggested by the inhibitory effect of this hormone on hair follicle regeneration and increased growth of interfollicular epidermis. In conclusion our data suggest that increased regenerative capacity can be caused by distinct cellular and molecular mechanisms.

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ERK1/2 are involved in acrosome reaction through its activation by FAK

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The sperm genome is highly compacted and consequently they do not show transcriptionand translation, therefore these cells depend on post-translational modifications to complete their maturation. In somatic cells one of the post-translational modifications more important is the phosphorylation that is carried out by kinases such as ERK1/2. ERK1/2 are present in mammalian spermatozoa, assigning roles in capacitation, motility and acrosome reaction, additionally these kinases might be involved in the sperm survival. Several signaling pathways have been suggested for ERK1/2 activation inmammalian spermatozoa; however, the ligand andthe receptor responsible of such activation are unknown in mammalian spermatozoa. One potential protein tyrosine kinase that may regulate ERK1/2 is focal adhesion kinase (FAK). The aim of this study was to determine the role of ERK1/2 in capacitation and acrosomal reaction, as well as participation of FAK on the regulation of ERK1/2during capacitation in guinea pig spermatozoa.The results obtainedby immunofluorescence showed thatERK1/2are dispersed throughout theacrosomeand themiddle piece, such locationchangedduring capacitation, migratingto theapicalacrosomalsegment and alongof the flagellum. Using an anti-p-ERK1/2 antibody, we describe the activation kinetics ofERK1/2, whichshowedthat these kinasesareactiveinearlycapacitation,reached its highestactivityat 10 minutes of capacitation. Direct inhibitionofERK1/2throughFR180204, a specificantagonist of ERK1/2, significantly inhibitedthe acrosome reactionbutnocapacitation, suggesting the possibility thatERK1/2participatein the regulation ofthe acrosome reaction. The results also showthat FAK inhibition, by PF573228-antagonist,significantlyreduced ERK1/2 activation. It is known thatFAKphosphorylationatY925 is required forERK1/2activation. Our results demonstratedthat Y925 phosphorylationoccurs duringcapacitation,from 3 minutes,and it was maintained untilthe60 minutesof capacitation.PF573228-antagonistalso inhibited the FAK phosphorylation at Y925.Together,the results ofthis study indicate thatERK1/2are activatedduring capacitation, although their effectsare visible onlyinthe acrosome reaction,andthatFAKis importantfor regulatingthe activity ofERK1/2inguinea pig spermatozoa.

Glial cell-derived Neurotrophic Factor favors the differentiation of mouse embryonic stem cells to motorneurons

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Glial cell-derived neurotrophic factor (GDNF) was first characterized for its capacity to promote dopaminergic neuron survival; afterwards, this effect was shown to be broader for other kind of neurons such as sensorial, cerebellar and motor neurons (MN). In the latter case, it is also proven that GDNF promotes the establishment of the neuromuscular junction and increases neurite length and synaptic vesicle size of MN. However, little is known about the role of GDNF during MN differentiation.Here we produced double transgenic mouseembryonic stem cells (ESC) that express: 1)*HB9*-driven GFP that is expressed in fully committed MN and, 2) EF1α-driven GDNF, constitutively expressed in pluripotent ESC.We differentiated MN by anembryoid body methodology, which briefly consist in growing cell aggregates in suspension for 6 days, with retinoic acid and Sonic Hedgehog agonistadded at the second day of culture.

Cells that overexpressed GDNF produced higher numbers of total cells at the end of the differentiation protocol. All fully committed MN should express at least one of the two so-called pan-MN markers,namely HB9 and Islet1.We found that GDNF overexpression increased the percentages of GFP+ cells from 20% to 50%, and also the proportion of Islet1+ cells from 16% to 46% of the total number of cells. Co-immunostaining of both markers resulted in higher proportions of double-positive neurons in GDNF-overexpressing cells (39% of total cells vs 12% in controls). GDNF also augmented β III tubulin-positive neurons from 35% (control) to 54% of the total cells.

These results demonstrate that GDNF is not only capable of increasing the survival of MN but also favors the differentiation process probably by expanding the number ofneural precursor cells committed to differentiate to MN.

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Glucose and sucrose have a differential impact on embryo axes during maize germination

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Sugars could have a dual role on plant cells, acting as signaling molecules as well as donor of carbon backbones and energy. Sucrose (Suc) can be sensed as a signal directly or a signal may arise via its hexose cleavage products: glucose (Glc) or UDP-Glc and fructose. It is well established that Glc triggers mitotic activity in developing tissues, while Suc is rather associated with the regulation of storage and differentiation-related processes.

The aim of this work was to analyze at the cellular level the effect of Suc and Glc on maize embryo axes during germination and post germination.

Embryos imbibed on control conditions (sugar starvation) for 7 days showed a marginal growing without differentiation. When Suc was included to the media there was an important embryo axes growing. Around day 4 protrusions of lateral roots was evident. In contrast, maize embryo axes on Glc showed a growing similar to Suc treatment but no lateral roots were observed.

At the cellular level, root meristems showed a smaller cell size and a higher cell quantity per area on embryo axes imbibed on Glc at 24 h than control, while on Suc the cells were bigger and fewer cells per area unit could be detected.

To evaluate the proliferative rate, EdU (a thymidine analogue) was incorporated to embryo axes in order to follow *de novo* DNA synthesis. At 24 h of imbibition cells at meristematic region showed an important EdU incorporation on Glc treatment compared to Control or Suc.

Overall, the results suggest that Glc stimulate cell proliferation while embryo axes under Suc stimuli are prone to differentiate more than to proliferate.

Ustilago maydis: A comparative microarray analyses at different stages of basidiocarps development.

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Basidiocarps are the characteristic fruiting bodies of Basidiomycota, where basidia are formed. They are classified into simple and complex structures. Simple basidiocarps have only a hymenium on its surface, while complex also contain pileus and stipe.

Species belonging to the Basidiomycota phylum are divided into the subphyla Agaricomycotina which form basidiocarps, Pucciniomycotina which do not form basidiocarps, and Ustilaginomycotina, in which members of the Exobasidiomycetes class form simple basidiocarps, and Ustilaginomyctes class which do not form basidiocarps (1).

In 2012, Cabrera-Ponce *et al*. described that under controlled conditions, the species *Ustilago maydis* is able to differentiate and form basidiocarps. In this process, a differentiation from the yeast form to mycelial shape takes place, further developing the basidiocarps.

To achieve a deeper understanding of this phenomenon, we proceeded to analyze the differential gene expression occurring in the process. A single-channel microarrays (Nimblegen) were used for these analyses.

We observe that during the process of young basidiocarp formation from mycelium, around 2100 genes, representing a third of the whole genome, were differentially regulated, about half of them being up-regulated. Using the Funcat (MIPS) program they were divided into different functional categories.

The categories containing a larger number of genes were Metabolism, and Non-classified proteins, but the categories of Interaction with the environment, Differentiation-cell fate, and Cell cycle were also represented by a substantial number of genes.

Homologues of some genes described in the literature as important for the development of fungal fruiting bodies were identified to be up-regulated during basidiocarp formation: hydrophobins, ortho-methyltransferase, helicase and fatty acid synthase (3, 4, 5). We are in the process of mutation of these genes.

Some important conclusions can be remarked: a) Contrary to the literature, members of the Ustilaginomycetes form basidiocarps, indicating that their evolution to a parasitic mode of life did not involved loose of genes, but only their silencing. b) The process of basidiocarp formation in Ustilaginomycetes probably involves similar mechanisms to the rest of Basidiomycota.

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The *Podospora anserina* endoplasmic reticulum-shaping proteins and their role in sexual development.

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The endoplasmic reticulum (ER) is a continuous membrane system composed of different structural and functional domains. The ER peripheral domains consist of an interconnected network of tubules and sheets that closely interact with most other organelles of the cell. The formation, function and dynamics of organelles like peroxisomes and mitochondria importantly depend on the ER. In the laboratory we are interested in understanding the dynamics and interactions of organelles during cellular development. We use the filamentous fungus *Podospora anserina* as model system, and we have found that deletion of different peroxisome biogenesis factors affects different steps of its sexual development. Moreover, we know that specific mitochondrial functions are also required for progression through sexual development, and that the dynamics and function of this organelle are affected by specific peroxisome assembly defects. We are interested in understanding how the ER participates in orchestrating the intracellular dynamics during development. Here, by studying the localization of the ER translocation channel protein Sec63, we aim first to understand the dynamics of the ER during sexual development. Then, we want to understand the role of the *P. anserina* reticulon and Yop1 proteins in this process. Reticulons and Yop1 are integral membrane proteins that maintain the tubular shape of the ER. They are present in all studied eukaryotes and posses a 200-amino acid domain, which forms a hydrophobic W-shaped hairpin that promote high-curvature ER tubules. These proteins are thus necessary to maintain the arrangement of the peripheral ER and, conceivably, they could be important to establish the different domains and interactions of the ER. Furthermore, reticulons and Yop1 have been implicated in mediating physical interactions between the ER and other organelles, namely peroxisomes. We have identified a *P. anserina* gene (Pa_1_22550), which presumably encodes for the single reticulon protein of this fungus, as well as two genes (Pa_2 _5730 & Pa_ 4_3260), which presumably encode isoforms of Yop1 (designated YOP1 & YOP2, respectively). Homologous recombination-based gene-deletion experiments are currently in progress to understand the function of these proteins in shaping the ER, and the relevance that this process has on the progression of the sexual development of this fungus.

Regulation of seed structure and root development by the glutamate-carboxipeptidase altered meristem program 1(AMP1)

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Abstract

AMP1 is a gene that encodes an endoplasmatic reticulum membrane-localized glutamate-carboxipeptidase involved in plant growth and development. Mutation of this gene cause early flowering, altered shoot apical meristem, constitutive photomorphogenesis, increased level of cytokinins and cyclin cycD3 expression, delayed germination and hypersensitivity to ABA. Here, we show that *amp1-10* has three classes of seed phenotypes: i) normal WT-like seeds, ii) raisin-like seeds and iii) seeds with protruding embryos. Interestingly, these different classes of seeds correlate with alterations in both embryo and seed coat structure, as revealed by electronic microscopy. Since the germination is a process regulated by internal and external factors that begins with capture of water by the mucilage present in the seed coat and finish with emergence of primary root and cotyledons, we tested whether AMP1 seeds have normal responses to both abscisic acid and gibberelic acid during germination. The delayed germination of *amp1* mutants previously described was attributed to high levels of ABA in these mutants, however, the phenotype alterations evidenced in our research were found to correlate with altered cell division in the root meristem. In fact, some individuals from raisin-like seeds and seeds with protruding embryos never germinate even with GA treatment, which remarks the important roles of the seed coat and proper embryo development on the germination process.

Molecular characterization of auxin transport inhibitors and their role in root morphogenesis

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Abstract

Many aspects of plant development are dependent upon the distribution of the phytohormone auxin. It is transported in a polar manner through plant tissues from shoots to roots with a local re-distribution in root tips. This polar transport causes an asymmetric distribution of auxin between cells that triggers developmental processes such as embryo and organ development, vascular pattering, apical dominance and tropisms.

Auxin transport inhibitors are essential tools for investigating the polar auxin transport and its effects on plant development. One of the most widely used inhibitors is 1-naphthylphthalamic acid (NPA), which acts as a specific IAA efflux blocker via targeting the ABCB transporters. However, because of the pleiotropic effects of NPA on root systems such as inhibition of primary root growth, gravitropic responses, and lateral root development, it is difficult to identify particular cellular targets of auxin transport and its local gradients. Therefore, the use of synthetic molecules, which are capable of affecting auxin homeostasis in a tissue or organ specific manner, may help to better understand the molecular mechanism of auxin action. In this work, we performed a screen of molecules analogous to NPA to find out compounds with less pleiotropic effects on root architecture. Our research identified naphthyl ethyl phthalamic acid (NEPA), which differs structurally from NPA due to an additional carbon and metil group. Unlike the effects observed when using NPA, plants treated with NEPA did not show agravitropic roots and the primary root growth was less inhibited. Interestingly, NEPA affected lateral root formation in a dose dependent manner, indicating that either lateral root initiation or elongation is a particular target of this compound.

Regulation of root hair development in *Arabidopsis thaliana* by *Pseudomonas aeruginosa* and related quorum-sensing signals

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Abstract

Root hairs are long, tubular outgrowths of specialized epidermal cells that play important roles in nutrient and water uptake, organic compounds secretion, and plant-microbe interactions. Because root hairs contribute as much as 77% of the root surface area, they provide the major point of contact between the plant and the rhizosphere, where roots interact with several bacterial communities. The *Pseudomonas* genus includes numerous species that live in association with plant roots, thus affecting developmental programs. Recently, it was demonstrated that in *P. aeruginosa*, the QS system *lasI* can modulate root hair initiation and elongation. Nevertheless, there is limited information about the regulatory networks underlying these organogenesis programs. Here we show that *P. aeruginosa* and QS mutant *lasI* modulate expression of genes involved in root hair development in *Arabidopsis*. Moreover, the *P.aeruginosa lasI*-triggered root hair initiation and elongation requires functional auxin-ethylene machinery. Our data indicate that hormonal factors mediate *Arabidopsis-Pseudomonas* interaction, which interfere with postembryonic root hair developmental programs.

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TRPV4 channel is necessary for appropriated establishment of the tight junctions in corneal epithelium and regulates its barrier function in combination with EGF.

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We are interested in identifying ionic channels that regulate the proliferation and/or differentiation of epithelial cells. In this work we used the spontaneously immortalized cell line RCE1-5T5, derived from rabbit corneal epithelium, because it reproduces *in vitro* early stages of differentiation of this tissue. After screening for ionic channels differentially expressed between proliferative and differentiated RCE1-5T5 cultures by RT-qPCR, WB, and confocal microscopy we observed that TRPV4 channel in proliferative cells is located in the nucleus and cytoplasm of the cells while in differentiated cells is in the apical membrane of the more external cells layer in the stratified epithelium. In spite of this, its mRNA levels remains constant and protein channel decreases 3.5 folds when cells differentiate.
TRPV4 is a cationic channel, activated by hypotonic cell swelling, heat, endogenous agonist like arachidonic acid and endocannabionoids and synthetic specific agonists 4-αPDD and GSK1016790A (GSK101). It has been reported that TRPV4 mediates the regulatory volume decrease behavior in human corneal epithelial cells. TRPV4 also regulates the epithelial barrier function of tight junctions (TJ) in skin keratinocytes, mammalian gland cells and lung endothelium. In epithelial cells, the development of tight junctions is a key step of differentiation, because, in addition to seal the intracellular space, TJ confer cell polarity and regulate the paracellular flow.
With these antecedents we first demonstrate by calcium imaging that TRPV4 is functional in RCE1-5T5 cells and induces an increment of intracellular calcium in the presence of its specific activator GSK101 (100 nM) which is avoided by treatment with RN1734 (30 µM) an specific blocker of TRPV4 or EGTA. We then analyzed the role of TRPV4 on TJ recording the Transepithelial Resistance (TER) of RCE1-5T5 cultures grown in presence of GSK101 or RN1734. We found that TRPV4 is necessary for the establishment of TJ. In mature epithelia, activation of TRPV4 increases the TER value. This effect seems to be mediated by an increase expression of claudin 1,2 and occludin.
It has been reported that TRPV4 as heteromer with TRPP2 forms an EGF activated channel at the apical membrane of renal cells. On the other hand, EGF depletion reduces the TER in RCE1-5T5 cells. Therefore we evaluated the possible participation of TRPV4 in regulation of TER in RCE1-5T5 by EGF. We found that activation of TRPV4 can enhance the values of TER in absence of EGF and the blockade of TRPV4 avoids the normal increase of TER even in presence of EGF.
In conclusion our results suggest that TRPV4 is necessary for the correct establishment of the barrier function of TJ in corneal epithelia. Additionally in differentiated epithelium activation of TRPV4 regulates the barrier function of TJ increasing the values of TER and it is also involved in the regulation of TJ by EGF.

UTP induces migration and EMT in ovarian cancer cells.

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Ovarian cancer is the 5th-leading cause of death in occidental countries, and only 30% of the patients survive 5 years after the initial diagnosis. This lethality may be related to its metastatic ability, since 70% of patients show disseminated metastasis in the peritoneal cavity. Enhanced motility and invasiveness are two basic properties of the cancer cells, and they are essential for the metastatic process; it has been proposed that the acquisition of this invasive phenotype is mediated by the *epithelial to mesenchymal transition* (EMT). It has been reported that Epidermal Growth Factor (EGF) is a potent inducer of EMT, and also that its receptor (EGFR) is subject to transactivation by cellular messengers acting mainly through G protein-coupled receptors (GPCRs).

ATP has important roles in intercellular communication mediating autocrine and paracrine signaling. Recent evidence strongly suggests that the purinergic system also participates in tumor growth via a regulatory loop that promotes proliferation through at least three mechanisms: 1) activation of tumor cell metabolism, 2) stimulation of ATP efflux into the tumor interstitium, and 3) increased expression of purinergic receptors. ATP can activate two different families of receptors, P2Y, which are GPCRs, and P2X.Other evidence links purinergic signaling to EMT and migration induction, and the P2Y2 receptor has been shown to induce EGFR transactivation. The aim of this work is to study the possible role of P2Y receptors in the induction of migration and EMT through transactivation of the EGFR in the carcinoma-derived cell line SKOV-3.

First, we studied the effect of different P2Y agonists on cell migration, and found a stimulatory effect of UTP (205 ± 38 % of basal); SKOV-3 cells express P2Y2, P2Y4, P2Y11, and P2X7; however, the responsiveness toward UTP indicates the participation of P2Y2 and/or P2Y4 receptors. This response was inhibited by preincubation with the EGFR inhibitor AG1478 (10 nM; 108 ± 19 % of basal) or with the PI3K inhibitor wortmanin (1 nM; 96 ± 19% of basal), suggesting the participation of EGFR and PI3K/AKT pathways. Also, 100 μM UTP was able to induce the phosphorylation of EGFR.

To investigate if this effect on migration involves the EMT, we also tested by immunofluorescence the effect of UTP on the expression level and cellular distribution of vimentin and E-cadherin. We found that UTP increases the expression of vimentin and diminishes the expression level of E-cadherin. Although control and UTP-stimulated cells differed in the abundance of E-cadherin, both showed a cytoplasmic distribution indicating a mesenchymal phenotype; the addition of the ectonucleotidase apyrase to the culture medium maintained the epithelial phenotype with E-Cadherin expression in the cell-cell contacts. All together, our data suggest that in SKOV-3 ovarian carcinoma cells, ATP induces an increase in cell migration involving EMT; it acts through P2Y2 and/or P2Y4 receptors by a pathway that requires EGFR kinase activity.

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Dystroglycan depletion inhibits the functions of differentiated HL-60 cells

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Dystroglycan, best known as a central component of the dystrophin associated protein complex in muscle, is a ubiquitously expressed cell adhesion molecule with crucial roles in the assembly of the basement membrane, muscle integrity, cell adhesion and signalling. Loss of these functional roles for dystroglycan give rise to distinct disease phenotypes including muscular dystrophies, severe neurological phenotypes and adenocarcinoma.

Dystroglycan has been characterized in blood tissue cells as part of the dystrophin glycoprotein complex. Recently, it was observed that upon neutrophil activation β-dystroglycan localized together with actin filaments in lamellipodia and pseudopodia, suggesting a role of β-dystroglycan in the migration and trafficking of neutrophils, but to date nothing is known of its role in the differentiation process of neutrophils. In this work we investigated the role of dystroglycan in the human promyelocytic leukemia cell line HL-60 differentiated to neutrophils.

Firstly, we performed western-blot, qRT-PCR and confocal analysis to evaluate the protein pattern expression, mRNA levels, and subcellular distribution of dystroglycans in differentiated and non-differentiated HL-60 cells. Immunoblot and qRT-PCR assays reveled that dystroglycan protein levels and mRNA expression were increased in differentiated cells compared to non-differentiated cells. To ascertain if dystroglycan is involved in the HL-60 cells differentiation process, we transfected HL-60 cells with a shRNA to knockdown dystroglycans expression, then, transfected cells were differentiated with DMSO and subjected to migration, differentiation, phagocytosis, morphological and respiratory burst assays. Differentiation and morphological analysis showed that knockdown cells fail to express CD11b (differentiation marker) and exhibited a diminution in the number of filopodia. Furthermore, migration, phagocytosis and respiratory burts activity were also diminished in dystroglycan depleted cells compared with control cells. Our results suggest that dystroglycan is a key protein for the maturation of HL-60 cells.

Notch-1 and Notch-2 show differential activity on cell proliferation and differentiation of rabbit corneal epithelial cells.

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Tissue homeostasis requires the maintenance of a rigorous balance between cell proliferation and differentiation. Such balance is regulated by highly conserved molecular signals. In particular, Notch receptors were identified as key components of the mechanisms that regulate either binary cell fate decisions, or the activation of cell differentiation and cell proliferation. To understand how this balance is maintained in mammalian corneal epithelia, we analyzed the participation of Notch and its ligands in controlling rabbit corneal epithelial cell proliferation and differentiation. As experimental model, we used the RCE1(5T5) cell line, which mimics the differentiation of corneal epithelial cells. End point RT-PCR showed that RCE1(5T5) cells express Notch-1 and Notch-2 receptors as well as their ligands Jagged-1, Jagged-2 and Delta-1. By qRT-PCR experiments, we evaluated the expression profile of Notch-1 and Notch-2 during growth and differentiation *in vitro* of RCE1(5T5) cells; and found that Notch-1 is mainly expressed during proliferative stage of the cultured cells, while Notch-2 prevailed in cell cultures that initiated differentiation and began to form a stratified epithelium. Immunostaining experiments revealed that Notch-2 had a suprabasal localization in K3-keratin positive cells, in agreement with the expression of its encoding mRNA; whereas Jagged-1 showed a nuclear localization in some cells, maybe explained through the proteolytic cleavage of its COOH-terminal end. The results are highly suggestive of a Notch pathway with a bidirectional effect on neighbor cells. The stimulation of RCE1(5T5) cells with a soluble form of Jagged-1 lead to a decrease in the proliferative rate and an increased expression of K3 keratin. Conversely, cell cultures treated with a soluble form of Notch-1 showed an increased proliferative ability. Our results suggest that Notch-1 and Notch-2 exert a differential effect on corneal epithelial cells: while Notch-1 modulates proliferative potential of corneal cells, Notch-2 seems to be directly involved in the expression of the differentiated phenotype.

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Unusual nuclear localization of TRPV4 ionic channel in renal epithelial Cells (MDCK)

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ABSTRACT

The Transient Receptor Potential Vanilloid 4 channel, TRPV4, is a Ca^{+2} and Mg^{+2} permeable cation channel involved in many different cellular functions such as osmoregulation, mechanosensitivity and nociception, among others. Several mutations on TRPV4 gene are related with skeletal dysplasias, and moto-sensory neuropathies. As an ionic channel, the expected subcellular localization of TPRV4 is in the plasma membrane. However, we have seen that TRPV4 is expressed in the nuclei of cultured canine kidney epithelial cells (MDCK) when the cells are grown in subconfluence conditions, whilst in confluence conditions, TRPV4 was observed in the cytoplasm and cilia. So far, there are no reports about the nuclear localization of TRPV4 in epithelial cells, although there are some reports that show the nuclear localization of membrane proteins structurally similar to TRPV4. For the above mentioned, the aim of this work was to analyze this unusual nuclear localization of TRPV4 in MDCK cells. We obtained protein extractsof nuclear and cytoplasmic fractions of MDCK cells after 24 h and 7 days of culture (subconfluence and confluence conditions) as well as a total protein extract. Thereafter, a series of Western Blot analysis were performed in order to visualize the expression of TRPV4. We identified TRPV4 expression in the total and cytoplasmic protein extracts from both conditions whilst in the nuclear extractsTRPV4 was only present in subconfluence conditions. Furthermore, we found that the TRPV4 expressed in the nuclei of subconfluent cells did not correspond to the expected molecular weight of 100 kDa but to a higher one suggesting a potential posttranslational modification. These results demonstrate the unusual nuclear localization of TRPV4, a completely novel characteristic for this well-studied protein. Additional work is necessary to determinate the possible biological function of nuclear TPRV4 channel in MDCK cells.

Assessing the nitric oxide production in the basal land plants: the moss *Physcomitrella patens* as model system

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Nitric oxide (NO) is a gaseous molecule involved in plant development and physiology. There are two enzymatic mechanisms for NO production in the plant kingdom: 1) the nitric oxide synthase (NOS)-like enzymatic activity which uses L-arginine to generate NO and citrulline and 2) the nitrate reductase (NR) enzyme which reduces nitrate to nitrite, and nitrite to NO. Most of the studies regarding NO production in the plant kingdom have been performed in green algae and higher plants. In green algae, NO is produced mainly by NR whereas in higher plants NO can be produce by both, the NOS and NR mechanisms. In the basal land plants (i. e. mosses, liverworts and hornworts) there is no information about NO production and its physiological roles. In order to gain insight about NO enzymatic synthesis in basal land plants, we adopted the moss *Physcomitrella* patens as a model system. With the use of electroparamagnetic resonance spectroscopy and confocal laser scanning microscopy we detected NO in protonemal tissue. Moreover, our results indicate that the moss *P. patens* produces NO in a NR-dependent manner, suggesting that this mechanism of NO production has been present during the Viridiplantae evolution.

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Analysis of the Biological activity of the Paired Domain-lacking isoform of the transcription factor PAX6 (PAX6ΔPD) in mammalian corneal epithelium differentiation

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Pax6 is the master control gene for eye morphogenesis; it codes for a highly conserved homeotic transcription factor. In mammals, three PAX6 isoforms were identified; of these, PAX6ΔPD lacks the paired domain (PD), which is one of the DNA binding domains. By now, the function of PAX6ΔPD is unknown, although it is possible that it acts as an antagonist of the canonic protein. We studied the function of PAX6, PAX6(5a) and PAX6ΔPD during corneal epithelial development using RCE1(5T5) cells as a model. Overexpression of PAX6ΔPD resulted in a significant increase in proliferation, in contrast with PAX6 or PAX6(5a) overexpression. As expected, cells overexpressing PAX6 underwent differentiation, since they were K3 keratin (CK3) positive, which is a terminal phenotype marker; on the contrary, PAX6ΔPD overexpression blocked differentiation leading to very low or nonexistent levels of CK3. PAX6ΔPD may affect Pax6 function through protein-protein interactions. Bimolecular Fluorescence Complementation assays (BiFC) showed the existence of strong interactions between Pax6ΔPD and the other two isoforms. As a result of this analysis, we detected high affinity between the homeodomain and the paired domain of Pax6 (HD vs. Pax6PD) and between homeodomains of all these proteins (HD vs. HD), suggesting the importance of HD for these bindings. In order to understand the interaction of PAX6ΔPD with other transcription factors, our future experiments will analyze the mutation of a conserved motif in HD, which causes the loss of the protein-protein binding capacity as occurs for the Antennapedia homeodomain.

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α6 integrin as a marker of rabbit corneal epithelial cells with migratory ability: Study in RCE1(5T5) cell line.

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Integrins mediate cell-extracellular matrix interactions, they are involved in 'outside-in' and 'inside out' signaling, as well as they regulate both physiological and pathological processes. α6β4 is one of the integrins expressed in the corneal epithelium. It participates in the hemidesmosomal assembly, adhesion processes, and probably in regulation of cell proliferation, and migration. We studied the expression of α6β4 integrin in cultured RCE1(5T5) cells which *in vitro* mimic the differentiation of mammalian corneal epithelium. Our results show that both isoforms of α6 integrin (α6A and α6B) are expressed in RCE1(5T5) cell line, being α6A the predominant isoform. Also, we observed that in proliferative cultures, the expression level of α6 integrin was high, as also seen for the transcription factor ΔNp63α which is a putative stem cell marker and a possible corneal epithelial cell proliferation marker. In contrast, K3 cytokeratin which is associated with the terminal phenotype was no detected. As cells underwent differentiation, α6 integrin and ΔNp63α expression decreased whereas that of K3 increased. Additionally, immunostaining of proliferative cultures revealed that α6 integrin, plectin and vimentin cytoskeleton partially co-distributed at the leading edge of the migrating cells. Taken together, our results suggest that undifferentiated corneal epithelial cells with high migratory potential express stem cell or early progenitor cell markers. It is possible that cell motility is dependent on vimentin cytoskeleton expression and its interaction with extracellular matrix through α6β4 integrin and plectin.

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The estructure and function of *Ustilago maydis* proteinase A

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Proteinase A (PrA or PEP4Um) is an aspartyl proteinase found in the vacuoles of *Ustilago maydis,* a Basidiomycete dimorphic fungal pathogen of maize *(Zea mays)* and causal agent of corn smut. The role of the PEP*4Um* in *Ustilago maydis* was investigated by generating deletion mutants in *a1b1* and *a2b2* using a hygromycin resistance cassette (Hyg^R) as the selectable marker.

pep4um is 1257 bp and is located in chromosome 15, encodes for a protein of 418 amino acids. The protein sequence contains the characteristic conserved motifs of aspartyl proteases in the active sites $VILD^{125}TGSSNLWV$ and $AAID^{307}TGTSLIAM$ and a putative N-terminal vacuolar targeting signal sequence of 22 amino acid residues.

The mutant strains were corroborated by PCR and Southern blot of yeast genomic DNA for interruption of *pep4*um. The mutants showed a slower growth rate than wild-type strains in YPD and YNB liquid media. Mutant strains were affected in dimorphism induced by a pH change and in the presence of palmitic acid. Additionally, we observed that these mutants exhibited reduced mating and pathogenesis in corn.

In order to demostrate the role of *pep4um* in dimorphism and pathogenesis, we performed complementation analysis with plasmid p*pep4*-pCbx122 carrying the wild-type *pep4um* gene and carboxine as selectable marker. Transformed *U. maydis* were recovered and confirmed by PCR. As expected, the wild-type phenotype was recovered. The complemented strains shown to be as virulent as the wild-type strains and the dimorphic transition was unaffected.

In summary, our data are evidence that PEP4Um is involved in cell growth, morphogenesis, and pathogenicity in *U. maydis*.

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Cellularlocalization of the RE-1 SilencingTranscription factor in LungCancercelllines

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ABSTRACT

Higher eukaryotes express many REST (RE1-SilencingTranscription factor) isoforms, which are differentially expressed throughout the development. Whereas non neuronal tissues require canonical REST expression to repress neuronal phenotype, in mature neurons truncated REST isoforms antagonize the REST function, allowingthe phenotype maintenance. In neuroendocrine Small Cell Lung Cancer (SCLC) the loose of REST function have been observed, however, the presence of REST isoforms is not determined. Here, we studied the expression profiles and localization patterns of REST in MRC5, A549 and H69 cells by means of western blot and confocal microscopy.Cell fractioning and western blot evidenced significant loose of nuclear REST and the presence of cytoplasmic truncated REST (tREST) isoforms in H69 cells, which was confirmed by confocal microscopy. Interestingly, canonical REST was localized in membranes. Altogether, our results in H69 cells depict the REST profiles observedinneuron progenitors during neurogenesis, suggesting its role for the neuronal fates acquisitionin SCLC.

Effect of PPAR agonist compounds on adipociteand myocitedifferentiation, and changes in energy metabolism ofmesenchymal stem cells from pigs'bone marrow.

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Mesenchymal stem cells (MSC) are multipotent, able to differentiate into several lineages. The aim of the study was to evaluate the effect of PPAR agonistson differentiation and metabolic features of porcine MSC induced to adipogenic or myogenic lineages.To determine the species'ideal age forcell isolation, two ages were used: 5-7days old (Exp.1) and 0-2days old (Exp. 2). Only the cells of Exp. 2 expressed MSC markers (Oct4, Nanog, CD90, CD44, CD45 and ABCG2). Cell growth curves during 15 subcultures were measured to determine proliferation capacity, with 4200 and 5300 total cells for Exp.1, and Exp.2 ($P \leq 0.0001$), repectively. We explored the conditions that presentedadipogenic (AD) or myogenic differentiation (MD). Messenger RNA expression of PPARγ (AD), MyHC (MD) and energy metabolism-related genes were measured by qPCR.For AD, cultured cells after 4thpassage in previous medium were transferred to adipogenic medium supplemented with rosiglitazone (1, 10 and 30µM), telmisartan (5, 10 and 20 µM) or conjugated linoleic acid (CLA)9-*cis* 11-*trans*and 10-*cis* 12-*trans* (12.5, 25 and 50µM). For MD, in 10thpassage myogenic medium were added with either bezafibrate (100, 200 and 400 µM), telmisartan or CLA 10-*cis*12-*trans*(12.5, 25 and 50µM), and L165 (10, 20 and 40µM). In both cases,the results showed an ability of CLA to promote adipose and muscle differentiation ($P \leq 0.0001$) depending on the isomer used, which is a clear sign of the opposite effects reported for these compounds. To determine if differentiated cells were functional,metabolic changes were measured by qPCR. Treatment with ALC 9-*cis*11-*trans* showed to be the most adipogenic and expression levels tended to beupregulated($P \leq 0.05$) for HX2, ACCAa, ATGL, LPL and G6PD, while downregulated for PFK and ACCAb ($P \leq 0.05$). For the MD the most myogenic treatment it was ALC 10-*cis* 12-*trans* and changes in expression for enzymes wereupregulated ($P \leq 0.05$) for PFK, ACCAb, CPT1, G6PD and remained unchanged for HX2 and ACCAb ($P \geq 0.05$). Our results suggest that differentiated cells exhibit a typical cell lineage metabolism and present higher efficiencies bothin anabolism and catabolism.

Sperm survival mediated by integrins

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The storage of sperm in female genital tract is a widespreadreproductive strategy in the animal kingdom. During reproduction, the sperm are stored in specialized organs, where they can remain viable for several days to years before fertilizing the egg. In mammals millions of spermatozoa are deposited during ejaculation in the female reproductive tract, but only a few thousand enter into the fallopian tube. In this arduous journey through the reproductive female tract towardsfertilization, spermare transiently adhered to the epithelial cells lining of the caudal isthmus, the lower region of the oviduct; this interaction has been suggested to extend its fertile life. Several molecules such as spermadhesins, sulfated glycolipids and lectins, found in the sperm rostral face, are suggested that can mediate the sperm-epithelial cells binding, but no explain as the sperm remain alive and fertile. Integrins are associated with various processes such as adhesion, migration, proliferation and cell survival;these transmembrane proteins have the ability to connect the cytoskeleton withextracellular matrix (ECM) proteins, forming focal adhesion contacts, which activate a series of signaling cascades that influence cell survival. Therefore, the objective of this work is to investigate the presence of integrins in guinea pig sperm andwhether these proteins mediate the sperm adhesion to ECM proteins such asfibronectin, increasing the sperm life.Our results show: 1) By Western blot theintegrins α3β1 and α6β1 were detected in guinea pig spermatozoa. 2) By immunofluorescence α3β1 and α6β1 were found in the apical acrosmal region and in the flagellum. 3) Sperm are able to bind to fibronectin matrix, such interaction was inhibited for: an anti-fibronectin antibody, previous sperm incubation with fibronectin and by the tripeptide sequence RGD, which competes bythe integrins site recognized by fibronectin. 4) Interaction of sperm with fibronectinincreased the sperm life (4-8 times) compared with sperm capacitated in suspension. In conclusion, integrins expressed in guinea pig sperm could be involved in the sperm interaction with ECMs proteins find in oviductalepithelial cells, such as fibronectin. Per se, sperm-fibronectin matrix interaction could also increase the sperm life.

AfeA, TmpA and TmpB are membrane enzymes that regulate asexual development in *Aspergillus nidulans*

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In the fungus *Aspergillus nidulans*, conidiation is triggered by environmental signals such as exposure to air, starvation for nutrients and the presence of self-generated chemical signals, depends on the activity of the Zn-finger transcription factor (TF) BrlA. The inactivation of genes *flbA* to *E*, *fluG* and *tmpA* results in similar *fluffy* phenotypes, characterized by delayed conidiophore and conidia formation. FlbA is a regulator of heterotrimeric G protein signaling. *flbB* and *flbC* encode transcription factors needed for proper *brlA* expression. *flbD* encodes a Myb TF that regulates both asexual and sexual differentiation. *fluG* and *tmpA* encode enzymes needed for the production of independent extracellular signals required for *brlA* activation.

Here we show that *afeA* as new gene whose inactivation causes a *fluffy* phenotype and decreased *brlA* expression. AfeA is a member the adenylate-forming superfamily most similar to unknown function coumarate ligase-like (4CL-Lk) and acetyl-coA ligase enzymes. Functional AfeA::mRFP and GFP::AfeA proteins were localized in globular structures along the cytoplasm, as well as in hyphae and the plasma membrane of hyphae and conidiophores. We also analyzed the localization of tmpA and tmpB, which are putative transmembranal proteins that also participate in asexual development. Tagged GFP::TmpA and TmpB::BFA showed a similar localization pattern, being detected in plasma membrane and septa in growing mycelium as well as in conidiophores. The plasma membrane localization of three different enzymes that regulate conidiation is consistent with their role in the production of extracellular signals that regulate conidiation.

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"Transcription-associated adaptive mutagénesis in DNA-repair deficient *Bacillus subtilis* cells"

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ABSTRACT

In nature, the soil bacterium *Bacillus subtilis* may face selective pressures that limit its growth. To overcome such conditions, this microorganism must keep transcription and protein synthesis active. The genetic changes that permit microorganisms to proliferate under growth-limiting conditions occur through a cellular process termed adaptive mutagenesis[1]. Several hypothesis have been postulated to explain such phenomenon; one of them proposes that populations with different physiological traits can be produced in a cell culture, including a hypermutagenic subpopulation where genetic diversity can be generated[2, 3]. It has also been suggested that in non-dividing cells, the RNA polymerase increases its error frequency generating mutant messenger RNAs that produce proteins with altered functions that allow the cell to exit from of its growth arrestment, replicate its genome and fix a heritable mutation[4]. Interestingly, experimental evidences have revealed that highly transcribed genes are the most vulnerable to suffer base alterations due to the formation of secondary structures in the non-transcribing strand, thus making transcription the nexus between physiology and mutagenesis[5].

The genetic and physiological basis of the transcription-associated-mutagenesis process in non-dividing cells and whether such phenomenon occurs in a specific subpopulation are currently unknown. Therefore, in the present work we investigated how changes in transcriptional levels affect the generation of mutations in non-dividing *Bacillus subtilis* cells proficient or deficient for guanine oxidized (GO) and base deamination (Ung, YwqL) repair systems. To this end, we developed a system to directly detect transcription associated loss and gain of function mutations in the reporter*gfp* gene. The levels of transcription in this system are controlled with riboswitches in stationary phase liquid cultures of non-replicating (Trp^-) *Bacillus subtilis* cells bearing a termoconditional mutation in the helicase-loader encoding *dnaB*gene and/or starved for tryptophan. We report here the characterization of this system using fluorescence microscopy, fluorometry and flow cytometry techniques; moreover, the strategy that will be employed by means of fluorescence activated cell sorting, to isolate the putative hyermutagenic subpopulations and its further molecular and physiological characterization will be also discussed.

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Analysis of Type I-like and Type II-like telomeres of telomerase-negative survivors of *Ustilago maydis* by PCR amplification and Bal31 digestion kinetics.

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Telomerase, the cellular reverse transcriptase, is a ribonucleoprotein enzyme- complex responsible of the telomere lengthening in most eukaryotic cells. It is composed by a catalytic protein telomerase reverse transcriptase (TERT) and a telomerase template RNA (TER). Mutations in any of the core components have profound effects onthe proliferative capabilityand the cell viability. Intelomerase- negative *est2* mutants of *Saccharomyces cerevisiae*, telomeres shorten, cells senesce, the replicative potential ceases and cells die.Eventually, survivors arise which replicate and maintain telomere function beyond senescence by alternative- lengthening recombination-based pathways. Based on the structure of the terminal restriction fragment (TRF) of chromosomes, survivors are classified in two types: Type I survivors, which exhibit tandem amplification of the subtelomeric Y' element followed by small tracts of telomeric $C_{1\text{-}3}A/TG_{1\text{-}3}$ DNA; and Type II survivors, which show very long and heterogeneous tracts of $C_{1\text{-}3}A/TG_{1\text{-}3}$ DNA at chromosome termini. The growth and maintenance of the two types of telomere structures have specific genetic requirements, involving more than a dozen genes. In post- senescent telomerase-negative mutants of *U. maydis* two mainTRF hybridization patterns that match with those of *S. cerevisiae* were obtained. However, to associate *U. maydis* TRF patterns with those of yeast, significant differences between chromosome termini of the two fungus must be taken into account:(a) In *U. maydis,* telomeric TTAGGG arrays shape homogeneous tracts whereas variations of the $C_{1\text{-}3}A/TG_{1\text{-}3}$DNA motif occur in yeast telomeres. (b) *U. maydis* has a conserved border adjacent to telomere repeats, of which the yeast chromosomes are devoid, (c) Telomere associated Y'-elements are conserved in sequence and length in yeast, whereas evidence indicates that *UTASa* subtelomeric element of *U. maydis* has length polymorphisms. To confirm the similarity in structure and organization of TRF in *U. maydis* survivors, we conducted a series of heterozygous crosses of Type I-like and Type II-like survivors with the wild type 520 strain. The telomere structure and organization of mutant survivors prevail in the postmeiotic progeny from heterozygous diploids in non-Mendelian fashion. The inherited telomeric structure is preserved in haploid progeny for many generations, in trt^+ or trt^-, regardless of the inheritance of the functional *trt1* allele in haploid cells, which suggest haploinsufficiency of Tert1. Time course of *Bal*31 digestion of the telomere DNA from 521 parental strain, *trt1*-disrupted mutant, and the W204 progeny showed that TRF in W204 is a combination of Type I and Type II survivor TRF, which was also more resistant to *Bal*31 digestion that those of parental or dirsrupted mutant. The hybridization TRF patterns suggest the Type I-like, and Type II- like survivors, could correspond to their counterparts in yeast.

Determination of lenghts of the amplified subtelomeric elements by sequencing and mapping, could give us further insights of nature and size of the Type I-like survivor amplicon.

Expression and subcellular localization of Dp71 isoforms in PC12 cells: colocalization with β-dystroglycan and α-syntrophin

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Several alternative splicing of dystrophin Dp71 have previously been described. Dp71, Dp71a and Dp71c, deriving from intact exon 78, are grouped in Dp71d; Dp71b, Dp71ab and $Dp71_{\Delta 110}$, with splicing of exon 78, are included into Dp71f group; Dp71e and Dp71ec, that retain part of the intron 77, correspond to the Dp71e group. Dp71a, Dp71ab and Dp71e also lack the exon 71 while Dp71c, Dp71ec and $Dp71_{\Delta 110}$ lack exons 71 to 74. Each group has a unique C-terminal that imparts specific characteristics to Dp71 isoforms. In this study, we explored the expression of Dp71 isoforms in PC12 cells at cDNA level as well as the localization and colocalization of recombinant Myc-Dp71 proteins. We found that PC12 cells express Dp71a, Dp71c, Dp71ab, Dp71e and Dp71ec isoforms. The localization and colocalization analyses, in undifferentiated and nerve growth factor (NGF)-differentiated cells, showed that Dp71a, Dp71ab and Dp71e localize and colocalize with β-dystroglycan and α-syntrophin in the cell periphery/cytoplasm. A faint staining of these Dp71 isoforms was observed into the nucleus and Dp71a was increased in NGF-differentiated cells as we previously reported. Dp71c and Dp71ec were mainly localized in the periphery of undifferentiated and differentiated cells and did not colocalize with β-dystroglycan and α-syntrophin. Interestingly, an increasing of Dp71e and Dp71ec was observed into the nucleus of NGF-differentiated cells. All Dp71 isoforms were also localized in the neurite extensions. In conclusion, PC12 cells express Dp71a, Dp71ab, Dp71c, Dp71e and Dp71ec isoforms. The cellular localization is determined by splicing of exons 71 to 74 and the specific C-terminal end. Dp71a, Dp71ab and Dp71e colocalized with β-dystroglycan and α-syntrophin.

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Detection of DNA of preys in the diet of a generalist predator: threshold in the number of detectable preys and time consumption

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The whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) is a major pest of tomato. Pest populations can be controlled using natural enemies under biological control programs. However, natural enemies do not always effectively control pests. To improve the effectiveness of natural enemies is important to clearly assess their trophic relationships. An effective method to determine trophic relationships is by finding DNA of preys in the digestive gut of predators by PCR. A first step of this method and the aim of this study is to determine which is the threshold number of detectable prey and the time in which preys are still detectable from consumption. As prey we used *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) and *T. vaporariorum*, as predator we used *Geocoris punctipes* (Hemiptera: Lygaeidae). We designed specific primers for the detection of prey. To assess the threshold number of detectable preys, we exposed an individual predator to one of the following treatments: 1, 3, 5 and 10 preys. To assess the threshold detection time from prey consumption, a predator was isolated after consuming 3 preys (without access to more food) to complete the corresponding test period and then was sacrificed. As a result, we analyzed predators after the following periods following consumption: 0 (i.e. sacrificed immediately after prey consumption), 3, 6, 12, and 24 h. Results show that the threshold number of detectable prey was 3 for both prey species. As for the threshold detection time from prey consumption we found that after
24 h DNA of prey was still detectable. The time at which 50% of predators were positive in detection of DNA of prey detection was 17.85 h for *T. vaporariorum* and 25.10 h for *E. eremicus*. These results will be useful in the next step of analysis to characterize the trophic relationships of the predator and prey considered.

ANALYSIS OF EXPRESSION OF THE GENES ζ-carotene desaturase (zds) and carotene isomerase (crtiso) IN DIFFERENT PLANT TISSUES OF *BIXA ORELLANA* L. BY RT-PCR REAL-TIME TECHNIQUE.

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ABSTRACT

Achiote (*Bixa orellana* L.) is a native plant from neotropical region. This plant has a great industrial importance, because it produces a characteristic red pigment (bixin). Achiote and its pigment are used at different levels, ranging from traditional medicine to a use as additive in foods, this led the industry be more interested in a further study of this pigment production in this plant. The bixin production in annato is based in carotenoids production. Carotenoids are 40C molecules that are originated from the union of two GGPP molecules.The substrate for bixin production is lycopene, which is a carotenoid generated from four desaturations and two isomerizations of phytoene by PDS, ZDS, ZISO and CRTISO enzymes. ZDS and CRTISO enzymes are coded by *zds* and *crtiso* genes, respectively. These genes expression is regulated through plant development by developmental and environmental signals. Each one of these signals act at different level depending of the tissue, so the *zds* and *crtiso* genes expression could be different in the different plant tissues. In achiote, bixin production has been directly related with the expression of genes involved in carotenoid biosynthesis. In the present study, expression of *zds* and *crtiso* genes were analysed in different plant tissues (seeds, flower buds and leaves), that in order to have a broader picture about these genes expression in photosintetic and no-photosintetic tissues in this plant. Results tell us that *zds* and *crtiso* expression levels in seeds could be related to the high production of bixin in this tissue; and that these genes could be regulated in a special way in flower bud and leaf tissues, where expression patterns contrast with those reported in other models.

Role of ribosomal protein S1 in the translation of adenine- or uracil-rich mRNAs

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Adeninenucleotides downstream the initiation codon promote protein synthesis and the mRNA binding to 30S ribosomal subunit. Ribosomal protein S1 is known for its high affinity for single-stranded AU-rich RNA stretches. On the other hand, S1 is involved in the docking of the mRNA to the 30S subunit. Thus, A-rich mRNAs may be efficiently translated through their interaction with ribosomal proteinS1.

In this work the role of ribosomal protein S1 in the translation of A-, U-, C- and G-rich mRNAs was tested.

First,we analyzed the role of ribosomal protein S1 in the mRNA-ribosome interaction, ternary complexes were formed with tRNA initiator, wild type and S1-deficient 30S subunits and synthetic transcripts containing adenine or guanine tandems downstream of the start codon and the same initiation codon and Shine-Dalgarno sequence. These messengers were 5' labeled with ^{32}P to quantify the messenger bound to the ternary complex.

Interestingly, synthetic messengers containing adenine and uracil nucleotidesdownstream of the initiation codon bound stronger to 30S subunit than messengers with guanines at the same positions. Furthermore, compared with the G-rich mRNA the enhanced binding of the adenine- or uracil-rich mRNAs was reduced to a greater extent when the ternary complex was formed with S1-deficient 30S subunits. The binding affinity was restored to wild type levels when S1-deficient subunits were reconstituted with purified ribosomal proteinS1. A-rich mRNAs showed higher affinity than u-rich mRNAs in the formation of these complexes.

To evaluate the effect of ribosomal protein S1in protein synthesis, a conditional mutant in S1 was employed, where S1 gene (*rpsA*) is under the promoter of arabinose to analyze the *in vivo* translation of A-, U- or G-rich*lac Z* messenger. Preliminary results showed a reduction in protein synthesis from A- and U-rich *lac Z*messenger compared to G-rich *lac Z* messenger under the S1-deficient condition. These results suggest that the enhanced translationofadenine- or uracil-rich mRNAsmay be due to direct interaction with ribosomal protein S1.

Expression of the green fluorescent protein in members of the *Sporothrix schenckii* complex

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The *Sporothrix schenckii* complex groups closely related fungi that causes sporotrichosis, a granulomatous infection mainly found in humans and other mammals. These organisms are an interesting model to study the biochemical, genetic, molecular and physiological basis of cell differentiation and morphogenesis. So far, there is a significant advance in the knowledge about their phylogeny,susceptibility to antifungal drugs andvirulence attributes. Despite this significant progress, there is limited availability oftools to study this organism with genetic and/or molecular approaches.
Here, we describe the transformation of *S. schenckii*and *S. brasiliensis*with a vector, which contains the sequence of the gene encoding the green fluorescent protein (GFP) under the *gpd*promoter. The plasmid integration was maintained even without selective agent, indicating the generation of stable transformants. As expected, GFP expression was detected in mycelia and yeast cells when analyzed under fluorescence microscopy.
The strains obtained will be used in *in vivo* experiments to assess the fungal distribution in a rat model of granulomatous sporotrichosis.

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RFLP-PCR'sstandardization to identify*CYP2C9's* allelic variants (** 2, * 3* and *SNP4)*in the Mexican population

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CYP2C9 enzyme belongs to CYP P450 2C hemoproteinsubfamily. *CYP2C9*codifies a 490 amino acidprotein (55 kDa) and metabolizes about10% of therapeutically important drugs (warfarin, tolbutamide, losartan, NSAIDS, etc.). *CYP2C9* expression is influenced by both environmental and genetic factors.

Single nucleotide polymorphisms (SNPs) have been described in the codifying sequences of *CYP2C9* and could contribute with the inter-individual variability of several drugs responses (adverse reactions, drug interactions or therapeutic failure). Variants **2, *3* and *SNP4* induce different functional alterations in enzyme activity. Reported *CYP2C9*allelic frequencies in Mexican-Mestizos (healthy volunteers) are**1*= 0.87, **2*= 0.1 and **3*= 0.03 (RFLP-PCR in a Monterrey sample); **1*= 0.915, **2*= 0.07 and **3*= 0.015 (Real-time PCR in aDurango sample); **1*= 0.910, **2*= 0.051 and **3*= 0.039 (Mexico City). Reported frequencies in acenocoumarol-treated patients are: **1*= 0.922, **2*= 0.078 and **3*= 0.043 (RFLP-PCR, Guadalajara).

Objective: To establish the optimal conditions for both polymerase chain reaction and enzymatic restriction in order to determine variants **2* and **3* as well as *SNP4* (not yet studied in the Mexican population) of *CYP2C9* in the Mexican population. Therefore, curves with different amplification and enzymatic restriction conditions were performed on DNA from healthy volunteers' peripheral blood, in order to identify variants **2* and **3* (oligonucleotides described by Llerena in 2004 and not used for samples of Mexican mestizos). In the case of *SNP4* (not described in the Mexican population), the oligonucleotides described by Fazleen in 2012 were used.

Results: to identify variant **2* at the reported conditions(Tm= 59 ºC, [oligo]= 60 pmol/μL, 2.5 U Taq and [Mg^{2+}]= 1.2 mM), variables were modified. In optimal conditions we obtained Tm= 59 ºC, [oligo]= 0.5 μM, 1.35 U Taq and [Mg^{2+}]= 2.0 mM. For enzymatic restriction, we used 20 μL of the PCR product and 10 U of *Ava II*. Starting from the aforementioned initial conditions, we identified variant **3* in the following optimal conditions: Tm = 59 ºC, [oligos] = 0.5, 1.5 U Taq and [Mg^{2+}]= 1.5 mM. For enzymatic restriction, 20 μL of the PCR product and 10 U of *Nsi I*were used. The optimal conditions to identify variant *SNP4* are: Tm= 54 ºC, [oligo]= 0.5 μM, Taq= 1.35 U and [Mg^{2+}]= 2.0 mM. For enzymatic restriction, 20 μL of the PCR product and 10 U of *Tfi I*were used.

Conclusion: The optimal conditions to identify *CYP2C9's* allelic variants *(*2, *3 and SNP4*)were found. Thesediffered from those described in the literature, mainly in the amount of Taq polymerase enzyme, magnesium and oliglonucleotides concentration, but not in the annealing temperature. For enzymatic restriction, optimal conditions were found by modifying the amounts of amplification and restriction products.

Genome-wide copy number analysis in Müllerian aplasia

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Approximately 0.02% of women are affected by Müllerian aplasia (MA), a congenital disorder of the female reproductive tract characterised by the absence of uterus and the superior third of the vagina. Affected individuals display normal secondary sex characteristics and gonadal function, and karyotyping often reveals a normal 46,XX complement.The complex etiology of MA is hitherto unknown. The most recurrent deletion affects *LHX1*(5.7% of cases), which codes for an embryonic high-hierarchy transcription factor (TF). *Lhx1*$^{-/-}$ female mice display Müllerian agenesis, andmutations in *C. elegans* ortholog *lin-11* lead to vulva-less and egg-laying defective phenotypes.These data suggest that *LHX1* regulates a conserved gene module.

In order to understand the molecular basis of uterine differentiation, in this work we carried out several approaches to identify and prioritize lower-hierarchy candidate genes for MA. We performed a conventional genome-wide copy number (CN) analysis in 13 Mexican MA patients using high-density microarrays. Then, we compiled an enriched candidate gene dataset by incorporation of data from previous studies on 175 patients, and from our data reanalysed at higher resolution (≥10 contiguous probes). An annotation-based analysis was performed to prioritize these genesby their structural and functional characteristics. Finally, we used a bioinformatics approach to predict LHX1 regulation targets, and the results were contrasted against the candidate gene dataset.

Novel candidate genes for MA were identified from the genome mapping analysis in Mexican patients: *NCOA2*, *EYA1*and*METAP2*. Over-represented functional categories were recovered from the subset of deleted and haploinsufficient candidate genes: nephric duct morphogenesis and pronephros development (adjP=0.0001), ErbB signalling pathway (adjP=0.0009), adherens junction (adjP=0.005), and others. Motif search on the promoter regions (-600 bp) offourdocumented *Lhx1* regulation-targets in *Xenopus* and their homologs in 5 other species revealed a highly conserved 50-nt signature (e-value=1.34e^{-36}). Several known TF binding sites are contained in such signature, including those of HOXA5and HNFB1, which have beenpreviously associated to MA. The refined motif was further searched against human promoter regions, and 45 putative target-genes of LHX1 were identified. Of these, 38 genes are expressed in uterine tissues, 19 during embryogenesis, and 15 in both conditions. Two of the predicted genes, *NPHP1* and *PIAS3,* overlap with the MA deletion set. In conclusion, we identified novel genes associated to MA and adapted an analysis strategy to prioritize candidate genes. Further validation of these findings by cellular or animal models will be required to understand their role in female reproductive tract development. Project supported by grants PAPIIT-IN219912 UNAM and CONACYT-133273.

Genome-wide identification of the genes mediating antagonistic pleiotropy of aging in the budding yeast

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In the 1950s the antagonistic pleiotropy theory of aging postulateda trade-off between early-life traits and longevity leading to the selection of genes with adverse effects duringlate life because of their beneficial effects early in life. Many genetic and environmental interventions had confirmed the trade-off between longevity and early-life history traits, but to datefew genes with an antagonistic pleiotropic behavior have been reported. Here, we identify a comprehensive genome-wide list of antagonistic pleiotropic genes using quantitative data of gene effects on cell growth and chronological longevity of the budding yeast. We found that antagonistic-pleiotropic aging genes typically have deleterious effects on fitness under different growth conditions, play a role in growth-related processes, and are notoriously involved in cytoplasmic translation and chromatin remodeling. Polysomal-profile analysis and endoplasmic-reticulumstress sensitivity testsin slow-growing yeast isolates showed that a decrease in ribosomal translation and a better unfolded proteinresponse promote longevity at the expense of growth in some long-lived isolates. Our results confirm that ribosomal translation is one of the downstream cellular mechanisms that underlies the antagonistic pleiotropy of aging and suggest that an important number of additional mechanisms underlie the cellular trade-off between growth and longevity.

Characterization of dystrophin mutant Dp40c-L170Pexpression in PC12 cells

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Dystrophins (Dp) are proteins related to muscular dystrophies,a group of heterogeneous and hereditary diseases involvedin progressive muscle degeneration. Thedystrophin Dp427 allows the connection between thecytoskeleton and the extracellular matrix in muscular cells. DystrophinsDp140, Dp71 and Dp40 have been related with cognitive problems in patients with Duchenne muscular dystrophy. Dp140 and Dp71areexpressed during fetal brain development.Besides, mutations in dystrophinexons 75 and 76 wereassociatedwith mental retardation in patients with dystrophinopaties.

Dp71 and Dp40 share the same promoter being Dp40 the shortest dystrophinreported to date. Dp40 is expressed in human fetal tissues: muscle, lung, liver, brain as well as in adult muscle.It has been shown that Dp40 interacts with a group of presynaptic proteins to form a presumptive novel complex in the mouse brain.In addition, our lab has found thatDp40 mRNA is expressed in PC12 cells, using recombinant proteins the wild type Dp40c is localized in the cell periphery, cytoplasm and in less extent in nucleus.

Site directed mutagenesis ofamino acid L170P changesthecytoplasm/ nucleus ratio of Dp40 leading to a predominant nuclear distribution in transient transfected PC12 cells. We have created aPC12 Tet-On cell line that expresses myc/Dp40c-L170Pin an inducible manner and subsequently clones have been isolated. All clones showed a higher number of cells expressingMyc/Dp40c- L170P compared tothose cells obtained by transient transfection; however, only few of themshownpredominant nuclear staining. Despite of the fact that it is aninducible system, basal expression was obtained in most of the clones analyzed by Western blot.Upon cellular differentiation with NGF we found that neurons-like cellsobtained from the clones expressing Dp40c-L170Phad longer and thicker neurites as well as larger cells bodiesin the presence and absence ofdoxycycline. This phenomenon was not observed in the cells transfected with the empty vector.

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Frequency of polymorphisms of five genes involved in athletic performance inanMexicanpopulation

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Abstract

Various reports have provided evidence that genetic variants in some genes have an enhanced adaptive response to endurance training and strength. In this study, we estimatedthe frequency of polymorphisms of five genes involved in athletic performance in a Mexican population and compared with the genotype frequencies of 14 populations reported in the database of 1,000 genomes project. We studied 321 individuals from an unselected mestizo Mexican population of Mexico City. Samples were obtained from the blood bank at the National Institute of Rehabilitation, genomic DNA was isolated by the CTAB-DTAB method, real-time polymerase chain reaction (PCR) allelic discrimination TaqMan assay was used for genotyping *MST* (rs1805085, rs1805086), *BDKRB2* (rs1799722), *FST* (rs1423560), *ACTN3* (rs1815739) and *ADRB2*(rs1042713)variants.Deviations from Hardy-Weinberg equilibrium (HWE) were tested using the chi-square Pearson test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl [20/04/2013]), p value <0.05 was considered as statistically significant. SPSS software v. 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.Genotype frequencies distribution was according to Hardy-Weinberg equilibrium (HWE). A Chi-square test was performed to search for differences in allele and genotype frequencies in populations (ASW: American of African Ancestry in SW USA; LWK: Luhya in Webuye, Kenya; YRI: Yoruba in Ibadam, Nigeria; CLM: Colombian from Medellin, Colombia; MXL: Mexican Ancestry from Los Angeles USA; PUR: Puerto Ricans from Puerto Rico; CHB: Han Chinese in Bejin, China; CHS: Southern Han Chinese; JPT: Japanese in Tokyo, Japan; CEU: Utah Residents (CEPH) with Northern and Western European Ancestry; FIN: Finnish in Finland; GBR: British in England and Scotland; IBS: Iberian population in Spain; TSI: Toscani in Italy) reported in the 1,000 Genomes Project (http://www.ensembl.org/index.html) and our data, showing difference with a p<0.05 as follows: rs1805085 for ASW, LWK, YRI, the rs1805086 ASW, LWK, YRI and MXL, the rs1799722 ASW, LWK, YRI PUR FIN and GRB, the rs1042713 CHS, CEU and TSI, and for rs1815739, rs1423560 was different for all populations.Information reported herein is of utmost important for subsequent association studies with possible implications for research in molecular genetics of exercise.

Comparison of mutation profiles in the Duchenne Muscular Dystrophy gene among populations: implications for exon skipping and stop codon read-trough therapies

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Several molecular approaches are emerging to restore dystrophin expression in Duchenne Muscular Dystrophy (DMD); a severe neuromuscular monogenic disease characterized by progressive muscle waste and weakness. Among those strategies skipping and stop codon read-trough rely on the type of mutation. The first is aimed to restore reading frame by blocking exons with antisense agents and the second avoids the effect of premature stop codons. Hence, potential applicability depends in part on the frequency of mutations in the different populations. Therefore we describe the mutation profile of Mexican patients with clinical diagnosis of DMD using Point Mutation-Multiplex Ligation Probe Amplification and High Resolution Melting as screening techniques.Then we compared mutations frequencies of exons that represent current targets for exon skipping among populations. In total 162 unrelated DMD patients from were included in the study. A search in LOVD DMD database was performed to include patients with DMD from different countries all analysed by MLPA. By comparing mutation frequencies, differences were found only for skipping of exon 44, ($P = 0.0002$). Being Mexico and Germany the countries with the highest percentage of patients that could potentially benefit from skipping of exon 44.

Functional divergence of duplicate genes coding DEAD RNA helicases in *Bacillus subtilis*.

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Proper cellular function of organisms depends on the ability to modify RNA structures, as in the case of the tRNA and rRNA. RNA helicases are potentially required in any process of RNA metabolism since they prevent RNA chains adopt erroneous folding or inappropriate interactions with proteins. DEAD box helicases are characterized by having a structural core (helicase) formed from at least 12 motifs comprising around 350 amino acids. *B. subtilis* has 4 genes coding for DEAD RNA helicases (*cshA, cshB, deaD, yfmL*), to which different functions have been attributed, such as assembly of ribosomes and binding to the degradosome. Some studies have reported that their expression is affected by environmental stress.

The objective of this work is to analize the role of differential regulation and the degree of functional divergence of duplicated genes coding DEAD box RNA helicases in *Bacillus subtilis*. There are different aspects to be considered in order to understand the participation of DEAD box helicases in RNA metabolism.

First, we analized, if there is compensation for gene expression when one gene coding for DEAD RNA helicases is mutated. The results show in most cases, there is not compensation in optimal conditon, so we are determinig the expression profile in cold stress.

Another important aspect is to understand if there are functional differences among the RNA helicases or if the apparent lack of complementarity between the RNA helicases is due to differences in their expression. To answer this, we inserted the DEAD RNA helicases genes under the control of the xylose promoter in wild type, *ΔcshA ΔcshB, ΔdeaD* and *ΔyfmL* backgrounds. This allow us to maintain genetic expression through growth phases of the response to under several types of abiotic stresses. We are determinig if the wild type phenotype is recovered by functional complementation.

Describing the epistaticinteractionsamong genes that extend yeast lifespan

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The ageing process is influenced by different pathways, which are regulated by environmental and physiological signals.These cellular functions underlie the gradual and progressive deterioration observed in ageing cells.Whit the help of model organisms such as yeast, worms, and flies, it has been possible to discover important genes and mechanisms that regulate the pace of ageing, some of which are conservedacross these model organisms. Recently, we reported the chronological-lifespan phenotypes of over 5,600 single or double gene knockouts yeast revealing that 20% of the genes affect lifespan of this organism; of these 7% extend lifespan. However, it remains unknown how these different longevity factors interact with one another. Here,we characterize the chronological lifespan over 300 double-knockouts to score the genetic interaction (epistasis) among 17mutations that extend lifespan. We will present the frequency of positive and negative genetic interactions, along with the functional associations of these genes. We focuson the genes that slow the aging process in the yeast and that have a human homologue. Our study will reveal to what extent yeast lifespan can be extended by the combination of different longevity factors and will inform on whether these genes typically act together or independent from one another.

Wnt signaling pathway alterations induced by *Trichinella spiralis* muscle larvae excretory-secretory products in primary myoblast cultures

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Trichinella spiralis is an intracellular parasite of mammalian skeletal muscles. Infection by L1 larvae triggers a series of changes in the infected muscle, which eventually transforms the infected cell into the nurse cell phenotype, a structure that allows parasite survival for long periods of time. In this process, the participation of excretory-secretory products (ESP) released by muscle larvae stage (ML) have been suggested. To elucidate the cellular processes altered by this parasite, we developed murine myoblast primary cultures as a model to evaluate the changes at genetic expression level induced by ML ESP during differentiation from myoblast to myotubes.

The hind limb muscles were collected from 2-4 day old BALB/c mice to establish myoblast primary cultures. After 8 days under differentiation conditions, total RNA was isolated from ESP-treated and -untreated myoblast primary cultures. cDNA microarray assay was performed to evaluate gene expression changes between treated and control cultures. Data analysis were performed by using different bioinformatics tools and some of the changes observed were validated by qRT-PCR.

ESP treatment produced a 50% reduction in myotube formation. In addition, shorter and thinner myotubes with fewer nuclei were observed. Among the 22,000 plotted genes, the expression of 1280 genes was up-regulated while 1140 genes were down-regulated by treatment with ESP. These genes were associated with different cellular processes like actin cytoskeleton regulation, cell cycle, apoptosis, MAPK signaling pathway, JAK/STAT signaling pathway and cell development. Interestingly, different genes from the Wnt signaling pathway, which is involved in muscle cell differentiation, were also altered.

These results suggest that the Wnt signaling pathway may be partially responsible for the changes induced by ESP treatment in myoblast primary cultures.

Functional characterization of *Candida tropicalis MNN4* and *OCH1*

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Candida albicans and *C. tropicalis* are yeast-like organisms that can cause severe infections in immunocompromised patients. The cell wall structure and composition are critical for the interaction with host tissues and components of the immune system. In *C. albicans*, the cell wall has an inner layer of chitin and β-glucans, and an outer coat composed of mannoproteins, i. e., proteins that are mannosylated by the glycosylation pathways. *C. albicans OCH1* is involved in the synthesis of the *N*-linked mannan outer chain, and is required for cell wall integrity and virulence. On the other hand, *MNN4* is involved in the regulation of the phosphomannosylation pathway that adds phosphomannan moieties to both *N*- and *O*-linked mannans. This cell wall component is required for proper phagocytosis by macrophages.

The *C. tropicalis* genome contains two putative genes with significant similarity to *C. albicans OCH1* and *MNN4*. Thus far, there is not information about the cell wall assembly and mannosylation pathways in *C. tropicalis*; therefore the study of the putative orthologs to *OCH1* and *MNN4* is relevant to understand how the mannosylation and phosphomannosylation pathways contribute to the virulence and immune sensing of *C. tropicalis*.

In the present study, we conducted the complementation of *C. albicans mnn4*Δ with *C. tropicalis MNN*4 under the control of *C. albicans ACT1* promoter. Our results indicate that expression of Ct*MNN4* restored the ability to bind the cationic dye Alcian blue, suggesting restoration of the phosphomannosylation pathway. In addition, we also performed the complementation of *C. albicans och1*Δ with *Candida tropicalis OCH1* under the control of *C. albicans ACT1* promoter. Experiments are in progress to assess whether this gene is the functional ortholog of *C. albicans OCH1*.

To investigate further the function of both genes, we are currently disrupting both genes using the Sat-1 flipper strategy

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Resveratrol increases expression of the sarco/endoplasmic reticulum Ca^{2+}-ATPase in mice hearts.

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In the heart, the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), actively transport Ca^{2+} towards the interior of the sarcoplasmic reticulum, regulating the process of contraction and relaxation of the cardiomyocyte. At least one functional copy of the SERCA2 gene (*Atp2a2*) is essential for life, and the loss of one allele (*Atp2a2*+/-) diminishes mRNA and protein in the heart, causing alterations in cardiac contraction-relaxation and leads to cardiac failure in the long term. The *Atp2a2* gene was targeted previously in mice by removing the promoter and first two exons, which eliminated expression of the mutant gene. Haploinsufficient (*Atp2a2*+/-) mice of a mixed background (50% 129/Svj, 50% Black Swiss) were crossed to obtain wild-type and *Atp2a2*+/- mice, and genotypes were determined by PCR analysis of tail DNA.

Resveratrol is a natural non-flavonoid polyphenol that has been previously demonstrated to have cardioprotective effect against damage in diabetic hearts, by ischemia/reperfusion, oxidative stress, among others. Resveratrol is an indirect activator of SIRT1, a histone deacetylase type III. A regular chow diet enriched with resveratrol (0.067%) was administered to wild-type mice and *Atp2a2*+/- haploinsufficient mice from 3 months of age during 3 months up to 1 year (100 mg/kg/day).

In this work, we found a decrease in SERCA2a mRNA expression in haploinsufficient mice compared to wild-type mice. Treatment with resveratrol of haploinsufficient mice showed increased expression (20-30%) of SERCA2a mRNA. Similar results were obtained in the expression of SERCA2a protein quantitated by western-blot. Taken together, the results demonstrate that the activation of SIRT1 in the hearts of mice fed with resveratrol participates in the up-regulation of the *Atp2a2* gene. Supported by grants DGAPA-PAPIIT IN213613 and CONACyT 164413.

Extrachromosomal complementation of *trt1*-disrupted mutant of *Ustilago maydis*

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Telomerase reverse transcriptase (TERT), the catalytic protein-subunit of the telomerase ribonucleoprotein complex, is necessary for the telomere maintenance. Mutations affecting *TERT* genes have dramatic effects on telomere length, chromosomal stability and cell viability. Previously the *TERT* gene (*trt1*) of *Ustilago maydis* was identified and interrupted. This gene is encoded on an open reading frame of 4,116 nt located at the *locus* um11198 from the *U. maydis* Database (Munich Information Center for Protein sequences; http://mips.helmholtz-muenchen.de/genre/proj/ustilago/). Trt1 exhibits the conserved motifs of all TERTs, at the same distances and sequence order. Previously, a disruption cassette containing a chimeric *hph* gene controlled by P_{HSP70} promoter was used to generate the *trt1*-disrupted mutants trt1-1 and trt1-2. Small, dry, hyper-pigmented colonies, exhibiting gradual telomere shortening, reduced replicative potential and lack of telomerase activity confirmed that *trt1* encodes the telomerase reverse transcriptase in *U. maydis*. In order achieve the genetic complementation of a *trt1*-disrupted gene in mutants, an autorreplicative plasmid (pTrt1), derived from pNEBUC GE77+ (CBx^r), was constructed to harbor a chimeric *trt1* gene controlled by the P_{HSP70} and T_{HSP70} signals from the *hsp70* gene *U. maydis*. Transformation of pTrt1 or pNEBUC GE77+ vector was conducted as described Tsukuda *et al.* (Mol Cell Biol. 1988. 8:3703) on the W204 strain, a trt^- F1 progeny from the trt1-1 X 520 cross, and on 521 strain. Transformats were selected by resistance to carboxin, and defied against hygromycin. The acquisition of pTrt1 into five W204 transformants was confirmed with drop-plate assays and by genetic re-transformation of total DNA extracted from clones in *E. coli* DH5. Amplification of DNA sequences of pTrt1 by PCR,and the transcriptional expression of chimeric *trt1* sought by RT-PCR was positive in the tested transformants (T1 to T3). Regaining telomerase activity in the transformant clones, as well as the fact that only pTrt1 but no pNEBUC GE77+ transform W204 strain confirmed the claim of the successful genetic complementation. Variations in telomerase activity (34 to 91%) after complementation, restoration of the replicative potential and growth rate to almost normal levels in the W204 strain and the decreased growth rate in the 521 strain, as well as the finding of a slight decrease in intensity in the telomere signals from the genomic hybridization patterns of the pTrt1-transformants will be presented and discussed.

Increased expression of Hedgehog (Hh) molecules and EMT-related Hh pathway downstream target genes in transgenic mice K14E7

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Cervical cancer is the second most common malignancy in females worldwide. However, it has now become a potentially preventable and curable disease due to advances in screening, diagnosis and treatment. It is well known that human papillomavirus (HPV) is the single most significant etiological agent in cervical cancer and its viral oncoproteins, such as E6 and E7, are the main factors linking to tumor progression. E7 is considered the major transforming protein given its ability to immortalize human epithelial cells as well as to induce cervical tumors [in cooperation with 17β-estradiol (E_2)] in genetically engineered mice.

The transformation from low-grade lesion to invasive cancer must involve cellular and genetic changes together with activation of signal transduction pathways. The Hedgehog (Hh) signaling acts as a key regulatory mechanism of cell fate determination during embryogenesis and plays a significant role in cancer development. However, the role of Hh signaling pathway in cervical cancer is rarely reported. A recent report has shown that the expressions of Gli1, Gli2 and Gli3, Ptch and Smo in Hh signaling pathway are up-regulated in uterine cervical carcinoma and its precursor lesions.

In this study we employed cervical tissue of 3 and 7-month-old K14E7 transgenic and FvB mice treated with E_2, as well as untreated mice to evaluate the expression of Hh molecules and EMT-related Hh pathway downstream targets, such as Snail, cd44 and Cyr61 by quantitative RT–PCR analysis and immunohistochemical. This analysis demonstrated that the expression of Hh components and EMT- related Hh downstream target genes are significantly elevated in K14E7 and K14E7+E_2.

E7 induced elevated Gli1 protein levels in the nucleus which increases its transcriptional activity. Estrogen treatment, only en some cases increased beyond the expression of these molecules.

NUCLEAR EXPORT OF β-DYSTROGLYCAN

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Dystroglycan is an essential component of the Dystrophin-Associated Proteins Complex (DAPC), which maintains the integrity of the sarcolemma and connects the extracellular matrix with the actin cytoskeleton. The DAPCalso participates in cell signaling processes. Dystroglycan is composed by α and β subunits, which are associated each other n a non-covalently manner. β-Dystroglycan (β-DG) is a transmembrane protein involved in cell signaling, cell adhesion, and cytoskeleton remodeling. Recently our research group demonstrated that β-DG is transported to the nucleus through a nuclear localization signal (NLS) located in the yuxtamembranal region of the protein.

In this study, we present the identification and characterization of a nuclear export signal (NES) in β-DG. *In silico* analysis identified a putative NES located in the residues^{763}ILLIAGIIAM^{772}. Treatment of C2C12 cells with leptomycin B, an inhibitor of CRM1 exportinresulted nuclear accumulation of both endogenous β-DG and GFP-βDG fusion protein, which implies that CRM1 exportinmediates the nuclear export of β-DG. Likewise inactivation of the NES by site-directed mutagenesis caused nuclear accumulation of GFP-β-DG. Finally we demonstrated *in vitro* interaction of CRM1 exportin with β-DG. Overall, our results suggest that β-DG has a functional NES that is recognized by CRM1 to export the protein from the nucleus to the cytoplasm. The nuclear export of β-DG may be important to modulate its levels in different cell compartments.

Getting a mutant gene in *Gallibacterium anatis* 12656-12 *qseC*

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The Gram negative bacteria *Gallibacterium anatis* belongs to the *Pasteurellaceae* family of gamma-proteobacteria. *G. anatis* is considered a member of the normal-flora which inhabit the genital and upper respiratory tract of wild and domestic birds. *G. anatis* produces gallibacteriosis a major disease afecting the reproductive system of hens, and the eggs laying, decreasing the production up to 80%. Despite the importance of this pathogen, little is known about *G.anatis* virulence factors involved in tissue colonization, biofilm formation, and the regulatory networks that control the expression of those virulence factors. Bacterial adherence to mucosal surfaces is the first step in the infection process, wich is followed by the bacterial proliferation and successful colonization of different host tissues. Biofilm plays a central role in the infective process; after initial host-pathogen interaction, the production of this structure, its growth and spread is driven by quorum-sensing (Q-S) signals. Q-S is a bacterial sinchronizing mechanism which responds to changes in cell density, and modifies the expression of genes throught the synthesis of an autoinducer (AI) or hormone-like compound. Q-S is produced when the AI concentration reaches an upper threshold. In *E. coli,* QseC is autophosphorylated upon detection of norepinephrine (NEI), epinephrine (EPI), or AI3, subsequently it interacts with QseB response regulator, triggering the control cascades of gene expression of virulence factors. *qseC*-null mutants of enteropathogenic *E. coli* are unable to activate the expression of key virulence genes and shows reduced growth in animal infection models. Here we describe the finding of sequences with high similitude to *qseC* in the genome databases of *G. anatis* UMN 179, 12656-12 and F149. Specifically in 12656-12 strain, the hypothetical *qseC* gene is located in the 200 kb contig12. Using this information, oligonucleotide primer pairs were designed to amplify 12656-12 genomic sequences. These sequences were useful to assemble a conventional one-step-disruption cassette. Genetic transformation was achieved by induction of natural competence in the 12656-12 strain as described Christensen (2012, Appl Environ Microbiol. 78: 4914–4922). Disruption of the putative *qseC* gene was verified by PCR amplification assays. The recent construction of those *qseC*-null mutants of *G. anatis* 12656-12 will allow us to determine the involvement of this gene in the control of virulence genes.

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Characterization of cis-elements that negatively regulate transcription of *EPA* genes through silencing proteins of *Candida glabrata*

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Candida glabrata is an opportunistic fungal pathogen capable of adhering to epithelial host cells. This adherence is mediated by some members of the large family of cell wall proteins encoded by the *EPA* (Epithelial Adhesin) genes. The majority of the *EPA* genes are localized in subtelomeric regions resulting in a negative regulation of transcription of these genes through an analogous mechanism to *S. cerevisiae* called subtelomeric silencing. *In vitro* adhesion to epithelial cells is mainly mediated by Epa1, the product of the *EPA1* gene which is localized 21 kb from the right telomere of chromosome E (E_{-R}) and forms a cluster with *EPA2* and *EPA3*. This particular telomere contains a cis-acting protosilencer element called Sil2126 localized between *EPA3* and the telomere and a negative-element (NE) downstream from *EPA1*. The protosilencer Sil2126 and NE negatively regulate the expression of *EPA3* and *EPA1*, respectively. Subtelomeric silencing at this telomere depends on Rap1, Rif1 and the Sir proteins, but surprisingly not on the yKu70/80 proteins.

To assess whether these elements act together to extend silencing in E_{-R}, we deleted Sil2126 and NE and tested *EPA1* expression using the reporter gene *URA3* under the control of the *EPA1* promoter. We found that *URA3* expression is slightly decreased in the absence of both Sil2126 and NE in comparison with a strain only lacking NE. These results could indicate that there is an additive but subtle effect of Sil2126 and NE on the *EPA1* promoter. We will determine whether *EPA2* and *EPA3* expression are affected by these deletions.

To determine if Sil2126 requires the presence of the NE to silence a reporter placed at 34 kb from the telomere, we are constructing reporter strains with single and double deletions of the NE and Sil2126 that contain a Sil-reporter system at –34 kb.

Association of variants in *NR3C* and *SLC6A4* genes and Major Depression in Mexican Mestizos

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Two of the most accepted biological correlates to explain the Major Depression (MD) are impaired serotonin (5-HT) function and altered hypothalamic–pituitary–adrenal (HPA) axis activity. However, both hypotheses alone are insufficient to cause depression. Now is becoming clear that the environment plays a crucial role in MD pathogenesis given certain genetic predisposition. Therefore in the present study we analysed environmental and genetic factors involved in depressive illness. In total, 400 subjects underwent a psychiatric interview and a whole blood sample was obtained for DNA extraction. Genotyping of rs41423247 and rs6198 polymorphisms was performed for *NR3C1* (glucocorticoid receptor gene) and rs1042173 for the*SLC6A4* gene by real time PCR. Measurement of NR3C1 and SLC6A4 in serum was also performed by ELISA in extreme phenotypes for patients and controls. The results differ from those reported regarding the association of the studied polymorphisms with major depression, since in any case we found statistically significant results, with the illness or any clinical feature of the disease. In all we obtained $p > 0.05$. Similarly, the receptors, no correlation between the disease and the concentration of protein was found. There was no association of the variants and Major Depression and serum levels of the proteins studied were not different between groups.

Pax8 produces aberrant transcripts in cervical tumors and derived cell lines

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Transcription factor PAX8 is important in embryogenesis and its expression has been used as a biomarker in different tumors. PAX8 gene produces, by alternative splicing, four isoforms with differential transactivation properties. Our aim was to evaluate PAX8 expression in cervical carcinomas and determine which isoform(s) were present.Six normal and 51 tumoral tissues were used to analyze PAX8 expression. After microarray hybridization, expression levels of PAX8 transcripts were analyzed by searching on the resulting database.PAX8 was expressed in 80% (41 out of 51) of the analyzed tumor samples while it was present in 3 out of 6 normal cervical epithelia samples.To our knowledge, there is no report of PAX8 expression in squamous cervical carcinoma.

In order to detect the PAX8 isoforms expressed in cervical carcinoma-derived cell lines, we used an antibody raised against the c-terminal region of PAX8, shared by its isoforms. We observed a 50 kDa band, apparently corresponding to PAX8; additionally, we detected around 9 lower molecular weight bands, which suggest that we had detected novel, shorter PAX8 isoforms.We performed 5' RACE experiments to obtain the sequence of PAX8 isoforms; however the analysis of sequence showed several aberrant transcripts. Intron/exon PCR results suggest that the genomic sequence of the PAX8 gene is at least partially disrupted, or that it harbors a considerable amount of mutations that impede amplification of exons 2 to 10.

Overall, we found a slight over-expression in cervical cancer tissues compared to normal cells, a pattern of aberrant transcripts produced by the PAX8 gene that encode shorter isoforms in cervical carcinoma-derived cell lines and tumors, as well as evidence hinting at genomic alterations of the gene. Further studies would shed light on the implications of these transcripts in carcinogenesis and their role as cause and/or consequence of other molecular phenomena.

Association between the BDNF val66met polymorphism and BMI in Mexican children

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Abstract

The BDNF p.Val66Met polymorphism (rs6265) is a gene variant that has been implicated with neurological as well as neuropsychiatric disorders. Evidence suggest that BDNF regulates eating behavior, food intake, energy metabolism and the control of body weight. The functional BDNF Val66Met single nucleotide polymorphism has been associated with eating disorders, BMI and obesity in adult populations. The aim of this study was to determine the possible association of BDNF Val66Met polymorphism and BMI in Mexican children (n=480) subdivided into a particular nutritional status according to their BMI percentile. BDNF Val66Met genotype was obtained by two methods, RFLP (n=369) and TaqMan SNP genotyping assay (n=111). The frequency of the Met/Met genotype compared to the Val/Val and Val/Met genotypes and to the Val carriers (combination of Val/Val and Val/Met genotypes) differed significantly between normal weight and the combined overweight and obese children (p=0.042 and p=0.037, respectively). It was also shown that individuals with the Met/Met genotype were almost seven times more likely to be classified as obese relative to Val carriers (OR= 6.6, 95% CI= 0.8-53.5, p= 0.07). This is the first report showing the significant association between the presence of the BDNF rs6265 Met/Met genotype and obesity (BMI percentile) in Mexican children.

Association of theR577Xvariant in theACTN3 gene withobesity in mexican mestizos

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Overweight and obesity are the result of abnormal accumulation of fat that represents a risk for health. In particular, in Mexico obesity is a major issue of public health. Sinceobesity is the main factor predisposing to cardio metabolic risk.ACTN3encodes a protein called α-actinin-3 expressed mainly in skeletal muscle where its role is anchoring the myofibrillar actin filaments, the variant R577X within this gene generates a premature stop codon that avoids production of the protein, the functional allele 577R associates to muscular force whereas the null allele associates to muscular resistance. The main objective of the present work wasto determine whether the R577X variantof ACTN3 gene associates to overweight, different types of obesity and/or normal weight in Mexican mestizos.A case-control study was performed on a sample of 321 individuals that were stratified according their Body mass index (BMI) using OMS criteria into the following categories normal,overweight,obesitytypes1,2 and 3. Samples were obtained from healthy blood donors from INR and were processed to isolate DNA using the CTAB y DTAB method. After that real time PCR was performedusing allele specific hydrolisis (TaqMan) probes.Statistical analysis was carried out usingχ^2 (Chi-square). Allele and genotype distributions were assessed to fit Hardy-Weinberg equilibrium (HWE). Statistical analysis confirmed that genotypes fit HWE ($p=0.26$). And association test showed significant difference between genotype frequecies among the groups of study. Differences were found for type 3 obesity compared to the other groups with p-values of $p=0.02$, $p=0.0003$ y $p=0.002$ respectively.Our preliminary data shows that the TT genotype of this variant is associated to type 3 obesity under the recessive model. This work may help for future studies of genes affecting body composition and the homeostasis of muscle and fat.

Molecular identification and phylogenetic analysis of mosses of semiarid area from Durango.

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ABSTRACT:

Mosses (Bryophyta) represent the second most species-rich group of land plants after angiosperms and are a distributed in all terrestrial habitats suitable for plants growth. Bryophytes are difficult to identify to species level due to their small size, few easily visible morphological characters and show considerable morphological plasticity in response to environmental factors. DNA barcoding by standardized gene regions may thus be of great help in the bryophyte species diversity determination. This study aims the molecular identification and phylogenetics of mosses using the discrimination ability of three barcode markers (one protein-coding gene*rbcL* and noncoding region*trnH-psbA* of chloroplasts DNA and ITS2 region of nuclear ribosomal DNA) on mosses sampled in semiarid area from Durango.42 mosses were sampled and DNA wasextracted by CTAB method. PCR amplifications of *rbcL*, *trnH-psbA* and ITS2 barcode markers were performed using specific primers and cleaned using the AxyPrepDNA gel extraction kit (Axygen) and the amplicons were sent to sequencing. Sequences were analyzed using GenBankand MEGA5for taxonomic and phylogenetic analysis. We extract DNA to 42 mosses samples.As preliminary results eight samples were achieve the amplification and sequencing of three DNA region markers.

An altered hydrotropic response (*ahr2*) mutant of Arabidopsis is tolerant to drought stress

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Plants are constantly exposed to environmental changes like poor light, gravity, obstacles and low water content in the soil and they have developed a morphological plasticity to adapt to those changes. One of the ways in which plants contend with the fluctuating environment is by developing tropisms. Plant roots show positive hydrotropism in response to moisture gradients, a feature that is important in controlling their growth orientation for obtaining water. The hydrotropic response has an important role in establishing the structure of the root system, and thus, has implications on the ability of plants to survive under limiting water conditions such as drought stress. In addition, it has been reported that ABA, a water stress hormone, is a modulator of the mechanisms that integrate the hydrotropic response. To better understand how Arabidopsis responds to moisture gradients, we isolated the *ahr2* semi-dominant mutant of Arabidopsis that displayed altered hydrotropic response in the screening system with a water potential gradient generated with glycerol (NM→WSM; NM, normal medium and WSM, water stress medium). Moreover, we used single sequence length polymorphism (SSLP) mapping to localize the *ahr2* locus to chromosome III and we determined that the semidominance of *ahr2* is likely a gain-of-function mutant. On the other hand, in a system with a mixed water potential gradient (NM→WSM→NM), the *ahr2* roots grew for about 19 days vertically towards the higher water potential sector of the plate (NM) placed at the bottom showing higher hydrotropic response than the wild-type roots that grew only for 7 days and do not reach the first frontier between NM and WSM. Furthermore, the *ahr2* mutant showed higher sensitivity to exogenous ABA on germination and seedling establishment compared to wild-type. Consistent with this result, the *ahr2* mutant grown under drought conditions showed improved tolerance compare to the wild-type. Therefore, we propose that the *ahr2* mutant could help us to establish the relation between ABA signaling with hydrotropic response and drought tolerance.

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Generation of membrane transporters´s deletion mutants of *Ustilago maydis* by the *BsaI* cloning system

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Introdcution. The membrane transporters are proteins with hydrophobic and hydrophilic domains capable of interact with the extracellular environment and move compounds inside and outside the cell[1]. In fungal, are related to different mechanisms such as nutrition, secretion of substances and drugs resistance among many other processes. Because they are related to such varied systems are good targets for studies on the mode of action of new antibiotics or substances capable of inhibiting the growth of fungi and bacteria[2]. On the other hand *Ustilago maydis*, a dimorphic basidiomycete, causer of the disease known as corn smut (Hutlacohe) is a potential model to the study of cellular and genetic responses versus different compounds. Using the *BsaI* cloning system[3], a recently published technique, the aim of this work was to generate mutants with deletion of membrane transporters of *Ustilago maydis*, with perspectives of this strains are the basis for subsequent mechanistic and physiological studies under conditions of nutritional or environmental stress.

Material and methods. The strategy of mutation was performed using the genome database MUMDB to *Ustilago maydis* (http://mips.helmholtz-muenchen.de/genre/proj/ustilago) and clone manager version 9 software. Mutation technique used was established by Terfrüchte *et al*. in 2014 and commercial strain FB2 was used. Transporters selected were: Mannose transporter (um01062, *mt*), high affinity glucose transporter (Δum11514, *gt*) and PNS1 protein (um12080). Were designed flanking regions of each gene of interest, of about 1 Kb of size in each case and they were added a cleavage site for the restriction enzymes *BsaI* and *SspI* (in the flanking region and ends respectively). Using a backbone with a hygromycin resistance cassette, the construction of plasmid was performed using PCR-generated flanks. Subsequently the plasmid was put into bacteria to generate many copies. Protoplasts of commercial strain of *Ustilago maydis* FB2 were transformed to generate the mutant by homologous recombination. The mutants were confirmed by PCR and Southern blot.

Results and discussion. The percentage of genetic homologous recombination in all cases was approximately 20%. Southerm blot shows the expected bands for the mutant and wild type, although nonspecific signals can be seen in all cases. The phenotype of the deletions strain are similar to wild type, when they are grown in complete medium. Specific growth rate of the sugar transporters mutants, is slower than the PNS1 mutant and wild type.

Conclusions. It is possible to obtain mutant strains of membrane transporters of *Ustilago maydis* using *BsaI* cloning system. **Acknowledgment**. This research was financially supported by SIP20130925 and 20141521 projects. Especial thanks to the CONACyT for the scholarship grant to the principal author with fellow number 231581.

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Gallibacterium anatis as model organism to study the CRISPR/Cas system in *Pasteurellaceae*

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The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the associated Cas (CRISPR associated) proteins provide to bacteria and archea the immune system to avoid the invasion by extra-chromosomal genetic elements such as phage and conjugative plasmids. The system is classified into Type I, II and III and twelve subtypes. The proteins Cas3, Cas9 and Cas10 are specific for each of the respective I, II, and III systems, whereas Cas1 and Cas2 are universal for the three systems. Other 40 proteins are also included as members of the Cas proteins. Recent reports revealed that the global repressor protein H-NS in *E. coli* and *S. thypi* regulate negatively the expression of CRISPR/Cas system, whereas the expression of CRISPR/Cas can be induced by triggering the SOS-system in response to DNA damage by UV irradiation, exposure to alkylating chemicals (such as mitomycin C) and to some antibiotics.

Avibacterium paragallinarum and *Gallibacterium anatis* are two worldwide distributed pathogens that cause serious economical loses in poultry farms. Both bacteria are fastidious and hard to manipulate; despite *G. anatis* poses all the genes required for genetic transformation, *A. paragallinarum* does not. *G. anatis* is refractory to to incorporate foreign DNA, and transformation frequencies obtained after induction to natural competence are low. This bacterium could be better studied at the molecular level if the machinery for genetic transformation were docile enough to allow its manipulation. CRISPR/Cas-based immune system could contribute to prevent genetic transformations in these organisms; to test this hypothesis, in this work we set up a system to study the role of CRISPR/Cas system in transformation frequency of *G. anatis* and *A. paragallinarum* with plasmids. The CRISPR arrays and Cas proteins were absent in *A. paragallinarum,* but were found *G. anatis* UMN179 and 12656-12 strains after a bioinformatics analysis using the CRISPR Finder and BLAST programs, and NCBI database; here the organization of the entire CRISPR/Cas loci are proposed: two loci were found in the *G. anatis* UMN179 genome sequences and three in the 12656-12 strain. Systems were classified as Type I subtype D. A bacterial growing kinetic was done in order to determine the optimal moment to analyze transcriptional expression of the Cas genes. Transcriptional expression of the *cas1, cas2* and *cas3* genes was found by RT-PCR procedure in the12656-12, interestingly they were constitutive at initial phases of growth. Transformation of 12656-12 strain with a heterologous plasmid from *Pasteurella multocida* render two useful transformant clones. U.V. irradiation of transformed and wild type strains, followed of PCR amplification and sequencing of the CRISPR locus to analyze acquisition of a new CRISPR spacer will be explained and results discussed.

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C. glabrata strains that express both types of mating information are more sensitive to oxidative stress

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Candida glabrata is an opportunistic fungal pathogen frequently isolated in nosocomial infections. *C. glabrata* is an asexual, haploid yeast that has a closer phylogenetic relationship with *Saccharomyces cerevisiae* than with other *Candida* species such as *Candida albicans*. Even though *C. glabrata* is an asexual organism, it has conserved orthologues of the genes that control mating in *S. cerevisiae*. In, *C. albicans* and *S. cerevisiae* the control of cell-type identity, which is essential for mating, is achieved through a regulatory circuit between the **a1**, alpha1 and alpha2 proteins encoded at the mating type loci (*MAT* or *MTL*). The **a1** and alpha2 proteins in *C. albicans* and in *S. cerevisiae* form a heterodimer that is crucial for cell-type identity control and for other processes like the morphological switch (in *C. albicans*) and the response to some types of stress (in *S. cerevisiae*). *C. glabrata* contains the genes encoding these proteins, however, it does not maintain cell-type identity. It is possible that a heterodimer is also formed in *C. glabrata* and that it is involved in the response to stress.

We have asked first whether *C. glabrata* can form a heterodimer (**a1**/alpha2) using bimolecular fluorescence complementation (BiFC). This technique also allows us to determine the intracellular localization of the proteins **a1** and alpha2. We have constructed a positive control strain that expresses the yKu70 and yKu80 proteins with amino and carboxyl terminus of the yellow fluorescent protein YFP respectively and in the opposite configuration. The results show a fluorescent signal in these strains, therefore validating the technique to detect protein-protein interactions in *C. glabrata*.

In addition, in order to determine whether the information at the *MTL1* locus (**a** or alpha), participates in the response to stress we have characterized phenotypically strains of *C. glabrata* expressing different mating information from *MTL1* under various stress conditions. Our results show that the strains expressing simultaneously both types of information are slightly more sensitive to several concentrations of H_2O_2 than the parental strain, suggesting that these genes may participate in the response to oxidative stress in *C. glabrata*. Moreover, we want to know if the strains containing different mating information have differences in its transcriptome through RNA seq.

Study of *KCNJ11* and *ABCC8* alelic variants from K_{ATP} channels in type 2 diabetic patients treated with sulphonylureas, biguanides or both.

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Introduction:
Diabetes *mellitus* type 2 (DMT2) is a high prevalence pathology, and a great public health problem in Mexico [1]. There are five main groups of antidiabetic drugs: biguanides, tiazolidinediones, α- glucosidase inhibitors, glinides (or metglinides), and sulphonylureas. On our country the most used on first level medical attention are biguanides and sulphonylureas.
Sulphonylureas are insulin secretagoges that inhibit K_{ATP} channels [2]. The K_{ATP} channels regulate physiological insulin release on β-pancreatic cells. There have been reported several single nucleotide polymorphisms (SNPs) on the genes codifying for these channels (*ABCC8 and KCNJ11*)
that are related with diabetes susceptibility [3] and possible with drug efficacy [4].

Objective:
To study the SNPs from the K_{ATP}: R1273R from *ABCC8* gene, and E23K from *JCNJ11* gene, in order to know the frequencies of each genotype in Mexican diabetic subjects, and compare them with other world diabetic populations.

Methodology:
DNA samples of 80 diabetic patients from Hospital Juárez de México were genotipifyed by PCR RFLP. Genotype frequencies were obtained by direct counting, and results were analyzed for Hardy Weinberg equilibrium using Chi square (X^2). The obtained frequencies were compared to published frequencies for other world populations by Fisher exact test comparing each population with our sample.

Results:
The sample analyzed was composed by 80 patients treated with glucose-lowering drugs (sulphomylureas, biguanides or both), six persons were excluded because were treated with insulin or a combination of insulin and drugs. The mean age of the sample was 58.59 ± 10.07 (S.E.M) and the evolution time was very variable (0 to 42 years). The cardiovascular risk biomarkers: body mass index (BMI) and waist height ratio (WHR) were elevated (Mean, 28.60 ± 4.62, and 0.62 ± 0.069 resp.); glucose and triglyceride values were out of normal ranges (192.22 ± 87.20, and 178.45 ±
86.04 mg/dL resp.), only cholesterol was in normal range (189.22 ± 39.36 mg/dL).
The frequencies of R1273R were in Hardy Weinberg equilibrium (X^2=0.1279) while E23K were not (X^2=7.79). We found that E23K genotype frequencies were significative different to Caucasic, Chinese and Turkish reported DMT2 populations (p> 0.01; CI = 95%). While A1273A allelic frequency
was significative different to Caucasic and Chinese (p> 0.01; CI = 95%), but not Indian population.

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Functional analysis of *pyr*4 gene encoding orotidine-5'-monophosphate deca rboxylase and its usefulness as a selectable marker in *Trichodermaatroviride* .

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Trichodermaatroviride is a filamentous fungus of great biotechnological interest du e to their production of enzymes and secondary metabolites, also is a mycoparasit e of plant parasitic fungus of commercial interest. So a comprehensive study of fun ctional genomic at different stages of its life cycle is very important for understandin g the role of different genes in its biology. Molecular strategies for the functional stu dy of its genome are scarce, mainly the selectable markers. In addition to a high co st, the selection markers, which provide resistance to antibiotics, have different effe ctiveness. For a long time auxotrophic strains have been obtainedwith the aim to u se their genes that complement the mutation as selectable marker, however, they have been generated by saturating mutagenesis (chemical or physical) causing ac cumulative changes into its genome.

Nowadays, the whole genome sequences for a number of filamentous fungi includi ng the model organisms are currently available. With the goal to improve methods f or functional genomic, in this work we are generating novel tools which will be an i mpact in gene study. Specific oligonucleotides were designed to amplify the 5 'and 3' flanks of *pyr*4 gene, excluding the complete open reading frame (ORF) in a first PCR reaction. In a second PCR, the fragments were joining to obtain DNA molecul es which lacking of *pyr*4 ORF (Δpyr4). Finally, a PCR was performed to amplify the mutagenic cassette using nested primers and then, it was used to transform proto plasts of *T. atroviride*.The transformants resistant to 5-fluororotic acid were selecte d on minimal medium (MM) supplemented with uracil and its use as genetic backgr ound in investigation is being evaluated.

To implement the *pyr*4 gene as a selectable marker, were designed several oligon ucleotides to amplify shorter versions of *pyr*4 gene with goal to determine the functi onal minimum promoter. The plasmids obtained were usedfor complementation tes t in auxotrophic strains. The size minimum of *pyr*4 gene, able to restore the auxotro phy,will be usedas a homologous selectable marker for future genetic manipulation s in fungi.

Escargot gene involvement in the establishment of gustatory neurons that respond to volatilized nicotine in *Drosophila melanogaster*.

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Summary

Through of screening of flies carrying insertions element P{GawB} random was obtained L4, the insertion of this element causedsensivityto volatilized nicotine, the time half of recovered (THR) is90 minutes, unlike the wtTHR is 30 minutes. Element insertion P{GawB} was localized gene escargot (*esg*) and encodes a transcription factor type zinc fingers. *esg* is expressed from early stages of development and plays an important role; neuronal differentiation, the correct formation of the tracheal system and maintaining diploidy the imagales discs. We have confirmed that *esg* is the gene that causes hypersensitivity nicotine using $esg^{35Ce\text{-}3}$ allele that exhibits a substitution of an amino acid due to a point mutation in the region of the third zinc finger and which fenocopiar sensitivity to nicotine displayed by L4. Employing interfering RNAs (dsRNA) against *esg* was found that low levels of Esg causes loss of proboscis. The loss of the proboscis in flies with a genetic background L4, causes are no longer sensitive to volatilized nicotine and present a wild phenotype, suggesting that this organ plays an important role in sensitivity to nicotine in the line L4. Proboscis is the main external organ of taste, which contains multiple sensillawich respond to different flavors. Each of these sensilla contains from 2 to 4 neurons and co-expressing different taste receptors. It has been reported that receptors Gr33a, Gr66a and Gr93a, detect bitter substances. We used a mutant with loss of function for the *Gr33a* gene and crossed the line L4. The progeny with both mutations, when exposed to volatilized nicotine no longer sensitive to nicotine as with flies they lack the proboscis. This suggests that *esg* could be involved in the development of gustatory neurons that detect bitter taste and through them is detected nicotine.

Analysis of serotonin 2A receptor gene in depression associated to Parkinson's disease in Mexican population

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Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder that affects ~2% of elderly people around 65 year old. Depression is a common symptom in Parkinson's disease (PD) and it is present in up to 40% of the patients, with adverse impact in their quality of life. There is extensive evidence of degeneration of serotonin neurons(5HT) in PD as well as depression in PD is related to disturbance of serotonin neurotransmission. Furthermore, differentstudies have shown an association between serotonin 2A receptor (5-HT2AR), encoded by *5-HTR2A* gene and major depressive disorder (MDD). However, the relationship between this gene and depression in PD in Mexican population has not been described.**Objective:**The aim of this work was to assesswhether a functional polymorphism (rs6313) in the exon 1 of the *5-HT2AR*gene is related withdepressionthat occurs in PD of Mexican population.**Methods:**We assessed rs6313 polymorphism using a case-control study in 30 PD patients with depression, 30 PD patients without depression and 30 controls without PD and depression. All participants presented similar age, gender and ethnicity (Mexican population). The frequencies of genotypes and alleleswere determined by DNA sequences andHigh resolution melting (HRM).**Results:**The distribution of allelic and genotypic frequency of rs6313 was similar between patients and control subjects.The analyzed results did not show significant association between rs6313polymorphism and PD or depression in PD.**Discussion and conclusions:**To date, these data suggest that rs6313 polymorphism is not a major factor of susceptibility inPD and depression associated with PD in a Mexican population.It is necessary to increase the number of subjects in the study andconsider factors such as additional allelic variants in the *5-HT2AR* gene, and gene-gene interactions to reach more conclusive results.

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Expression and subcellular distribution of β-dystroglycan in prostate cancer

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The dystrophin glycoprotein complex, DAPC is a membranal protein complex involved in the structure and function of the plasma membrane during the muscle contraction-relaxation process. Key proteins of this complex are α- and β-dystroglycan, which provide a link between the extracellular matrix and the cyto skeleton trough their interaction with extracellular matrix and cytoskeleton proteins respectively. β-dystroglican is imported to the nucleus via recognition of its nuclear localization signal (NLS) by impotins. Nuclear β-dystroglican interacts with the nuclear envelope proteins emerin and lamins A/C and B1.β-dystroglycanhas a molecular weightof43 kDa, but a 30 kDafragmentis generated through its proteolytic cleavageby the extracellular matrixmetalloproteaseMMP9.

β-dystroglycan and lamins A/ C and B1 have recently been related to certain types of cancers. Particularly, nuclear accumulating of 30 kDaβ-dystroglycan as well as an altered expression of lamins B1 and A/C have been revealed in prostate cancer. In the present study we analyzed the subcellular distribution of β-dystroglycan in different prostate cancer cell lines with varying degrees of invasiveness, using immunofluorescence assays. We noted that β-dystroglycan accumulates in the nucleus and nucleolus of the cancer cells with less invasiveness, and interestingly, the nuclear localization of β-dystroglycan disappeared in the more invasive cells. Then, we will examine whether the loss of the nuclear function of β-dystroglycan is related to the progression of prostate cancer.

ERCC1 genotypes in Mexican lung cancer patients.

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The nucleotide excision repair pathway (NER) removes a patch of single-stranded DNA containing a lesion in a stepwise manner. In this repair process participates ERCC1 which together with XPF, forms a complex that cleaves one strand of DNA at position 5'of the DNA lesion. Polymorphism in ERCC1, have been identified, and the genetic differences have been associated with lung cancer susceptibility and cis-platin chemotherapy response. There are several studies of ERCC1 genotypes in Asian and Caucasian tobacco smoker lung cancer patients but non in our population.

The aim of the present study was to identify ERCC1 genotypes in the hispanoamerican primary lung adenocarcinoma patients. Blood DNA was obtained from 132 lung cancer patients and 131 healthy subjects. The frequencies ERCC1 genotypes were examined by real time PCR using TaqMan probes. The X^2 test that combines the 2 x 2 contingency tables was used to determine the association between ERCC1 genotypes and lung cancer. We observed that ERCC1 rs2589 (Arg280His) C/T genotype (p = 0.05; OR = 1.72, 95% CI: 1 – 3.11), rs25487 (Arg399Gln) C/C genotype (p = 0.00002); OR = 3.22, 95% CI: 1.79 -- 5.89) and rs11615 (Asn118Asn) A/A genotype (p = 0.003; OR = 5.89 CI: 1.56 – 26.11) were associated with lung cancer risk in our population. The analysis of cancer patients according to risk factor showed that wood smoke exposed patients (W) had an association of rs2589 C/T genotype (p = 0.005; OR =1.99, 95% CI: 1 – 4.93) that was not identified in tobacco smokers (T) and patients in whom no association with a known risk factor could be establish (N). On the contrary groups T and N had a significantly overall risk of cancer associated with rs11615 A/A genotype but not W patients. The 3 groups of lung cancer patients had an association with rs25487 C/C genotype particularly W subjects ($p = 2.2 \times 10^{-5}$; OR = 4.99, 95% CI: 2.14 – 11.74).

In conclusion there are ERCC1 genotypes associated with lung adenocarcinoma in our population that are different among W and T lung cancer groups.

"Recopilación de Encuestas y Muestras Biológicas Humanas de Estudiantes y Trabajadores del Campus Chontalpa de la Universidad Juárez Autónoma de Tabasco (UJAT), como primera etapa para identificar la asociación entre la infección por Dengue y el genotipo del Tabasqueño"

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Universidad Juárez Autónoma de Tabasco, Campus Chontalpa,

Resumen La asociación entre dengue y fenotipo Tabasqueño.

Objetivo: Recolectar Muestras Biológicas humanas (suero y sangre) y aplicar encuesta a individuos del campus Chontalpa-UJAT, para identificar asociación entre Dengue y el fondo Genético de individuos originarios de Tabasco.

Metodología: De Diciembre 2013 a Junio 2014 se capturómuestra biológica de individuosvoluntarios invitados a participar en el estudio. Todos los sujetosllenaron una encuesta para determinar origen, lugar de residencia, edad, género, talla, peso y sobre todo parentesco de familiares originarios de Tabasco (tres generaciones originarios de Tabasco).
El diseño de la encuesta también consideró datos confusores como enfermedades crónico-degenerativas, medicación y consumo de alcohol. Algunas encuestas faltantes se lograron recopilar por 6 estudiantes a través de una dinámica muy audaz por parte de las mimas. El vaciado de datos lo realizaron15 estudiantes de la carrera Químico Farmacéutico Biólogo de 2º Semestre de la Materia Biología Celular y Molecular de la División Académica de Ciencias Básicas del campus Chontalpa-UJAT. El vaciado de datos tanto de los análisis clínicos como de las encuestas fue revisado más de 5 veces y en diferentes ocaciones por distintas personas para asegurar la verasidad de los resultados. Todos los datos y muestras fueron manejados en doble siego en concordancia con la ética laboral de los laboratorios de análisisclínicos. En lo que respecta a la muestra biológica se recolectó en dos tubos por individuo por sistema Vacutainer; el primero con EDTA para Biometría Hemática; por su función anticoagulante, y el segundo sin anticoagulante para la recuperación del suero. Se realizaron análisis clínicos básicos como; niveles de triglicéridos, colesterol, glucosa y la determinación de grupo sanguíneo. El resto de las muestras biológicas congelaron a -20°C para extracción de ADN en la segunda etapa del proyecto.

Resultados: El total de individuos participantes en el estudio fue 422, de los cuales se logró obtener un total de 330 encuestas, de las cuales solo de 321 se logró el llenado de las encuestas, la recolección de la muestra y el análisis clínico completo. se obtuvo que el 61.51% de los individuos cumplieron con los análisis químicos completos, el 38.18% solo con dos partes de los análisis clínicos y el porcentaje restante (0.31%) con un solo tipo de análisis clínico.

Discusión: El número mencionado de encuestas corresponde al 78.38% de los folios totales; 435 folios, pero solo 330 fueron útiles por tener las encuestas fisicas. Del 100% de encuestas: el 97.27% arrojó datos completos con la toma de muestra. Se recuperó cerca de un 11.87% de encuestas que se habían perdido (corresponde a un 19.00%) y el 61.51% dio el resultado completo (con análisis clínico). Lo que indica que pese a errores humanos o falta de organización el trabajo en equipo con actividades particulares según las diferentes habilidades permite la recuperación de los objetivos de un proyecto, que en este caso se trató de las encuestas no aplicadas.

Conclusión: La primera fase del proyecto obtuvo un éxito general del 80%, considerando la prematura incorporación a actividades formales de investigación, más tratándose de alumnos de 2º semestre de la carrera de QFB, lo que demuestra que las labores de investigación pueden ser llevadas a la par del desarrollo académico y promover talentos científicos que serán parte de nuestras futuras generaciones.

Establishment of endogenous control genes in *Sporothrix schenckii* for data normalization in Real-Time PCR

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Sporothrix schenckii is a dimorphic fungus that causes sporotrichosis, a lymphocutaneous disease of cosmopolitan distribution, more prevalent in tropical and subtropical areas. In Mexico is endemic on central and west-central regions. Nowadays, there are few basic studies about this organism due to limited availability of strategies and molecular tools to manipulate it. Recently, a gene silencing methodology was established, but gene expression analyses are required to validate such approach. In addition, the gene expression assays are also important to assess how cells respond to external stimuli, and thus to determine its adaptation to several environmental conditions. There are different methods to quantify mRNA abundance, but regardless the chosen methodology, it is required a result normalization using endogenous control genes, which are expected to have minimal expression variation under development and different experimental conditions. Housekeeping genes are used to achieve this, but the set of "housekeeping" genes useful as endogenous controls for expression is not the same in all organisms thus far analysed. Here, we are aiming to identify genes whose expression is not significantly different during the dimorphic transition of *S. schenckii* and during its growth in different experimental conditions. So far, we have identified three potential housekeeping genes with no significant changes in their expression in yeast cells and hyphae and one of them has constant expression when cells are grown in different culture media. The usefulness of these genes for data normalization has been successfully demonstrated in quantitative RT-PCR experiments. Therefore, these genes represent a new molecular tool to perform expression analysis in *S. schenckii*.

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Functional characterization of three mannosyltransferases of *Saccharomyces cerevisiae* by complementation in *Candida albicans*

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Glycosylation is a post-translational protein modification that starts in the endoplasmic reticulum and continues in the Golgi complex. The biosynthetic pathway of protein glycosylation has been thoroughly studied in the baker yeast *Saccharomyces cerevisiae*, and nowadays it is well known the identity of the proteins involved in the elaboration of *O*-linked glycans, and most of the enzymes participating in synthesis of*N*-linked glycans.The *KRE2/MNT1* gene family participates in these biosynthetic pathways and is composed of nine members, whose products have the sequence signatures of mannosyltransferases.The majority of the family members have been studied and their rolein glycan elaboration has been established. Our group is interested toassign a role to the three uncharacterized members: Ktr4, Kt5 and Ktr7. Null mutants in these genes have no obvious phenotypes, so they have been useless in establishing the role of these genes in protein glycosylation. Since partial redundancy has been observed in *Candida albicans*, it is possible to use this organism to get insights about the function of *KTR4*, *KTR5* and *KTR7*. Here, weshow that heterologous expression of *S. cerevisiae* genes in *C. albicans* null mutants lacking members of the *KRE2/MNT1*restores the glycosylation pathway defects, providing us a biological platform to evaluate and assign functional roles to the *S. cerevisiae* genes in the different glycosylation pathways.

This work is supported by National Council of Science and Technology (CONACyT) (CB2011-166860) and Universidad de Guanajuato.

A DNA damage dependent mechanism regulates the return of *Bacillus subtilis* spores to vegetative growth

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In response to DNA damage, cells activate checkpoint-signaling mechanisms to control cell cycle progression and elicit DNA repair to maintain genomic integrity[1].Oxidative stress-induced damage, including 8-oxo-guanine andapurinic/apyrimidinic (AP) DNA lesions, were detected in dormant and outgrowing *Bacillus subtilis* spores lacking the AP endonucleases Nfo and ExoA[3]. Spores of the □*nfoexoA*strain exhibited slightly slowed germination and greatly slowed outgrowth that drastically slowed the spores' return to vegetative growth. A null mutation in the *disA*gene, encoding a DNA integrity scanning protein (DisA), suppressed this phenotype, as spores lacking Nfo, ExoA, and DisA exhibited
germination and outgrowth kinetics very similar to those of wild-type spores. Overexpression of DisA also restored the slow germination and outgrowth phenotype to *nfoexoAdisA*spores. A *disA-lacZ*fusion was expressed during sporulation but not in the forespore compartment. However, *disA-lacZ*was expressed during spore germination/outgrowth, as was a DisA-green fluorescent
protein (GFP) fusion protein[2]. Fluorescence microscopy revealed that, as previously shown in sporulating cells[1], DisA-GFP formed discrete globular foci that colocalized with the nucleoid of germinating and out-growing spores and remained
located primarily in a single cell during early vegetative growth. Finally, the slow-outgrowth phenotype of *nfoexoA*spores was accompanied by a delay in DNA synthesis to repair AP and 8-oxo-guanine lesions, and these effects were suppressed following *disA*disruption. We postulate that a DisA-dependent checkpoint arrests DNA replication during *B. subtilis* spore outgrowth until the
germinating spore's genome is free of damage[2].

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Evaluating the functionality of Gpn1carboxy-terminal domain and nuclear export signal in *Saccharomyces cerevisiae*.

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Gpn proteins belong to a new family of highly conserved small GTPases. The three paralog genes, GPN1, GPN2 and GPN3, are present in all eukaryotic organisms whereas there is a single GPN gene in *Archaea*. The 3D structure of the archaealGpn protein reveals a homodimericcomplex with a canonical GTPase domain. Eukaryotic Gpn1 contains a carboxy-terminal extension that is not present in *Archaea*. Molecular modeling of human Gpn1 indicates that the amino-terminal GTPase domain and the carboxy-terminal extension organize as two independent structural domains joined by a very long and flexibleα-helix. Although the function of these proteins is unclear, it has been demonstrated that Gpn proteins are essential for life in yeast and human cells, and are required for optimal activity of RNApolymerase II (RNAPII).In yeast, a reduction in Npa3/Gpn1 function leads to chromatid cohesion and cell cycle defects, as well as cytoplasmic mislocalization of several RNAPII subunits. Our lab identified previously a nuclear export signal (NES) that is sufficient for Gpn1 nuclear export. This NES is located at the carboxy-terminal domain, exclusive of eukaryotic Gpn1.

Hence, we have special interest in defining the functional relevance of the NES and carboxy-terminal domain for Gpn1 function in*Saccharomyces cerevisiae*. We replace by homologous recombination the endogenous *GPN1* genewith a truncated *GPN1*lacking the carboxy-terminal domain, in yeast strains expressing several GFP-tagged proteins known to beinvolved in RNAPII nuclear import.We demonstrated by fluorescent microscopy that the carboxy-terminal domain of Gpn1 is necessary for the nuclear export of RPAP2, a recently described phosphatase for the RNAPII CTD, and for optimal cell growth.Our results support a model where the primary function of Gpn1 is to mediate the nuclear export of RPAP2, which in turn mediates nuclear import of RNAPII.

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Cartel.

Area 13: Regulación genética y epigenética.

InsightsintotheevolutionanddomainstructureofAtaxin-2 proteinacrosseukaryotes.

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Ataxin-2 is an evolutionarilyconserved protein first identified in humans as responsible for spinocerebellar ataxia type 2 (SCA2). The molecular basis of SCA2 is the expansion of a polyglutamine tract in Ataxin-2, encoding a Lsm domain that may bind RNA and a PAM2 motif that enables interaction with the poly(A) binding protein.Although the association with SAC2 has been verified, a detailed molecular function for Ataxin-2 has not been established. We have undertaken a survey of Ataxin-2 proteins across all eukaryotic domains. In eukaryotes, except for vertebrates and land plants, a single ortholog was identified. Notably, with the exception of birds, two Ataxin-2 genes exist in vertebrates. Expansion was observed in land plants and a novel class lacking the LsmAD domain was identified. Large polyQtracts appear limited to primates and insects of the orders Hymenoptera and Diptera. A common feature across Ataxin-2 orthologs is the presence of proline-rich motifs, formerly described in the human protein. Our analysis provides valuable information on the evolution and domain structure of Ataxin-2 proteins. Proline-rich motifs that may mediate protein interactions are widespread in Ataxin-2 proteins, but expansion of polyglutamine tracts associated withspinocerebellar ataxia type 2, is present only in primates, as well as some insects. Our analysis of Ataxin-2 proteins provides also a source to examine orthologs in a number of different species. This work is supported bythe grant CB177528 from SEP-Conacyt

Changes in internalization and regulators of virulence gene expression after*S. aureus*pre-treatment withTNFα and IL-10

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Staphylococcus aureus is a Gram-positive bacterium that is a potentially lethal opportunistic human and animal pathogen.The pathogenic success of *S. aureus* can be attributed to a diverse array of virulence factors that include a large number of cell-surface binding proteins like adhesins that are expressed during colonization of the host, and secreted proteins (e.g. hemolysins, proteases, lipases) that are required in acute infections. During infection, the host environment may change markedly, modulatingvirulence genes expression. It has been reported that when bovine umbilical vein endothelial cells (BUVEC) cells are preconditioned with pro-inflammatory cytokines, changes in *S. aureus* internalization are observed. We propose that *S. aureus* is also able to sense these host signals during the pathogenic process. The aim of this work was to evaluate the internalization *in vitro* of *S. aureus* into BUVEC, and to analyze the expression levels of global virulence genes regulators (*agrA*, *rnaIII* and *sigB*),after pre-conditioning of *S. aureus*withthe cytokines TNFα (pro-inflammatory) and IL-10 (anti-inflammatory).*In vitro* infection assays were performed to evaluate internalization and qPCR to analyze global virulence gene regulators expression.The internalization of *S. aureus* in response to IL-10 was decreased while in response to TNFα it was increased with respect to the control. The response to both cytokines also showed differences in the patterns of gene expression. *agrA*was induced by IL-10 and repressed by TNFα (P<0.005).*rnaIII*was induced by both cytokines (P<0.05).*sigB*was differentially repressed by both cytokines (P<0.005) when *S. aureus*was pre-conditioned until the early exponential phase of growth. Taking together this results suggest that*S. aureus*is able to detect and respond to host signals involved in the pathogenic processprobably through *quorum sensing*- two component systems as suggested by our results with *agrA* and *rnaIII*, thus modifying its invasion potential.

Role of PARP/PARG [(Poly-ADP-ribosyl) polymerase/glycohydrolase] in the pathogenic fungus *Fusarium oxysporum f.sp. lycopersici*

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Fusarium oxysporum f. sp. lycopersici is a fungal plant pathogen for tomato, present in the soil, causing the disease called "vascular wilt". In response to damage, the plants are able to producing substances that may be genotoxic, fungi have evolved mechanisms to reverse this damage. In yeast and humans these pathways of DNA damage response have been characterized, all together make a network that, in addition to controlling cell cycle responses to genotoxic injury, preventing the spread of damaged genomes when the injury is irreversible.

In higher eukaryotes has been reported that poly (ADP-ribosyl) polymerase (PARP) is activated in response to DNA breaks generated by genotoxic agents such as reactive oxygen species, ionizing radiation, alkylating agents and/or free radicals. This modification of nuclear proteins with the poly-(ADP)-ribose (PAR) is a type of posttranslational modification required for activation of cellular processes, and is degraded by the enzyme poly (ADP-ribosyl) glycohydrolase (PARG). PARG has endo and exo glycohydrolase activity giving ADP-ribose free as product. The genome of *Fusarium oxysporum f. sp. lycopersici* has a gene encoding a protein orthologous to the human-PARG. In this work we showing the presence and function of this protein in this fungus and its possible role in physiology and/or pathogenesis. Previously, we amplified and cloned ORF from DNA and mRNA FOXG_05947. The fragment was expressed as His6-PARG. The recombinant soluble enzyme was purified, and its activity evaluated by assaying enzyme activity revealing that the purified enzyme exhibits activity of poly ADP-ribosyl glycohydrolase, and is inhibited by DEA (6,9-Diamino-2-ethoxyacridine lactate monohydrate) and tannic acid both reported as inhibitors of PARG.

In order to evaluate its function in the fungus, we generated a Δ*parg* mutant by using Double-joint PCR technique. This mutant was grown in presence of some mutants such as Methyl methanesulfonate, Ethyl methanesulfonate and Hydroxyurea. In addition a double mutant Δ*parg*-Δ*parp* was obtained and was grown in the same mutants above-mentioned. In this experiments a delay in growth was observed to the mutants. To test expression of the *parg* gene mycelia was grown under no inducing conditions and determined by qRT-PCR with gene-specific primers. Total RNA was obtained from mycelia of the wild- type, Δ*parg* and Δ*parg*-Δ*parp* grown for 24 h. Higher levels of qRT-PCR product corresponding to the *parg* transcript were detected in mycelia of the wild-type grown. By contrast, no *parg* expression was observed in the Δ*parg* and Δ*parg*-Δ*parp* mutant.

Chromatin Immunoprecipitation Analysis by RT-PCR in the yeast *Saccharomyces cerevisiae.*

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In order to set up chromatin immunoprecipitation analysis by RT-PCR, binding of *RTG3* and *NRG1*-encoded modulators to some of their target promoters was studied:
1.- Rtg3 determines the retrograde response and it has been shown that its action is required for *CIT2* expression, and that as well as Rtg1, it binds to *CIT2* promoter.
2.- It has been previously shown that Nrg1, alternatively mediates *ALT1* or *ALT2* repression, depending on the intracellular concentration of either alanine or of a product of its catabolism. Nrg1 occupancy of the *ALT1* and *ALT2* promotors was observed in both promoters. Analysis of the obtained chromatin immunoprecipitates, was carried out by traditional PCR. Nrg1 was bound to bind *ALT1* and *ALT2* promoters.

To analyze *GDH1* and *GDH3* promoter occupancy by.Rtg3 and Nrg1, myc^{13} tagged mutants were constructed. Chromatin immunoprecipitation experiments were performed on extracts obtained from exponential or stationary grown cultures on glucose-proline, YPD or etanol-glutamine cultures of the pertinent strains,*CIT2*, *ALT1* and *ALT2* were used as positive controls. Amplification of *GRS1* or *PHO5* coding sequences were used as negative controls.

Results showed that Rtg3 and Nrg1 bound *GDH1* and *GDH3* promoters in the tested growth conditions. However, conclusions from the RT-PCR analysis, were not straightforward since a large standard deviation was observed. It was thus not possible to determine whether Rtg3 or Nrg1 bound more efficiently to *GDH1* or *GDH3* during exponencial or satationary phases .Further experiments will have to be conducted in order to establish the experimental conditions that will allow to obtain reproducible results with lower standard deviations.

SPATIO-TEMPORAL ANALYSIS OF THE *Pv*KNOLLE PROMOTER ACTIVITY IN *Phaseolus vulgaris* TRANSGENIC ROOTS INOCULATED WITH *Rhizobium tropici*

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The symbiosis between legume and rhizobia is characterized by the formation of a nitrogen-fixin nodule nitrogen fixing nodule. The development of the nodule is a complex process that involves several steps: 1) the interaction rhizobia-root hair 2) the formation of thread formation (IT), 3) cell division of cortical cells, adjacent to the responsive root hair, to generate the nodule primordium and 4) the delivery of rhizobia from the IT into nodule cells to further differentiate to bacteroid and acquire the ability to fix nitrogen. Genetic and molecular analysis of this complex process have revealed that the formation of the nodule involves the recruitment and adaptation of root development molecular programs, namely cell division, formation of vascular bundles, the organization of the outer and inner cortex and the nodule,
among others[1].

In order to identifiy genes required for the cortical cell division in nodule organogenesis, we have performed a bioinformatic analysis of the *Phaseolus vulgaris* genome using as query sequences of proteins involved in the cell plate formation. One of the genes identified with this approach is KNOLLE.In *Arabidopsis thaliana* the expression of KNOLLE is tightly regulated during the cell cycle. KNOLLE codes for a syntaxin specifically associated to cytokinesis, it mediates the fusion of vesicles at the site of the cell plate formation during cell division[1]. By RT-qPCR analysis that the *Pv*KNOLLE transcript is abundant in the root apex, as well as in root hairs and stripped roots (with no root hairs, no apex). In order to explore the involvement of the *Pv*KNOLLE gene in *Phaseolus vulgaris* nodule organogenesis, we have PCR-amplified 1800 pb of the sequence upstream the *Pv*KNOLLE coding sequence. The fragment was cloned as a transcriptional fusion to the sequence coding for the chimeric reporter protein, GFP-GUS in the plant expression vector pBGWFS7. The corresponding construction is currently being used to generate *P. vulgaris* transgenic roots and perform a detailed analysis of the spatio-temporal activity of the*Pv*KNOLLE promoter during the nodulation process.

[1]Touihri *et al*., (2011) The Plant Journal 68:755-764.

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Comparison of the effect of HPV 18 and 11 (high and low risk types) E6 oncoproteins on the proliferation and migration of C33A cells

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Cervical Cancer (CC) is the second cause of death among Mexican women and is strongly associated with high-risk Human Papillomavirus (HPV) infection . Papillomaviruses are small double-stranded DNA viruses coding 8 genes;the E6 gene encodes an oncoprotein that contributes to cell transformation because it is expressed at early stages of viral infection and associated with the most important regulators of cell fate such as apoptosis,proliferation, oncogene transcription and tumor suppressor gene inactivationDue to the continuous expression of the E6 gene duringCC progression, it is necessary to precisely understand the effects of thisoncoprotein in host cells.With this aim, C33A cells (cervical carcinoma-derived, HPV negative) were transfected with plasmids containing the open reading frames of the HPV 18 and 11 E6 genes, high and low risk respectively. We found a differential effect on expression of important proteins for cell growth, such as p53 and caspase 3, in addition to differences associated with the repression of other target genes in cell migration and proliferation.

Thus, we have developed an*in vitro* model to analyze the differences between the molecular alterations caused by high- and low-risk HPV types

The role of the CDK8 module of Mediator in vegetative development of *Arabidopsis thaliana*

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Plants develop progressively, going through distinct growth phases. The transitions from the juvenile to the adult vegetative phase, and from the vegetative to reproductive phase, are tightly regulated by a genetic pathway involving the microRNAs miR156 and miR172, and the miR156-targeted *SPL* transcription factors. Knowledge about miR156 pathway has increased a lot recently, but the genes that regulate miR156 expression are currently unknown.Recent results in our laboratory have demonstrated that *MED12/CCT* and *MED13/GCT* act upstream of the miR156-SPL-miR172 pathway (Gillmor et al., in review). Thus, in addition to regulating developmental timing during embryogenesis (Gillmor et al., *Development*, 2010), *MED12* and *MED13* also regulate the timing of vegetative and reproductive development.

CCT/MED12 and GCT/MED13 form, with HEN3/CDK8 and its partner Cyclin C, a complex called “CDK8 module”, that represses the transcriptional activator core Mediator. The main goal of this work is to understand the role of the CDK8 module in controlling vegetative development.

Our results show that *hen3/cdk8* mutants also exhibit a delay in flowering time and a delay in the acquisition of some adult traits, particularly *hen3* mutants exhibit leaves with less serrations and longer petioles than wild type plants,suggesting that in addition to MED12 and MED13, CDK8 also plays an important role in regulation of vegetative development.

RT-PCR expression analysis in wild type, *cct*, *gct*, and *hen3* mutants has revealed that only certain*MIR156*genesand their *SPL* targets are transcriptionally regulated by the CDK8 module.I have seen that expression of some MIR156 genes is increased in *cct*, *gct* and *hen3* mutants, but to a different extent depending on the mutant. Interestingly, expression of some *SPL* genes is decreased in *cct* and *gct* mutants, but remains unaltered in *hen3* mutants, which is consisting with some transcriptome data, and could explain the less severe phenotype of *hen3* mutants.

We are currently carrying out experiments to further characterize the spatio-temporal expression of some genes of the MIR156 pathway in wild type, *cct*, *gct*, and *hen3* mutants. We are also generating double and triple mutants to determineif reduced function of MIR156 can rescue the wild type phenotype in mutants of the CDK8 module. Lastly, we are pursuing ChIP and GR strategies to determine which genes of the miR156-SPL-miR172 pathway are direct transcriptional targets of CDK8 module genes.

Construction of the recombinant proteins for the NMD putative factors Upf1, Upf2 and Upf3 of*Ustilago maydis*

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The regulation of gene expression is the process by which the information contained in the genome is used to generate the necessary proteins according to the requirement of each organism at a given time. Messenger RNA (mRNA) mediates the transfer of genetic information from the cell nucleus to ribosomes in the cytoplasm, where it serves as template for protein synthesis; for this reason, mRNA is submitted to various mechanisms of quality control. Nonsense-mediated mRNA decay (NMD) is the best characterized mRNA surveillance mechanism by which PTC-containg transcripts are selectively recognized and dowregulated to avoid the expression of truncated polypeptides. This surveillance mechanism is ubiquitous among all eukaryotes, it has been found in yeast, plants, nematodes flies and vertebrates. The Upf1, Upf2 and Upf3 proteins comprise the core NMD machinery; these proteins are highly conserved between species. *Ustilago maydis* is a basidiomycete that causes the carbon disease in corn, commonly known in Mexico as “cuitlacoche”, which has been used as a biological model to study various molecular and cellular processes. It is currently unknown if NMD occurs in this fungus. In order to study the mechanism of NMD in *Ustilago maydis*, theputative Upf proteins were identified in the genome of the fungus and compared with their homologous in *Homo sapiens*. Subsequently, primers were designed to amplify coding regions of the homologue genes identified. PCR products were cloned in the pPROEX HT vector (Invitrogen), which is specific for the production of recombinant proteins. The success and the orientation of the cloning were verified by restriction assays and sequencing. The induction of recombinant proteins was performed with 0.6 mM of IPTG in the strain DH5α of *E. coli*. The obtention of such recombinants was visualized by a denaturant polyacrylamide (SDS-PAGE) gel electrophoresis. The degree of conservation between *Homo sapiens* and *Ustilago maydis* for the identified proteins andthe presence of the homologs in the genome of the fungus, allows us to suppose that the NMD mechanism takes place in the basidiomycete. In the future it will be necessary to perform functional assays with the recombinant proteins obtained in this work to corroborate that this process is similar to the mechanism of NMD that occurs in humans.

Interaction of NFAT, AP-1, NF-kB, Sp1 and STAT3 protein and its involvement in the expression of IL-10 in U937 monocytes stimulated with LPS/PGE2.

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The immune response cause activation of pro-inflammatory cytokines by an antigen, in parallel an anti-inflammatory system is developed which regulates and stops the pro-inflammatory molecules limiting the tissue damage. Macrophages have a broad of functions, one of them is involved in the inflammation process. Activated macrophages synthesize and release molecules that attract monocytes to the zone which increase the expression of adhesion molecules in monocytes. IFN-gamma, GM-CSF and LPS stimuli contribute to the M1 macrophages subpopulation (pro-inflammatory phenotype); on the other hand, M-CSF, IL-4 and IL-3 stimuli induce M2 subpopulation (anti-inflammatory phenotype). M2 function is the production of IL-10, which is an anti-inflammatory cytokine, its deficiency or expression may enhance the inflammatory response and lead to the development of pathologies. The use of bioinformatic software has identified potential binding elements of transcription factors in the promoter of IL-10 gene, eg GRE,CRE, AP-1, C/EBP, NF-kB, STAT3, Sp1 and NFAT, among others; the promoter of IL-10 is very complex, which represents a potential study. In our laboratory, it was determined that LPS/PGE_2 stimuliinduce the expression of IL-10 in macrophages. *In vitro* studies of DNA- protein interaction demonstrated the presence of complexes in NFAT, AP-1, NF-kB, Sp1 y GREsites; also showed that c-Jun protein is presentin the proximal composite NFAT/AP1 (-181/-161bp), and the presence of the p50 and p65 proteins in the NF-kB distal site (-1973/-1964bp); indicating that this stimulus can activate different transcription factors that bind to its site in the IL-10 promoter.

With this evidence, the aim of this work is tostudy whether the interactions of NFAT, AP-1, NF-kB, Sp1 and STAT3 proteins regulated the expression of the IL-10 in U937 monocytes stimulated with LPS/PGE_2, and if this regulation is mediated by the formation ofmultiproteincomplexes.By testing Chromatin Immunoprecipitation technique (ChIP), we are determining the functional interactions of N-FAT, STAT3, Sp1 and AP1 proteins in the promoter region -827/+45bp and the NF-kB protein in the region from to -2519/-1557bp. In our model study we have standardized the cross-linking condition among transcription factors to DNA (0.5% formaldehyde), and the conditions of fragmentation of chromatin conditions (10 pulses, 45”ON/59”OFF) to obtain range DNA between 250-1000bp.Furthermore, by immunoprecipitation assay (IP) with antibodies against proteins of study, we are identifying potential multiprotein complexes that may participated in the activation of the expression of IL-10 under the stimulus with LPS/PGE_2; for this, by western blot assays verified the specificity of the antibodies in total protein extracts. The IP permormed withprotein extract from $2X10^6$ cells and anti-STAT3 antibody revealed two bands with a size between60 and 70KDa, suggesting that STAT3 may interact with other proteins to form a multiprotein complexes.

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Methylation analysis of genes for the T-type voltage-dependent calcium channels in cervical cancer derived cell lines

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Cervical cancer is the second cause of death in women worldwide. A positive correlation between increased gene methylation and increased pathological changes exists during the pathogenesis of cervical cancer. Previously, it was demonstrated that intracellular calcium control allows orderly cell cycle progression, and play a vital role in growing and cell proliferation. The voltage- dependent calcium channels control calcium influx through the membrane, which is an important factor in the early stages of cell death such as apoptosis and ischemia. T type calcium channels, a type of voltage gated calcium channel, have a unique electrophysiological characteristic that allows them open at low membrane potential. It plays a fundamental role in the regulation of intracellular calcium and homeostasis in non-excitable cells. This calcium channels are composed of several heterodimeric subunits: α, β, γ, δ that are encoded by multiple genes. Among them, the α1 subunit is the largest, and includes regulatory sites of secondary messengers, drugs and toxins. It also includes the voltage sensor and the device gate. There are three isoforms of α1 subunits: Ca_V 3.1 (α1G), Ca_V 3.2 (α1H) and Ca_V 3.3 (α1I). The expression of the T type calcium channels isoforms has been reported in a wide range of cells including cancer cells, probably providing an altered conduit calcium influx in response to the growing demand for calcium during its rapid proliferation. Our study has been aimed to evaluate the methylation in the promoter and regulatory regions of two genes encoding the main isoforms of T-type calcium channels: Ca_V 3.1 (α1G) and Ca_V 3.2 (α1H), and its correlation with their expression in cervical-cancer cells. Previously, it was reported no methylation of the T-type calcium channels in normal breast epithelial tissue, bone marrow, colon mucosa and placenta tissue suggesting that Ca_V 3.1 gene methylation is cancer specific. Regarding the role played by the Ca_V 3.2 gene, some studies have suggested that this gene is an oncogene candidate and is possibly regulated by hypomethylation. Using standard RT-PCR, the mRNA expression of the Ca_V 3.1 channels was verified in advance. Preliminar results demonstrated the expression of this channel in CALO and its absence in the other cervical cancer cell lines analyzed: C-33A, SiHa, HeLa and INBL. However, the mRNA expression of the Ca_V 3.2 was present only in C-33A, but absent in the other cell lines analyzed. After bisulfite DNA conversion the percentage of methylation will be established, and correlated with the expression of each one of the genes. Separately analysis of Cav 3.1 and Cav 3.2 genes will
distinguish their possible individual roles in the development of cervical cancer pathogenesis.

Transcriptional regulation of *GDH3* in *Saccharomyces cerevisiae*.

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Theyeast*Saccharomycescerevisiae*, experimented a whole-genomeduplicationeventabout 100 millionyears ago. It has been suggested that genome duplication lead to speciation, because it promoted genome innovation and adaptations that allowed the yeast to occupy new niches. The study of the conserved duplicated genes, known as paralogues, constitutes an interesting approach to understand their physiological roles and functional diversification that could explain their retention.

NADP-dependent synthesis of glutamate in *Saccharomyces cerevisiae* is mediated by the paralogues enzymes Gdh3 and Gdh1, they play a central role in nitrogen metabolism, and connect nitrogen metabolism to carbon metabolism through α-ketoglutarate.

Previous studies in our laboratory have shown that *GDH3*expression is repressed in glucose and derepressed when glucose is exhausted. It depends on the interaction of *cis* sequences present in the promoter, and *trans* elements we have not yet identified. Importance of the *GDH3* native promoter was confirmed, when GDH3 coding sequence was fused the bacterial glucose insensitive promoter PtetO7. Under this condition, *GDH3* was expressed throughout the growth cycle even in the presence of glucose

Northern analysis and determination of Gdh3 NADP glutamate dehydrogenase activity showed that*GDH3*expression is not regulated by Hda1, Tup1 and Sir2 chromatin remodelers.

The role of *NRG1/NRG2*as a repressor in the expression of *GDH3*was confirmed by Northern blot analysis; it was observed that in *nrg1Δnrg2Δ* double mutant the expression of the *GDH3* transcript was 2-fold increasedeven in the presence of glucose. The analysis of a *mig1Δ* is underway. Rtg3 was confirmed as an activator of *GDH3* by Northern blot analysis in ethanol grown-cultures.

Chromatin organization of the *GDH1* and *GDH3*promoters in glucose or ethanol-grown cultures is being analyzed through Nucleosome Scanning Assay (NuSA). Preliminary results show that *GDH1* promoter has a different re-positioning of nucleosomes in glucose or ethanol preparations, and thatthe binding sites for some transcriptional factors that were reported to regulate GDH1 expression are located in the nucleosome free region (NFR) of the promoter.

PHENOTYPE ANALYSIS OF *Phaseolus vulgaris* TRANSGENIC ROOTS EXPRESSING *Pv*CLC2-RNAi OR *Pv*CLC3-RNAi

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Phaseolus vulgaris (common bean) is one of the models for the study of legume-rhizobia symbiosis, a tightly regulated and complex process that leads to the formation of a nitrogen-fixing nodule, new organ in the root. Nodule formation involves several stages of development: root hair infection, infection thread (IT) formation, cortical cell division to form a nodule primordium, rhizobia delivery from the IT into nodule cells and differentiation to bacteroid which acquire the ability to fix nitrogen. Nodulation is initiated by a molecular dialogue between rhizobia and the legume root, which triggers series of coordinated physiological, cellular and molecular responses in root hair cells. Only actively growing root hairs are susceptible to interact with rhizobia. Polar growth of root hairs is restricted to the apical zone of the cell and depends on a dynamic activity of the apical cytoskeleton, vesicular trafficking, exocytosis and endocytosis.

To investigate the role of clathrin-mediated endocytosis (CME) in the root hair infection with rhizobia, we have decided to perform a functional characterization of *P.vulgaris* genes coding for the clathrin light chain (CLC) subunits. As a first step, we determined the profile of transcript accumulation of *Pv*CLC genes, in sections of *P. vulgaris* seedling root: apex, root hairs and stripped roots (with no root hairs, no apex). We have found that *Pv*CLC2 and *Pv*CLC3 mRNA displayed the higher accumulation in root hairs (48 and 32%, respectively). As a second step we applied a reverse-genetics approach to address the role of these genes during the nodulation. We have generated *P. vulgaris* transgenic roots expressing RNAi to specifically silence either *Pv*CLC2 or *Pv*CLC3 gene. The phenotype analysis of these roots challenged with *Rhizobium tropici* is currently in progress.

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Unraveling the function of *Phaseolus vulgaris RbohD* during nodulation

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Plant homologs of membrane-associated NADPH oxidases (Rbohs; respiratory burst oxidase homologs) have been associated with plant defense against pathogens (Torres, et al. 2002). Recently, it has been reported that during the legume-rhizobia symbiosis, some NADPH oxidases regulate the earliest steps of the bacterial infection in *Phaseolus vulgaris* (Montiel, et al. 2012) and impair nodule functioning in *M. truncatula* (Marino, et al. 2011). Nine members of the *P. vulgaris* Rboh gene family have been identified and named *PvRbohA-PvRbohI*. *PvRbohA*, *PvRbohB*, *PvRbohC* and *PvRbohD* transcripts were abundant in all organs tested, while the others were barely detected. We focused in studying the functional role of *PvRbohA*, *PvRbohB*, *PvRbohC* and *PvRbohD* during the symbiotic interaction. Previously, we found that overexpression of *PvRbohB* significantly enhanced the formation of ITs, nodule biomass, and nitrogen-fixing activity, and increased the number of symbiosomes in nodules, and the size and numbers of bacteroides in the symbiosomes; furthermore nitrogen fixation was enhanced (Arthikala, et al. 2014). However, down-regulation of *PvRbohA* trancripts resulted in a decreased number of nodules and size and mature nodules were devoid of rhizobial infected cells (Arthikala, manuscript in preparation).

Accumulation of *PvRbohD* transcript was greatest in roots and nodules (Montiel, et al. 2012). Therefore, we propose to unravel the role of this gene during the first stages of the *P. vulgaris-Rhizobium tropici* symbiotic process by overexpressing it. Herein, we present the data on the phenotypic characterization of the *PvRbohD* overexpressing transgenic roots after rhizobia inoculation.

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Analysis of the expression patterns of the Mitogen Activated Protein Kinase Kinases (MAPKKs) encoded on genome the *Arabidopsis thaliana.**

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Mitogen Activated Protein Kinase (MAPK) cascades are signal transduction modules used by all the eukaryotes to response to environmental and developmental signals. A typical MAPK module consists of three kinases (MAPKKK, MAPKK and MAPK), which activate sequentially by phosphorylation. Then the last component of the module (a MAPK) phosphorylates other proteins in the cytoplasm or nucleus to affect their activity or to impact the gene expression. On the genome of *Arabidopsis* are encoded 60 MAPKKKs, 10 MAPKKs and 20 MPKs, thus implying promiscuity and functional redundancy. In this scenario a valid hypothesis is that the signaling specificity of a particular MAPK cascade should be maintain by strict transcriptional and/or posttranscriptional regulation mechanism. Recently we have demonstrated that the MAPK6 is involved in the signaling pathway controlling the embryo and root development programs of *A. thaliana* (1) and current research in our laboratory is focused to identify the up- and down-stream components of this MAPK cascade. To develop this idea, the *in silico* predicted promoters (http://www.dna.affrc.go.jp/PLACE/) for the ten MPKKs genes annotated on the *A. thaliana* genome are being isolated and cloned in plant expression vector to regulate the expression of GFP and GUS reporter genes. The expression patterns directed by the regulatory regions of the MAPKKs genes will be analyzed on transgenic plants expressing these transgenes. The progress of our research will be discussed at the congress.

1) López-Bucio J. S., *et al*. 2014. *Arabidopsis thaliana* mitogen-activated protein kinase 6 is involved in seed formation and modulation of primary and lateral root development. Journal Experimental Botany, 65(1): 169-183.

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Analysis of the interaction between the yKu, Abf1 and Rap1 proteins of *Candida glabrata* and the *cis*-acting regulatory elements in the E-R telomere

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Candida glabrata is an emergent pathogen,resistant to traditional antifungals, responsible of the 15% of the systemic and mucosal candidiasis worldwide. In México it is responsible for 13% of the systemic infections. The ability of *C. glabrata* to cause disease lies in part in the fact that itcan adhere tightly to the epithelial host cells. This adherence depends on the expression of at least some members of the family of *EPA* genes that encode major adhesion, cell surface proteins. The Epa proteins are lectins that recognize host glycans and are members of the class of glycosylphosphatidylinositol-anchored cell wall proteins (GPI-CWPs). *In vitro* adhesion of *C. glabrata* to mammalian epithelial cells is mediated primarily by Epa1, and in the reference strain BG2, there are 23 paralogues of the *EPA1* gene. Most of the *EPA* genes are transcriptionally silenced due the fact that they are encoded in subtelomeric clusters where several regulatory proteins including Rap1, Sir, Rif1, Ku70, Ku80 and Abf1 act at telomeres and subtelomeric regions of the chromosomes and form compact chromatin structures. In *C. glabrata* however, this repression may vary between the individual telomeres and it depends on the interaction between regulatory proteins and *cis*-acting elements. In this study we are interested in analyzing the interaction *in situ* of the yKu70, yKu80, Rap1 and Abf1 proteins with the right telomere of chromosome E (Ch E-R) of *C. glabrata*. In this region the *EPA1*, *EPA2* and *EPA3* genes are located and some *cis*-elements that include a negative element, and a protosilencer have been characterized. To do this we are generating tagged versions of the functional proteins to use them in chromatin immunoprecipitationassays (ChIP) with the subtelomeric region of the ChE-R of *C. glabrata*.

"Site specific DNA methylation analysis of the RXRA promoter region from umbilical cord blood and its relationship to maternal nutritional status during pregnancy"

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Environmental factors during pregnancy, such as maternal nutritional status, may lead to changes in epigenetic regulation. There are critical windows during embryonic development that are sensitive to changes in DNA methylation patterns and influence the phenotype of the offspring.

The Body Mass Index (BMI) is an indicator of the nutritional status. During pregnancy, we can also measure gestational BMI and weight gain desirable. Direct evidence of the relationship between maternal nutritional status and changes in DNA methylation is well less established in humans compared with animal models. Godfrey et al. associated maternal carbohydrate intake in early pregnancy with the methylation status of CpG in the promoter of RXRA gene from umbilical cord. Then, the measurements of perinatal DNA methylation were related to later childhood adiposity.

The Retinoic Acid X-Receptor (RXRA) is a transcriptional factor that binds to other factors regulating the genes expression. Those genes are involved in energetic metabolism pathways such as adipogenesis and fatty acid synthesis, among others.

The present is a pilot study that aims to analyze the maternal nutritional status during pregnancy and the relationship of it with changes in DNA methylation patterns in a site specific promoter region of RXRA gene extracted from umbilical cord blood from women with normoevolutive term pregancies.

From patients records anthropometric, biochemical and nutritional values were recorded in a database. Analysis of these data allowed us to evaluate and classify the patients into groups according to their nutritional status.

Samples of DNA from umbilical cord blood were modified with sodium bisulfite in order to analyze them using the Methylation Sensitive - High Resolution Melting Point technique (MS-HRM) studying methylation patterns in a site specific of the RXRA promoter region and establish if there is a correlation with the study variables.

This work contributes to determine whether the weight gain during pregnancy relates with a DNA methylation pattern in a specific RXRA promoter region.

Methylation-sensitive high resolution melting analysesto site specific LEPR gene promoter methylation from umbilical cord related with the maternal BMI and weight gain during pregnancy

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The body mass index (BMI) and weight gain during pregnancy are maternal health indicatorsthat have being suggested to be influencing offspring phenotype and late-onset diseases. Genome-wide association studies (GWAS) on obesity led to the identification of leptin (LEP) gene as one of the genes associated to the central regulation of feeding.

LEP is an adipokine characterized for its pleitropic actions in development and physiology. This hormone binds to LEPR receptors that are expressed in many tissues such as brain, placenta and umbilical cord, among others. The LEPR Gln223Arg polymorphism has been related with obesity in Mexican adolescents turning it into studio target in our population.

LEP promoter DNA methylation has been associated with maternal and infant perinatal outcomesandtissue specific effects.LEPR promoter DNA methylation is poorly understood and hasbeing well less characterized in association to developmental programming.

In thispilot studywe analyzed a normal pregnancy women cohortin which women were classified according to pre-BMI andweight gain during pregnancy and a site-specific LEPR promoter DNA methylation was compared among them. DNA extracts from cord were modified with sodium bisulfite and submitted tomethylation-sensitive high resolution melting (MS-HRM) analyses.

MS-HRM melting curves were normalized with the comparison of different fluorescence levels of methylation through methylated and un-methylated controls. Differences between methylation levels were assessed by covariance using mother's pre-BMI and weight gain as covariates and contrast vectors for individual mean pairs.

In conclusion, LEPR DNA methylation is a plausible mechanism to be involved in fetal metabolic programming. Changes on LEPR promoter methylation patterns from umbilical cord could be an indicative for predisposition to obesity.

mir-26: A Putative Epigenetic Regulator in Iflammation-Associated Colorrectal Cancer Development

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Colorectal cancer (CRC) is the fourth most common cancer-related death cause worldwide. It has been observed that patients with chronic inflammatory bowel disease are more likely to develop this kind of cancer. Studies have shown decreased expression of tumor suppressor genes such as p53, GSK3 - B, APC, CDKN1A, PTEN and Rb1. Moreover, new evidence suggests that microRNAs could have an oncogenic function and promote tumor development. Using a bioinformatic analysis we identified miR-26 as a potential regulator ofGSK3-B, APC, PTEN and Rb1. To analyze the relation between mir-26 and its potential target genes, we developed an inflammation-associated CCR mouse model, through Azoxymethane / Dextran Sodium Sulfate administration . Histological sections were performed to validate the development of neoplasia. We measured the expression level of miR- 26 and its target genes by qRT-PCR and found miR-26 overexpression in the mouse model, while expression levels of its target were diminished. To verify whether this results were similar in humans, we analyzed it in the HCT116-C cells, with consistent results. However, by western blot we observed decreased GSK3 -B and Rb1 protein levels in the mouse model; this decrease may be associated with the increment of miR- 26. To determine whether miR- 26 directly regulates these two genes, we cloned the specific region of interaction with mir- 26 of each 3'UTR into the pMIR–REPORT vector and are currently undertaking experiments in HCT116 cells. The pMIR-REPORT system uses a decrease in luciferase activity as a reporter for miRNA-derived messenger degradation.

SILENCING THE *PvKEULE*GENES IN *Phaseolus vulgaris* TRANSGENICROOTS: EFFECT ON THE NODULATION

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The legumiosa-rhizobia symbiosis is a complex series of cellular events that take place in the root of the legume and lead to the formation of nitrogen-fixing nodule. Development of the nodule involves two main processes: 1) infection of the legume root with bacteria belonging to the Rhizobiaceae family (rhizobia) and 2) the nodule organogenesis. Infection involves the interaction between rhizobia and root hair to form the infection thread (IT), the progression of the IT towards the nodule primordium and the delivery of rhizobia from the IT into nodule cells to further differentiate to bacteroid and acquire the ability to fix nitrogen. Nodule organogenesis implies the cell division of cortical cells adjacent to the responsive root hair to form the nodule primordium, the formation of the nodule vascular bundles the connect the root central cylinder with the nodule vasculature and the cell differentiation and organization leading to the formation of the central and the peripheral tissues.

In order to gain insights into the molecular mechanisms regulating the formation of the nodule primordium, we have performed a search of *Phaseolus vulgaris* genes whose function may be related cortical cell division in the root. Among other candidates, we have identified three *P. vulgaris* genes as orthologs of *Arabidopsis thaliana* KEULE. We have named them *Pv*KEULE43, *Pv*KEULE54 and *Pv*KEULE99. The KEULE proteins are members of the family Sec/Munc18. In *A. thaliana* cells, KEULE, in complex with the syntaxin KNOLLE, regulates cytokinesis and cell plate formation. Furthermore, and independently of KNOLLE, KEULE is involved in root hair development[1]. RT-qPCR analysis showed that although the three *Pv*KEULE genes are expressed in the root, their transcripts are abundantly accumulated in root hairs. To determine whether PvKEULE genes play a role in nodulation, we have generated *P. vulgaris* transgenic roots expressing RNA interference (RNAi) specific for either*Pv*KEULE43, *Pv*KEULE54 or *Pv*KEULE99. We are currently studying the corresponding phenotype.

[1] Park *et al*., (2012) Developmental Cell 22:989-1000

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Analysis of genes involved in polar transport, signaling and metabolism of auxin during primary root development of *Pachycereus pringlei* (*Cactaceae*)

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The phytohormone auxin is a key factor in root growth and development. Many genes involved in auxin synthesis, perception and polar transport are identified, and their role in root cell division, elongation and differentiation is examined for a few plant species. Roots of most vascular plants exhibit indeterminate growth, that is, they grow and possess root apical meristem (RAM) for an extended time. However, in many species from *Cactaceae* family, including cardón *Pachycereus pringlei*, primary and lateral roots only grow for a short time: the RAM is consumed soon after germination and all cells in root tip differentiate. We are interested in deciphering mechanisms of determinate root growth in Cactaceae, as until now it is the only reported case of determinate growth of the primary root. For this, we employed mRNA-seq, de novo assembly and analysis of virtual gene expression in *P. pringlei* root tip on initial (when RAM is still exist) and terminal (when RAM is exhausted) phases of the primary root development. CLC Genomics Workbench program was used for the RNA-seq analysis. We have found numerous *P. pringlei* homologs of genes involved in synthesis, transport and perception of auxin in other plant species. The deduced amino acid sequences for *P. pringlei* PIN-FORMED (PIN) and AUX/LAX auxin transport carriers have more than 70% identity with those reported for other plant species. The three closest homologs of PIN1 and PIN2 auxin efflux carriers and most of AUX/LAX influx carriers were up regulated during initial phase similarly to the up-regulation of the homologous genes in the *Arabidopsis* RAM. Putative *P. pringlei* YUCCA (YUC) proteins involved in auxin synthesis showed maximum identity with *Arabidopsis* YUC10 *and* YUC11. Two of them were down-regulated on the initial growth phase. Unexpectedly, the three homologs of *AUXIN BINDING PROTEIN1 (ABP1)* were up regulated during terminal growth phase. Inferred similarities and differences in genetic regulation of auxin related genes in root tip of *P. pringlei* and other plant species will be reported. This work was supported by UNAM-DGAPA-PAPIIT, grant IN204912.

Genetic and transcriptional analysis of Zygotic Genome Activation in early embryogenesis of *Arabidopsis thaliana*

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Plant embryogenesis is a highly coordinated process that culminates with an embryo –embedded in the seed– which contains most tissues present in the mature plant. Despite crucial importance for developmental biology and crop breeding, many aspects of early seed development are still mysterious. In our laboratory, we study Zygotic Genome Activation (ZGA), the process in which the zygote becomes transcriptionally active after a period of maternal developmental control, a phenomenon collectively known as the Maternal to Zygotic Transition (MZT).

Using a functional analysis of gene activity, we have recently contributed to solving a long standing controversy regarding ZGA in plants (Del Toro De León et al., *Nature*, in press). To do this, we used 49 embryo defective mutants (*emb/+*) with clear preglobular mutant phenotypes to evaluate the ability of their wild-type paternal alleles to complement maternally inherited mutant phenotypes. Just after fertilization, we observed mutant embryos in our crosses between most *emb/+* plants and wild-type pollen, demonstrating that the paternal alleles are not immediately functional. In addition to isogenic crosses in the ecotype Columbia, we also tested the effect of hybridization on gene activity, by using pollen from 4 different ecotypes in our crosses with *emb/+* mutants. Epigenetically distant hybrid combinations resulted in faster complementation of maternally inherited mutations, suggesting a role for epigenetic marks in ZGA. In summary, we demonstrated that the paternal genome is not fully functional just after fertilization, that the maternal genome makes the dominant contribution in these early stages of development, and that hybridization can have a large effect of gene activity in early embryogenesis of Arabidopsis.

In order to understand the transcriptional basis our observations, we are currently investigating whether common signatures in the sequences of the maternally dominant genes are the responsible for their functional behavior. In addition we are exploring the transcriptional basis of the ZGA by analyzing motifs that could trigger the early transcriptional programs of embryogenesis in plants. Aside from *in silico* analysis, we are also performing functional assays by deletion and swap experiments in promoter regions of reporter constructs for selected genes. Our findings will contribute to understanding of gene regulation plant embryogenesis, and establish a basis for studying heterosis at this stage of plant development.

Ca^{2+}-dependent pathways control calsequestrin-2 expression in cardiomyocytes.

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Calsequestrin (CASQ) is the main Ca^{2+}-binding protein inside the sarcoplasmic reticulum (SR) of cardiomyocytes. The human *CASQ2* gene encodes the cardiac specific CASQ2 isoform. The first 140 bp of *CASQ2* gene promoter are highly conserved among species, containing a TATA-Box, binding sites for MEF-2 (Myocyte Enhancer Factor-2) and SRF (Serum Response Factor) transcription factors. Previously, we demonstrated that the MEF-2 and SRF binding sites within this region are functional in neonatal rat cardiomyocytes.
Sequence analysis of the human *CASQ2* gene promoter revealed potential binding sites for NFAT (at -1869 bp and -230 bp) and MEF-2 transcription factors (at -1665 bp and -133 bp). By EMSAs, we found that γ-^{32}P-labeled DNA oligonucleotides containing the sequences of MEF-2 sites formed specific complexes with proteins present in nuclear extracts from rat cardiomyocytes.
In cardiomyocytes, SR Ca^{2+} depletion induced by SERCA2a inhibitor thapsigargin (Tg) or the inhibition of protein processing with tunicamycin significantly increased the mRNA levels of endoplasmic reticulum (ER) stress markers Grp78, XBP1 and RCAN-1, but reduced CASQ2 mRNA levels up to 50%.
Functional assays with two *hCASQ2* promoter constructs (-3102/+176 and -288/+176) showed that ER stress induction with Tg and tunicamycin reduced the luciferase activity of both *hCASQ2* promoter constructs up to 50%. On the other hand, the inhibition of NFAT dephosphorylation with cyclosporine A or with INCA-6 reduced the luciferase activity of both *hCASQ2* promoter constructs up to 50%. Additionally, NFATc3 overexpressing cardiomyocytes showed a three-fold increment in the luciferase activity of both *hCASQ2* promoter constructs. Taken together, these data demonstrate than ER stress
and Ca^{2+}-dependent pathways, such NFAT and MEF-2, control the expression of the *CASQ2* gene in cardiomyocytes. Currently, we are investigating the molecular mechanism responsible for *CASQ2* gene down-regulation induced by ER stress response. Supported by grants DGAPA- PAPIIT IN213613 and CONACyT 164413.

Downregulation of SERCA3 expression in gastrointestinal cancer cells is modulated by Sp transcription factors

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Intracellular calcium concentration ($[Ca^{2+}]_i$) plays an important role in the physiology of gastric and intestinal epithelium regulating several signaling networks controlling cell growth, differentiation and apoptosis. The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) regulates $[Ca^{2+}]$ inside the endoplasmic reticulum and cytosol in most cell types. Normal colon and gastric epithelial cells express high levels of the SERCA3 isoform, which is selectively lost in cancer cells. Previously, Gelebart et al. found that SERCA3 protein expression is induced when gastric and colon cancer cells undergo *in vitro* differentiation.

In this work, we induced differentiation of KATO-III human gastric cancer cells and human Caco-2 adenocarcinoma cells in culture using HDACs inhibitors and confluence, respectively. We found a large increase in mRNA expression of SERCA3 (up to 50-fold), which was completely abolished when we add Actinomycin D, showingthat *ATP2A3* gene expression is regulated by a transcriptional mechanism. We measured transcriptional activity of pGL3- h*ATP2A3*-gene-promoter constructs in both cancer cell lines and found a marked increase in the activity up to 30-fold after induced differentiation. We narrowed the responsive elements to the region -135 to +142 bp of the 5'-flankig-region of the human *ATP2A3* promoter. This region is inside a CpG island and contains 8 consensus Sp-like elements.The functional analysis of these putative elements by site directed mutagenesis in -135/+142 construct showed minor transcriptional activity than wild type construct (0.5 fold). The Sp mutants also showed incomplete transactivation by HDACs inhibitors treatment, showing that these elements are key regulators inincreased SERCA3 expression afterinduction of differentiation of human gastrointestinal cancer cell lines.

Spis a family of transcription factors that includesSp1, Sp3 and KLF members. Previously,was shown an important role of Sp factors in transcriptional regulation of the *ATP2A3* genein mouse endothelium and human lymphocytes. High level of KLF4 is expressed in intestinal epithelium where is essential for terminal differentiation of normal tissue. Besides, it has been reported that both the expression of KLF4 and SERCA3 is decreased in gastric and colon cancer, and that both proteins and mRNAs increases after induced differentiation of these cancer cells in culture. We demonstrated a direct and specific interaction of KLF4, Sp1 and Sp3 with their target sequences in the human *ATP2A3*gene promoter by DNA-protein binding assays. We found that KLF4 binding increases in differentiated cancer cell lines, suggesting that might function as an activator of *ATP2A3* gene expression. In contrast, the results suggest that Sp3 functions as a repressor in tumor cells keeping low *ATP2A3* expression. We conclude that *ATP2A3* expression is regulated at the transcriptional level, positively by KLF4 and negatively by Sp3, which binds to the proximal promoter. Supported by grants DGAPA-PAPIIT IN213613 and CONACyT 164413.

Proximal CAAT box mediates SERCA2 increased transcription by thapsigargin

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The cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2) is vital for the proper contractile function in the heart. Decreased levels of SERCA2 mRNA and protein have been extensively demonstrated in animal models of cardiac hypertrophy and in patients with heart failure; however, the molecular mechanisms that mediate this altered expression have not been elucidated. The SERCA2 specific inhibitor thapsigargin (Tg) increases cytoplasmic calcium concentration ($[Ca^{2+}]_c$), induces endoplasmic reticulum stress (ERS) and has been associated with increased SERCA2a expression in cardiomyocytes. In this work, we show that Tg and A23187 increased SERCA2a mRNA and transcriptional activity of human *SERCA2* gene promoter constructs in primary cultures of neonatal rat cardiomyocytes. The calcineurin inhibitor cyclosporine A (CsA) prevented Tg induced SERCA2a expression, suggesting that NFAT transcription factor positively regulates *SERCA2* gene transcription. To confirm the role of NFAT in transcriptional activity of the *SERCA2* promoter, we overexpressed the transcription factor NFATc3 and found a 2-fold increase in transcriptional activity of a human *SERCA2* gene construct containing -254 bp of promoter sequence and 329 bp of 5´-untranslated sequence. Functional analysis of a mutated putative NFAT binding site located at +43 position of the human *SERCA2* gene, showed lower basal transcriptional activity compared to the wild type construct and no increased activity in response to NFATc3 overexpression. Since Tg induces ERS, we mutated a CAAT-box that is part of a putative ERS response element located in the proximal *SERCA2* promoter and assessed response of the mutated construct. Mutated construct showed lower basal activity compared to the wild type construct and did not respond to Tg treatment but was repressed by CsA. As the CAAT-box resulted essential for Tg induced SERCA2 transcription we used different pharmacological approaches and identified the CHOP transcription factor as a possible way of transducing stress exerted by Tg into increased SERCA2 transcription. Taken together, these results show that the NFAT binding site at +43 bp position and the CAAT box at -78 bp position of the human *SERCA2* gene are necessary for proper basal transcription of *SERCA2* gene and suggest a complex transcriptional regulation of the gene by increased $[Ca^{2+}]_c$ and also by ERS.

Analysis of human eIF3f phosphorylation by CDK1/cyclin B and its role in the control of protein synthesis

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Abstract

Gene expression is regulated at different levels from the chromatin structure to posttranslational modifications. It has been shown that altered expression of genes involved in proliferation and/or cell death might be involved in cancer development. Subunit f (eIF3f) of the eukaryotic initiation factor 3 has a modulatory role in translation initiation and progression of the cell cycle. Studies on eIF3f expression in malignant neoplasms show that eIF3f deregulation has a certain incidence on oncogenesis. Also, *in vivo* and *in vitro* studies show eIF3f as a Cap-dependent protein synthesis inhibitor. The correct cell cycle progression is verified during the phase transitions G1/S and G2/M, and metaphase. The Mitosis Promoting Factor is the key regulatory component of the G2/M transition and it is constituted by the catalytic subunit Cdk1 and its regulator cyclin B, which phosphorylates serine or threonine residues to various target proteins, modulating its activity. Our research group previously reported that eIF3f is a cell division-related protein whose expression peaks in the G2/M transition phase of the cell cycle. Thus, the goal of this work is to identify in human eIF3f the probable CDK1/CycB phophorylating site(s), as well as its (their) role in protein synthesis regulation. Since little is known about eIF3f postranslational modifications, we performed a bioinformatic analysis to identify possible CDK1/CycB phosphorylation sites in the eIF3f protein. We found two probable sites, which were site-directed mutagenized by designing point mutation-specific oligonucleotides. Resulting mutants were verified by sequencing. Native and mutated proteins were produced by *in vitro* transcription and translations protocols, and were subjected to phosphorylation by CDK1/CycB, also *in vitro*. Afterwards, we assayed how native and mutated proteins (phosphorylated or unphosphorylated) influence protein synthesis, simultaneosly, in both Cap-dependent and IRES-dependent mRNAs through a dual genetic construct that harbor Cap-Luciferase/IRES-Renilla. The results will be presented and discussed.

Towards the functional characterization of Brf1, a subunit of transcription factor TFIIIB, in the parasite *Leishmania major*

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Leishmaniamajor is a flagellated protozoan that produces leishmaniasis in humans. The parasite is also important because it presents atypical mechanisms of genetic expression, likepolycistronic transcription. Our group is interested in the study of transcription by RNA polymerase III (Pol III), which is responsible for the synthesis of RNA molecules that are essential for cell viability, such as tRNAs and 5S rRNA. A transcription factor required for the function of Pol III is TFIIIB, which is composed of three subunits: Brf1, Bdp1 and TBP. In *L. major* the Brf1 coding sequence (LmBrf1) is located on chromosome 25. A multiple sequence alignment of LmBrf1 and homologs in other eukaryotes showed that LmBrf1 contains the three conserved domains located in the N-terminal half of the protein: a zinc ribbon motif and two cyclin repeats (also known as TFIIB-related repeats). Northern-blot analyses with RNA isolated from cells growing in the presence of actinomycin D showed that the half-life of the LmBrf1 mRNA was around 60 min in mid-log phase cells, while the half-life of α-tubulin mRNA was estimated to be around 4 h. Thus, the half-life of LmBrf1 mRNA is relatively short. To study the role of LmBrf1 in transcription, we are in the process of generating LmBrf1 null mutants by homologous recombination. To accomplish this goal, we have made knockout vectors containing puromycin (pac) and hygromycin (hyg) antibiotic-resistant markers. A single-knockout cell line was obtained by transfecting *L. major*with the targeting cassette from the pac vector. Cellular clones were selected in the presence of puromycin and analyzed by southern blot to verify the replacement of one LmBrf1 allele by the pac gene. We are currently generating the double-knockout cell line. By nuclear run-on assays we will analyze Pol III transcription in the LmBrf1 null mutants.

Expression of MicroRNA-143 In Epithelial Cell Lines

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MicroRNAs (miRNAs) are small non-coding RNAs which regulate the expression of mRNAs containing complementary sequences. The disruption of miRNA function is associated to many human diseases, including cancer. Cervical cancer is a public health problem in Mexico and the world. The infection with high-risk human papilloma virus is the major risk factor for the subsequent development of cervical cancer. However, HPV infection alone is not enough to produce a malignant phenotype. Therefore, it has been proposed that aberrations at the miRNome must contribute to malignant progression. The miR-143 is down-regulated in a range of cancers including cervical cancer. Moreover, miR-143 plays an important role in promotingthe differentiation of smooth muscle cells and adipocytes. In addition, several other mRNAs related to carcinogenesis have been shown to be direct targets of miR-143. Our previous work shown that overexpression of miR-143 decreased proliferation and anchorage-independent growth in a cervical cancer cell line, suggesting that this miRNA could function as tumor suppressor. In this study, RT-qPCR analysis showed that miR-143 is not expressed in immortalized and tumorigenic epithelial cell lines. Moreover, we evaluated the relative expression of K-ras(a known miR-143 target) by immunoblotting and, as expected, we found high expression levels in most of tumor cell lines but low levels in immortalized cell lines. Further experiments expressing miR143 on immortal cells may elucidate if miR-143 functions as tumor suppressor in cervical cancer.

Analysis of the histone deacetylation on *Ustilago maydis* dimorphism

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Ustilago maydis (DC) Corda is a dimorphic and pathogenic fungus specific from teozintle and maize (*Zea mays* L.). In maize *U. maydis* cause the disease know as common smut or “huitlacoche” that is world-wide distributed. Typical symptom of disease is the flesh, edible galls on corn ears. Epigenetic mechanisms, like histone deacetylation, regulate gene expression and control the cell specialization through the development of organisms; including cell differentiation processes as the fungal pathogenesis.

We employed two different histone deacetylase inhibitors, valproic acid (VA) and sodium butyrate (SB) to assess the role of histone deacetylation on mating and dimorphism of *U. maydis* FB2 strain. We tested several concentrations of both inhibidors (1, 5, 10 and 20 µM). Only 20 µM VA affected the fungal growth rate on minimal media pH7 and was significantly different to the rest of treatments according to Tukey test. Microscopical morphology of fungal cells as macroscopic morphology of fungal colonies on complete and minimal medium revealed the abscenceof alterations with respect to the untreated strain. In the presence of 1 µM of SB on minimal medium pH3 the mycelium formation was inhibited aprox 75%, whereas when the fungus was grown on 5 µM SB, inhibition almost totally. In the other case, fungal growth on 0.5 and 1 µM of VA revealed yeast-like form. In relation to mating when the last concentrations was used formation of germ tubes was slightly delayed with respect to cells without histone deacetylase inhibitors. These findings are consistent with the filamentous constitutive phenotype of a defective strain in a histone acetyltransferase gene, and suggest the participation of acetylation/deacetylation mechanism on *U. maydis* dimorphism.

Transcription Factor Sp1 Regulates T-Type Ca^{2+} Channel $Ca_V3.1$ Gene Expression

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Voltage-gated T-type Ca^{2+}(Ca_V3) channelsmediate a number of physiologicalevents in developing and maturecells, and are implicated inneurological and cardiovascular diseases. In mammals, there arethreedistinct T-channel genes (*CACNA1G*, *CACNA1H*, and *CACNA1I*)encodingproteins ($Ca_V3.1$–$Ca_V3.3$) thatdiffer in theirlocalization as well as in molecular, biophysical, and pharmacologicalproperties. The*CACNA1G*is a large gene thatcontains 38 exons and islocalizedin chromosome 17q22. Onlybasiccharacteristics of the*CACNA1G* genepromoterregionhavebeeninvestigatedclassifyingit as a TATA-lesssequencecontainingseveralpotentialtranscription factor-bindingmotifs. Here, wecloned and characterized a proximal promoterregion and initiatedtheanalysis of transcriptionfactorsthat control$Ca_V3.1$ channelexpressionusingthemurine*Cacna1g*gene as a model. Weisolated a~1.5 kb 5'-upstream region of Cacna1g and verifieditstranscriptionalactivity in the mouse neuroblastoma N1E-115 cell line. In silicoanalysisrevealedthatthisregionpossesses a TATA-lessminimalpromoterthatincludestwopotentialtranscriptionstartsites and fourbindingsitesforthetranscription factor Sp1. Theability ofone of thesesitestointeractwiththetranscription factor wasconfirmedbyelectrophoreticmobilityshiftassays. Consistentwiththis, Sp1 over-expressionenhancedpromoteractivitywhilesiRNAmediated Sp1 silencingsignificantlydecreasedthelevel of CaV3.1 protein andreducedtheamplitude of whole-cell T-type Ca^{2+}currentsexpressed in the N1E-115 cells. Theseresultsprovide new insightsintothemolecular mechanismsthat control $Ca_V3.1$ channelexpression.

The role of the histone demethylase KDM4A in a cancer model.

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Lysine methylation is an epigenetic component that can be associated with relaxation or chromatin compaction; it was believed that histone lysine methylation was a stable and irreversible modification however, several enzymes capable of removing this mark have been discovered, among them KDM4A. This demethylase removes the methyl groups from H3K9me3, associated with heterochromatin, and H3K36me3, related to transcription elongation by RNA polymerase II. The deregulation of histone methylation and demethylation appears to be involved in cancer, for example, it has been observed an abnormal expression of KDM4A in breast cancer, suggesting that this may be involved in this disease. *CHD5* gene is inactivated in several types of cancer; it is reported that low expression levels of *CHD5* are associated with the development and progression of breast cancer. *CHD5* is a target gene of KDM4A, this demethylase binds to the region located at + 741 pb regarding the transcription start site (TSS) of *CHD5*; the dissociation of KDM4A causes an increase in the expression of this gene. Actually it still unknown the mechanism by which KDM4A negatively regulates *CHD5*, therefore the main objective of this work was to evaluate the role of KDM4A, the tri and dimethylation of the lysines 9 and 36 of histone 3 (H3K9me3/2 and H3K36me3/2) in the regulation of *CHD5* expression. In our study, we demonstrate that KDM4A mRNA levels increase in the breast cancer cell lines MCF7, MDA-MB-231 and the endometrial cancer cell line, HeLa, compared with a non-cancerous breast cell line MCF 10A. By immunofluorescences (IF) it was observed the presence of the KDM4A protein in cancer cell lines but not in MCF-10A; on the other hand, CHD5 protein was found in the cell line MCF-10A. These data suggest a relationship between the presence of KDM4A and the absence of CHD5. Chromatin immunoprecipitation assays (ChIP) showed that the union of KDM4A to the region located at +741 pb in CHD5 gene in cancer cell lines, but not in the MCF 10A. The presence of KDM4A correlates with the reduction of H3K36me3 and H3K9me3 modifications and enrichment H3K36me2 and H3K9me2. Moreover, K3K9me2 and H3K9me3 modifications are in this region in the cancer cell lines, which could be associated with repressive chromatin. These data suggest that the binding of KDM4A to the region +741 pb of *CHD5* is related with CHD5 protein depletion, suggesting that KDM4A could be involved in the reduction of H3K36me3 involved in the process of transcription elongation by RNA polymerase II. Our findings suggest a possible new mechanism epigenetic inactivation of the *CHD5* gene where it could change the process of elongation of transcription by RNA polymerase II.

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Interaction of lncRNA transcribed from α satellite repeats and the protein HP1α in a colorectal cancer model

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Centromere is a region necessary for the correct segregation of chromosomes during mitosis and meiosis for this reason the maintenance of this region is necessary to ensure the success of the segregation mechanism. HP1 is a protein that contributes importantly in the establishment and maintenance of heterochromatic domains that can be found in pericentromeric chromatin. *In vitro* studies show that HP1α has RNA- binding activity in his hinge region which is homolog to the amino acid sequence of CENP-C protein through which interacts with ncRNA transcribed from α satellite centromeric repreats and they both can be observed in nucleolus. This suggests that HP1α binds to the ncRNA transcribed from α satellite. Therefore, the goal of this work is evaluate if there is an interaction between the α satellite ncRNA and the HP1α protein in a colorectal cancer cell line. We evaluated the expression of this transcript by RT-qPCR in different cancer cell lines. Moreover, we evaluated the transcription of the ncRNA with a dose curve in HCT116 and WI-38 cells. Then, we standarized Immuno RNA-FISH assay in TSA treated cells to determine the cellular localization of the ncRNA using a set of RNA probes based on the sequence of our transcript of interest. Finally, we evaluated the interaction of the transcript with HP1α and other centromere proteins using RNA-ChIP assay. We observed that the transcription of this ncRNA is not dependent on the cell tissue origin. Also, we observed co-localization of the α satellite ncRNA and a nucelolar protein when it is overexpressed with TSA. This data in conjunction with RNA-ChIP assay results could suggest a role of the ncRNA transcribed from α satellite repeats in protein localization.

Regulation of ABA-INSENSITIVE (ABI) 4 transcription factor in *Arabidopsis thaliana*.

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The ABscisic Acid-Insensitive 4 (ABI4) transcription factor (TF) is essential for many processes during plant life. This TF is required for correct ABA and sugar signaling, lipid mobilization into the embryo, salt tolerance and nitrate/sugar-mediated root growth. Recently, ABI4 has also emerged as a central player in chloroplast to nucleus communication.

Diverse evidence demonstrated that *ABI4* is regulated at different levels. At the transcriptional level our current analysis, using transgenic plants that express the GUS reporter fused to different fragments of the ABI4 promoter, demonstrated that there are important elements for the proper expression of this TF during early development of plant. We also found that *ABI4* expression requires the positive regulation of the TF LEC2 during seed and early seedling development.

However, it is known that ABI4 transcript accumulation doesn't correlate with its protein levels, supporting a post-transcriptional regulation. Towards understanding the mechanism and identifying sequences involved in the regulation of ABI4, we select three motifs highly conserved among different plant species named as AP2-associated, LRP and PEST motifs. The function of these motifs was analyzed through mutagenesis or deletion in the Arabidopsis ABI4 protein using transient assays in Arabidopsis mesophyll protoplast. We found that deletion of the AP2-associated motif is required for proper nuclear localization. In contrast the LRP motif is important, but not essential, for the regulation of ABI4 transcriptional activity. Finally, we demonstrated that the PEST motif directly modulates ABI4 protein stability through the 26S proteosomal pathway. In addition to those post-transcriptionally regulatory mechanisms, our recent studies support that ABI4 might be also regulated by a mechanism that involves microRNAs. Our current advances in this aspect will be presented.

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Quantitative analysis of the telomere position effect on gene expression

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The expression of a gene can be affected by its position on a chromosome, notoriously by the susceptibility to inactivation of genes near the eukaryotic telomeres, namely the telomere position effect (TPE). Studies in subtelomeric regions in *Saccharomyces cerevisiae* have provided valuable insights into the mechanisms of the heterochromatin formation and epigenetic gene silencing, but we are still lacking a general picture of the way in which different subtelomeric regions and silencing factors underlie TPE. Here, we present a quantitative characterization at single-cell level of the transcriptional silencing at diverse subtelomeric regions. We developed a microscopy method and image analysis pipeline to detect the fluorescence intensity differences of a reporter integrated at fifteen subtelomeric positions. Such analysis allows to detect single-cell differences on gene expression, a fundamental aspect to describe a potentially variegated process. Our results showed widespread variation of silencing patterns at different subtelomeric loci, both in terms of expression level and expression-level variance. Furthermore, we want to describe the way in which subtelomeric silencing is affected by mutations in some of the main genes that are known to mediate this phenomenon. For instance, we observed differential effects of mutations in the SIR complex, specifically *sir3Δ*. Our study provides a framework to describe the genetic logic of subtelomeric silencing and to understand a wide range of biological problems associated to epigenetic gene silencing, including genome organization, survival mechanisms, pathogenesis, aging, and disease.

Analysis of the small RNA RgsA in *Azotobacter vinelandii*

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Azotobacter vinelandii is a nitrogen-fixing soil bacterium that produces alginate and polyhydroxybutyrate (PHB), two polymers of industrial interest. Also is a bacterium that undergoes differentiation to form cysts that are resistant to desiccation. Upon induction of cyst formation, the bacterium synthesizes alkylresorcinols that are present in cysts but not in vegetative cells. Production of these metabolites is regulated for the two-component system GacS/GacA, GacA control the expression of small regulatory RNAs (RsmZ, RsmY) that interact with RsmA protein, RsmA bind to their target mRNAs acting as translational repressors.
RgsA is a small non-coding RNA (ncRNAs) in Pseudomonas species. In the plant pathogen *Pseudomonas syringae* pv. tomato strain DC3000, RpoS regulates the expression of RgsA and these sRNA plays a role in the bacteria's susceptibility to oxidative stress and resistance to heat stress.

In this study an *rgsA* homolog was identified in the *A. vinelandii* genome. The function of RgsA in *Azotobacter vinelandii* is not known. In this work, we generate *rgsA* mutants in *A. vinelandii* strain AEIV (mucoid) and UW139 (non mucoid) strains. Levels of PHB, alginate and alkylresorcinols were determined in *rgsA* mutants and wild type strains. Also we determined the resistance to oxidative and heat stresses, as well as, biofilm formation.

To determine whether GacA and RpoS regulate *rgsA* expression, we constructed transcriptional *rgsA-gusA* gene fusions and measured its expression in *A. vinelandii rpoS* and *A. vinelandii gacA* mutants. RgsA sequence showed a single RsmA binding site, to demonstrate if RsmA protein binds to *rgsA* ncRNA, we generated a 143 nt transcript corresponding to RgsA and purified RsmA to perform RNA gel mobility shift experiments.

Effect of resveratrol in the expression of SERCA2b and SERCA3 in breastcancercelllines.

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Abstract

Ca^{2+} is a highly versatile intracellular signal that operates over a wide temporal range to regulate many different cellular processes such as differentiation, growth and cell death. The involvement of Ca^{2+} in so many fundamental cell processes naturally demands its efficient and precise control. The Ca^{2+}-ATPases from the sarco/endoplasmic reticulum (SERCA) are fundamental for maintaining intracellular Ca^{2+} homeostasis by pumping Ca^{2+}into the ER.It werereported alterations in the expression of SERCA pumps in different types ofcancer: oral, lung, colon, stomach, central nervous system, thyroid, breast, and prostate.

Resveratrol (RSV) is a phytoalexin produced by a wide variety of plants in response to stress situations. It can modulate multiple cellular processes, including apoptosis, cell cycle progression, inflammation, and angiogenesis. It affects the processes underlying all three stages of carcinogenesis; namely, tumor initiation, promotion and progression.

It is known that there are epigenetic mechanisms which regulate SERCA pumps expression such a methylation of clusters of CpGs called ‘‘CpG-islands’’ in the promoters of genes; or post-translational modifications on histones. However, SERCA2 and SERCA3 promoters contain a lot of response elements for transcription factors, and due that there is a differential expression of genes induced by RSV in human breast cancer cell lines(including genes correlated with apoptosis, cell cycle control, transcription factors, cell adhesion, metastasis, signal transducers and angiogenesis)is possible that a regulatory protein of SERCA transcription could be different levels after treatments with resveratrol, increasing or decreasing its expression.

We have used the breast cancer cell lines MCF-7 and MDA-MB-231 to quantify the levels of mRNA expression of SERCA2b (an ubiquitous isoform) and SERCA3 (an isoform expressed mainly in epithelial and hematopoietic cells). Results show a differential expression depending of cell line. While SERCA2b expression remain constant in both cell lines, SERCA3 expression increased in MDA-MB-231 cells in a transient way, with a higher value after 72 h of treatment with 50 μM of RSV highlighting the importance of SERCA3 expression in epithelial tissues.

Transcriptional regulation of *ipdC*and *hisC1* genes involved in indole-3-acetic acid production in *Azospirillumbrasilense*Sp7 strain.

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Indole-3-acetic acid (IAA) is an important phytohormone with the capacity to control plant development in both beneficial and deleterious ways. The ability to synthesize IAA is an attribute that many bacteria including plant growth-promoters among these is the bacterium *Azospirillumbrasilense*. There are three main pathways through which IAAis synthesized; the indole-3-pyruvic acid (IPyA),indole-3-acetamide and indole-3-acetonitrile pathways. In IPyA pathway initially, L-tryptophan is deaminated to IPA by an aminotransferase (AAT). Subsequently, a decarboxylase enzyme (PPDC) converts IPyA into indole-3-acetylaldehyde (IAAld), which is then oxidized to IAA by aldehyde dehydrogenase, mutase or oxidase enzymes[1]. Genes encoding for AAT are encoding for *hisC*and *hisC2*genes, while PPDC is coding by *ipdC*gene[1,2].
Currently, regulation of IAA synthesis in bacteria is not completely understood at the molecular level, although it is clear that synthesis responds to environmental cues. Acidic pH and anaerobic conditions, often encountered in the rhizosphere, increase*ipdC* expression in *A. brasilense*Sp245. High levels of IAA accumulate in culture media only after entrance into the stationary phase in *A. brasilense* Sp7 and Sp245[1,2,3,].In order to analyze the regulatory protein that controlled the *hisC1* and *ipdC* expression, we constructed a *tyrR::gm*^{r}mutant. IAA production was determined; mutant showed 25% of residual IAA compared to wild-type strain, suggestingTyrR may regulate IAA production. To further investigate TyrR-dependent *hisC1* and *ipdC* activation,bothpromoter-driven reporter gene assays were performed,the measurement of β-galactosidase activity in *ipdC::lacZ* and β-glucuronidase activity in *hisC1::uidA* transcriptional fusions.

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Genetic regulation of nitrogen metabolismin*Lachancea kluyveri*

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All living cells monitor their environment to ensure that sufficient nutrients are available to complete a full cell cycle. Adaptation of growth and metabolism to the nitrogen supply is a major issue for all organisms. Yeasts are able to utilize differentially available nitrogen compounds in the medium where they grow. A rich nitrogen source is defined as one that is well transported into the cell and requires fewer steps to reach metabolic glutamine or glutamate. Conversely, a poor nitrogen source is one that is not easily transported and requires more steps to form glutamine or glutamate. The ability to preferentially use rich nitrogen sources over poor ones is known as Nitrogen Catabolite Repression (NCR).In *S. cerevisiae*, NCR is regulated by the Ure2 protein and by four GATA family proteins: two activators (Gln3 and Gat1) and two repressors (Dal80 and Gzf3).Our interest is to study how different yeasts use diverse nitrogen sources and how this process is regulated.Using sequence analysis comparison, we found that *Lachancea kluyveri*has orthologous genes encoding for GATA transcription factors Gln3 and Gat1 and also for the Ure2 protein. We have generated a collection of single and double mutant strains in these genes in order to evaluate its role in nitrogen assimilation when cells were grown on glutamine, ammonia or proline as sole nitrogen sources. In order to elucidate if there is a NCR -like mechanism in *L. kluyveri* we are going to measure, by qRT-PCR, the expression of some of the genes involved in transport and catabolism of some of thenitrogen sources tested. Preliminary results showthat both *LkGAP1* and *LkMEP2* are regulated by GATA transcription factors Gln3 and Gat1 but this regulation has interesting differences with respect to how these orthologous genes are regulated in *S. cerevisiae*.

Pseudogenization and reactivation of gene expresión by gain or erosion within promoter regions during the expansion of gene families in plants.

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Gene duplication events exert key functions on gene innovations during the evolution of the eukaryotic genomes. Between 50-80% of the total gene content in plants arose from duplication events, of which at least15% corresponds to tandem duplication.Ubiquitin ligases or E3 enzymes are components of the ubiquitin proteasome system that function during the transfer of the ubiquitin molecule to the substrate. In plants, several E3s have expanded in their genomes as multigene families.The ATL family of ubiquitin ligases is an excellent model for studies of gene family expansion in plants. To gain insight into the consequences of gene duplications on the expansion and diversification of E3s, we examined the evolutionary basis of a cluster of six genes, *duplC- ATL*s, which arose from segmental and tandem duplication events in Brassicaceae.The assessment of the expression suggested two patterns that are supported by lineage. We hypothesized that two *duplC-ATL* genes underwent pseudogenization by erosion at the promoter region and that, in one case, promoter activity is probably regained in members of the Camelineae lineage after the retention of a short direct repeat duplication at the 5′UTR. We are confirming our hypothesis by examining the effect of adding and/or removing presumed TATA-box and other elements of the promoter region.Our findings provide insights into the evolution of gene families in plants, defining key events on the expansion of the ATL family of E3 ligases, as well as an unprecedented example of a novel acquisition of gene expression during evolution.

*both authors contributed equally to this work. Supported by the grant CB177528 from SEP-Conacyt.

A new lncRNA is regulated by the bidirectional promoter of *CatSper1*.

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The Cation sperm 1 (*Catsper1*) gene is exclusively expressed in sperms cells and produces a monomer for theCatSper channel. This channel is essential for sperm hyperactivation and necessary for oocyte fertilization.The regulation mechanisms of this gene remain elusive, so our research group recently started to characterizethe promoter region. It was found that this promoter has transcriptional activity both in sense and antisense asbidirectional promoters do. Therefore, we hypothesized that this promoter is of bidirectional nature andregulates the expression of a new gene whose expression could be related with CatSper1. The aim of this work was to analyze its expression and to identify thenew murine gen.

We performed an *in silico* analysis to predict elements of the new gene in the non-annotated 10kb regionupstream of *Catsper1* gene. The presence of a putative gene was predicted by identification of twotranscriptional start sites, a possible terminator sequence, 5 exons and a 4.4 Kb mRNA. The promoterregion of the *CatSper1* gene was transfected into HEK-293 and MSC1 cells in both sense and antisense toanalyze the transcriptional activity in vitro. We observed an increased transcriptional activity of the antisensepromoter in both cell lines. Two transcriptionalstart sites were determined by rapid amplification of cDNA ends (RACE5´). The expression profile of the new transcript was analyzed in a panel of mousetissues. The new gene was expressed only in male mouse liver and testis but not in liver or ovary of female mice. A RT-PCR analysis on purified germ cells showed positive expression in pachytene spermatocytes,round and elongated spermatids. On the other hand, the new gene was also expressed in embryonic stage on day11.5 post-coitum and in newborn testes. The full length of this transcript was found by RACE3´. This transcript was shown to be an unsplicedpolyadenylated transcript. The in silico analysis did not reveal any open reading frame (ORF), suggesting that this transcript could be a noncoding RNA.

These data indicate the presence of a divergent gene to *CatSper1*, whose expression is regulated by a bidirectional promoter and is not unique to spermcells. Silencing of this new transcript will allow to evaluate whether there is a functional relationship between*CatSper1* and the new gene.

Functional characterization of polyamine oxidase-2 (PAO2) uORF from*Arabidopsis thaliana*

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The 5´-untranslated region of an mRNA contains control signals that modulate thetranslational efficiency that can result in rapid changes to the proteome. Approximately 30% of Arabidopsis cDNAs possess an upstream open reading frame (uORF); nevertheless their impact on translation has been studied in detail inrelatively few cases. The flavin-containing polyamine oxidases (PAOs; EC 1.5.3.3)catabolize oxidative deamination of spermidine and spermine, thus contributing topolyamine homeostasis as well as in diverse biological processes through their reaction products. In *A. thaliana* genome five genes encoding PAOs have been identified, of these *AtPAO2* and *AtPAO3* transcripts possess anuORF. In this study, we characterized the uORF of *AtPAO2* gene using the *GUS* reporter gene. Transgenic lines harboring the constitutive CaMV 35S promoter showed that the uORF negatively affects GUS expression. Exogenous applications of polyamines positively modulate GUS expression, thus alleviating the negative effect of AtPAO2 uORF, while treatments with MGBG inhibitor show an opposite effect. In addition, transgenic lines inoculated with Trichoderma, a plant growth promoter, show an increase in GUS expression, which also suggests an increase in polyamine levels. These results propose that the uORF-mediated regulation of the AtPAO2 is modulated by polyamines. Moreover, the fact that uORFs are commonly found in polyamine pathway suggests that uORF-mediated control mechanisms might finely modulate the intracellular concentration of PAs in plant development and also in stress responses.

Effect of arsenic exposure on DNA methylation in brain areas

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DNAmethylationisa majorepigenetic mechanism thatregulates the expressionof genesand is crucial for neuronal plasticity. However,various environmentalagents canmodifyepigeneticpatterns leading tothe silencingorover expression of certaingenes. Arsenic, which causes cognitive deficitsin children exposed, could alterthe epigenetic mechanismsinvolved in learningandmemory.Therefore, we assessed the effect of intrauterine and postnatal arsenic exposure onDNA methylation in brain areas. Animals were exposed to arsenic in drinking water (3 and 36 ppm) from gestation until 4 months of age, and DNA methylation in brain cells was determined by flow cytometry and immunohistochemistry at 1, 2, 3 and 4 months of age. We found that the percent of 5-methylcytosine+ brain cells were similar between control and exposed animals, but a significant increase of 5-methylcytosine+ cells was associated with the age of the animals in all groups. Furthermore, we observed a significantly higher global DNA methylation at the first month in cortex and hippocampus in arsenic exposed groups compared to control group, followed by DNAhypomethylation at the third and fourth months but only in the cortex at high arsenic exposure. Our data demonstrates alterations on DNA methylation inbrain areas involved in memory formation by arsenic exposure, which may contribute to memory deficit induced with arsenic at the same doses.

Analysis of *Macrophomina phaseolina* (Tassi) Goid. pathogenicity genes considering several epigenetic backgrounds

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Epigenetics has been defined as the study of mitotically or meiotically heritable changes in gene function that cannot be explained by alterations in the DNA sequence. Epigenetic mechanisms, like DNA methylation and histone modifications, regulate gene expression and therefore they are responsible for the establishment of the phenotype in eukaryotes. Moreover, these mechanisms control the cell specialization through the development of organisms; it is the case of fungal pathogenesis which involves cell differentiation. In the absence of mutant strains, the use of chromatin remodeling enzyme inhibitors is a useful strategy to inquire about the biological consequences of chromatin modifications in fungi. *Macrophomina phaseolina* (Tassi) Goid. is a cosmopolitan and aggressive plant pathogenic fungus with a broad host range (more than 500 plant species) including common bean (*Phaseolus vulgaris* L.). Common bean is the most important grain legume crop for human consumption in the world and it is particularly affected by this fungus in water stress and high temperature conditions.

We used separately the histone deacetylase inhibitor trichostatin A (TSA) and the histone acetyltransferase inhibitors CPTH2 and garcinol like epigenetic backgrounds in the interaction assays between *M. phaseolina* and *P. vulgaris*. We evaluated the *MAC1*, *PMK1* and *LIPK* gene expression on infecting mycelium at 0, 24, 48 and 72 hours of interaction, with or without presence of inhibitors. These genes represent the PKA, MAP kinases and PKC pathways respectively, which are associated to the phytopathogenic process in many fungal species. We found a possible deregulation on some of these signal transduction pathways as a result of inhibitors action. We considered important the increased expression of *PMK1* and *MAC1* by both TSA and garcinol inhibitors, with respect to the expression levels recorded in control samples.

These results suggest a possible participation of the histone acetylation/deacetylation mechanism in the *M. phaseolina* pathogenic process on *P. vulgaris* and hence in their cellular differentiation processes.

Role of the RNA binding protein UmRrm75 from *Ustilago maydis*

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The RNA binding proteins (RBP) play important roles in post-transcriptional process such as: 5´CAP, pre-mRNA splicing, polyadenylation, and RNA stability and transport. These steps are highly regulated allowing spatio-temporal gene expression in order to synthesize proteins exactly where and when they are required. It has been shown that fungal pathogenicity and morphology are depending on post-transcriptional regulation. Until now three RBP´s from thephytopathogenic fungus *Ustilago maydis*have been studied. The K-homology domain protein Khd4 participates in regulation of mRNA stability, cell morphology and pathogenicity, the protein-contaningthree RNA recognition motifs (RRM)Rrm4 participates in polar growth and pathogenocity, and previously in our group was demonstrated that the UmRrm75 protein also affect filament formation and pathogenicity. This RBP (UmRrm75) present four RRM´s and a SR (serine/arginine) N- terminal domain and also it is glycine rich protein.

Now we have found that△umrrm75 haploid yeast-like FB1 and FB2 and the solopathogenic SG200 *U. maydis* strains are cold sensitive, suggesting that this protein could have a role as mRNA chaperone. Additionally these strains are also sensitive to the congo red cell wall stressor, suggesting that the cell wall biosynthesis is compromised.*U. maydis* mutants in class V myosin chitin synthases also fails to infect maize plants, so UmRrm75 protein could be involved direct or indirect in the transit of this chitin synthases to the hyphal tip, where they are localized. For example, the RRM4 protein from *Ustilago* is involved in the transport of the endochitinase 1(Cts1) to the filament tip, through microtubules. Additionally the RNA binding proteinsof SR type from *Saccharomyces cerevisiae* and *Candida albicans* are involved in mRNA nuclear export, since UmRrm75 might participate in long distance mRNA transport.

Can miR396 and its target genes regulate leaf development in *Phaseolus vulgaris* L. under drought conditions?

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Plants are continuously exposed to adverse environmental conditions because of their sessile nature. MicroRNAs (miRNAs) are~21 nt small endogenous RNAs that regulate gene expression at the post-transcriptional level in animals and plants. In plants, there are several deeply conserved miRNA families that regulate important processes such as development, biotic and abiotic stress responses. The miR396 family is conserved among monocot and dicot plants, and targets Growth-Regulating Factors (GRFs) a family of transcription factors that is involved in control of cell proliferation in *Arabidopsis* leaves. In *Arabidopsis,* miR396 is encoded intwo loci (*MIR396a* and *MIR396b*) which regulate seven (*AtGRF* 1-4 and*AtGRF* 7-9)of the nine genes encoding GRFs. It is known that miR396 is expressed in leaf primordia at low levels but accumulates preferentially in the distal part of young developing leaves and in consequence attenuates cell proliferation in plants under normal conditions of growth. Because the inhibition of leaf growth is one of the first responses of plants upon water deficit, we are interested in investigatingthe role of miR396 and of its targets in mediatingthe inhibition of leaf growth in *P. vulgaris*plants under drought stress conditions. It is therefore that previously our research group found that leaves of common bean plants reduce significantly both the leaf area and leaf number per plant upon drought conditions and that the expression of miR396a and pre-miR396a decreased in the first and second trifoliate leaves. Thus, the analysis of the role of GRFs and miR396 in regulating the leaf growth under drought conditions will provide new information about the molecular mechanisms that govern this developmental process under stress conditions.

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FUNCTIONAL STUDY OF NEUTRAL TREHALASE "*NTH1* GENE" IN *Ustilago maydis*

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The non-reducing carbohydrate trehalose (α-D-glucopyranosyl- (1→1) -α-D-glucopyranoside) plays a dynamic cellular role, it is useful as carbon storage and besides it works as a molecular chaperone, protecting the cell against several environmental stresses. Even is not well understood how it functions, it has been proposed that it could be replacing water molecules, stabilizing proteins and membranes in this way. For its qualities, is not surprising that trehalose is present in a wide variety of organisms, including, bacteria, invertebrates, lower and higher plants, archaea, fungi and yeast. In lower eukaryotes, the effort to elucidate the biological role of trehalose has been focused mainly in the yeast model *Saccharomyces cerevisiae*, nevertheless in fungal phytopathogens it is poorly understood, for example in the case of *Magnaporthe grisea* the catabolism of trehalose is important after plant cuticle penetration. It is well know that during the different stages of colonization/infection the plant responds with several defense mechanisms making an stressful situation for the fungal pathogen, in order to protect itself from the attack, some pathogens quickly increases the amount of intracellular trehalose to protect themselves.

Ustilago maydis is well known in the world as the causal agent of the corn smut disease, commonly called in Mexico as "Huitlacoche", which is considered as a delicacy. In the present study, we would like to try to understand the performance of trehalose's catabolism in this basidiomycete, mainly during plant infection and its general physiological response to stress. In yeast, there are two encoding trehalases genes, *NTH1* and *NTH2*. By in silico analysis in *U. maydis´*genome database, we identify a unique putative *U.maydisNTH1* (Um11661.1) neutral trehalase gene. We have now obtained the corresponding *nth1* mutants, we are analyzing its phenotype regarding its general response to abiotic stress, its ability to produce disease in corn plants and the amount of intracellular trehalose synthesized.

DIFFERENTIAL EFFECT OF THE MEMBERS OF MIR-34 FAMILY ON SIHA CELL PROLIFERATION

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MicroRNAs (miRNAs) play pivotal roles in controlling cell proliferation, apoptosis and invasion. Aberrant miRNA expression is now recognized as a molecular mechanism for many human tumors including cervical cancer. Infection with high-risk human papillomavirus (i.e. HPV-16 and 18) has been causally associated with the onset of cervical cancer. Furthermore, expression of cellular miRNAs has been linked to cervical cancer independently or associated to HPV expression. HPV-16 E6 protein inactivates and destroys p53 leading to a p53-null phenotype. P53 transcribe the members of miR-34 family formed by miR-34a-5p, miR-34a-3p, miR-34b-5p, miR-34b-3p, miR-34c-5p and miR-34c-3p. This family is transcriptionally regulated by p53 in response to cell damage and oncogenic stress. The ectopic expression of the miR34 family members recapitulates the biological effects of p53. In this work we analyze the function of miR-34 members on SiHa cell proliferation. Over-expression of miR-34a-5p and miR-34a-3p in cervical carcinoma cells causes 30% inhibition of SiHa cell proliferation. MiR-34b-5p mimic causes 30% inhibition of SiHa cell proliferation, however, miR-34b-3p mimic record no effect. MiR-34c-5p and miR-34c-3p reach 85% inhibition of SiHa cell proliferation. Our results show that the miR-34 family regulates cell proliferation at different extent and this knowledge could be used as therapeutic or diagnostic/prognostic tool in cervical cancer.

Transcriptional study of non-coding small RNAs genes *rsm Z1, Z2, Z3, Z4, Z5, Z6, Z7, Z8* and *Y* in *Azotobacter vinelandii.*

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Azotobacter vinelandii is a soil bacteria that belongs to *Pseudomonadacea* family, possess a postranscriptional regulatory system that has two components, the first component, is a regulatory protein called RsmA. RsmA has the ability to bind near of mRNA Shine Dalgarno sequence of its regulatory targets and promoting its degradation. The second component, non-coding small RNAs (sRNAs) releases the represion of the mRNA target, by binding the protein RsmA in a stem-loop structure characteristic of this sRNAs. *A. vinelandii* has nine sRNAs belonging to two different subfamilies of the Rsm sRNAs family, eighth *rsm*Z sRNAs and only one belong to *rsm*Y subfamily . Therefore it becomes the bacteria with more sRNAs described until now.

The sRNAs in *A. vinelandii*, *rsm Z3*, *Z4*, *Z5*, *Z6*, *Z7*, *Z8* and *rsmY* genes have a GacA binding site in its regulatory region, near of the transcription start point (50 bp). GacA is the response regulator (RR) of the GacS/GacA two component system. Interestingly, *rsmZ1* and *rsmZ2* genes have a large region between the GacA box and the transcription start point. The regulatory region of *rsmZ1* has a high percent of AT content, this fact suggest that a global regulator as HNS, could play a regulatory role in the expression of this sRNA. Moreover, *rsmZ2* has two putative IHF binding sites. As a result we have a regulatory network with nine sRNAs that allows having mRNA targets available for protein translation.

In this study we investigate both: the differential expression of sRNAs genes and the environmental factors that influence the expression of this regulatory elements. In order to get a global picture about how these elements are being regulated in different growing conditions, we construct transcriptional fusions of these nine sRNAs using a cromosomal integrative vectors with *gusA* activity. Furthermore, since *A. vinelandii* is a nitrogen fixing bacteria we explore the expression of these sRNAs in diazotrophic conditions and we found that all sRNAs have been expressed in differential way.

Down-regulation of *Phaseous vulgaris PvRbohD* gene during the symbiotic interaction with rhizobia

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Plant NADPH oxidases (respiratory burst oxidase homologs: RBOHs) are involved in numerous plant cell signaling processes, and have critical roles in the symbiosis between legumes and nitrogen-fixing bacteria. Our interest is focused in the symbiotic interaction between the model legume *Phaseolus vulgaris* (common bean) and *Rhizobium tropici,* a nitrogen-fixing bacteria. Recently, we reported a family of nine Rboh gene members in the *P. vulgaris* genome (Montiel, et al, 2012); they were named *PvRbohA, PvRbohB, PvRbohC, PvRbohD, PvRbohE, PvRbohF, PvRbohG, PvRbohH* and *PvRbohI,* based on their homology to the *Arabidopsis thaliana Rboh* genes. *PvRbohA*, *PvRbohB*, *PvRbohC* and *PvRbohD* transcripts were abundant in all organs tested, while the others were barely detected. The functional role of *PvRbohA*, *PvRbohB*, *PvRbohC* and *PvRbohD* during the symbiotic interaction is under study. Recently, we reported that down-regulation of *PvRbohB* suppresses ROS production and abrogates rhizobial infection thread progression (Montiel, et al., 2012). These and additional data indicate that *Pv*RBOHB is necessary for both infection thread (IT) progression and the maintenance of cortical cell division during nodule primordia development. Down-regulation of *PvRbohA* resulted in the abortion of ITs progression and affects bacteria release from IT; nodule size and number also decreased. Mature nodules were devoid of rhizobial infected cells in central tissues of RNAi samples (Arthikala, et al., manuscript in preparation).

Herein, to evaluate the role of *PvRbohD* in the nodulation process in bean transgenic roots (Estrada-Navarrete *et al*. 2007) a specific RNA interference (RNAi) construct was expressed. A phenotypic characterization of these transgenic roots after rhizobia inoculation will be presented and discussed.

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Transcriptomic analysis of photoconidiation process in *Trichoderma atroviride*

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Filamentous fungi can reproduce both sexually and asexually generating specialized structures. In asexual reproduction they have a conidiation process which generates spores. In *Trichoderma atroviride* conidiation is triggered by different stimuli such as a pulse of blue light, mechanical injury, and nutrient deprivation. This way of reproduction acts as a mechanism for survival and dispersal.[1]
Different stages of development are present before mature conidia; 4 hours after the stimulus aerial hyphaeare generated by 12 hours branched conidiophores with phialides have been formed, andby 24 hours after the stimulus mature conidia are observed.[2]

In order to study each response to environment in detail, different elements have been identified, specifically conidiation induced by blue light is carried out by blue light receptors, namedblue-light response proteins in *T. atroviride*, which are homologues of the *Neurospora crassa*white-collar 1 & 2.
After a blue light pulse a strong transcriptional response has been observed within minutesassociated with *de novo* transcriptional activity.

We decided to study the transcriptional status during the entire photoconidiation process after blue light pulseto recognize the changes and maintenance patterns of transcriptomic profiles during photoconidiation. In order to assess this goal, we have sequenced transcriptome from early stages (30 minutes), maturation stages (3 hours, 6 hours, 12 hours) andmature conidia (24 hours) of *T.atroviride* those samples will be compared with controls in darkness (36 hours and 60 hours).In total 14 libraries were sequenced (two replicates) with ~10 million reads by sample.Preliminary results showed hundreds of genes differentially expressed genes in 30 minutes and 24 hours after blue light pulse.

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Pirfenidone down regulates inflammatory mediators and modulates endocannabinoids receptors in non-alcoholic steatohepatitis induced by high fat/carbohydrate diet

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Non-alcoholic fatty liver disease begins with liver accumulation of triglycerides, which produce inflammation that eventually lead to unbalance between anti-inflammatory and pro-inflammatory cytokines leading to non-alcoholic steatohepatitis (NASH) and finally liver fibrosis. 5-methyl-1-phenyl-2-(1H)-pyridone (PFD) is indicated for chronic inflammation and fibrogenesis. Furthermore pharmacological modulation of cannabinoid type 1 receptor (Cnr1) and TGFβ1 is associated with inflammatory liver damage reduction. By contrast cannabinoid type 2 receptor (Cnr2) activation in chronic inflammatory diseases diminish inflammation by reducing IL17 synthesis.
We aimed to determine modulatory effect of PFD in inflammatory liver damage induced by experimental steatosis. Male C57/BL6 mice were fed with isocaloric normal diet (ND) or high-fat/carbohydrate diet (HFHC) (60% fat, 18% protein, 22% carbohydrate, and 55% fructose/45% sucrose in drink water) for 13 weeks. For both diets, PFD 100 mg/kg/d or vehicle was administered intragastrically.
Compared to HFHC mice, HFHC+PFD mice had significantly less weight gain, lower blood glucose, triglycerides and hepatic steatosis. ALT and AST level caused by HFHC diet was significantly reduced by PFD regimen. Furthermore, PFD attenuated the synthesis and secretion of Cnr1, IL17, COL1A1, CD11b, TNFα, MCP1, IL6 and TGFβ1 but elevated that of interleukin-10 in HFHC mice, which is associated to liver inflammation diminishment. Also PFD displayed a slight Cnr2 increase, which may explain IL17 reduction and improvement of liver markers. These results suggest that protection from HFHC diet-induced steatohepatitis by PFD is likely associated with the capacity of reduce inflammatory mediators, where IL-17 expression correlates with NASH□related liver diseases and fibrosis. Key words: Pirfenidone, steatosis, IL-17, endocannabinoids receptors.

Effect of the different sizes of dsRNA in the efficiency of NADHoxidase gene silencing in *Giardia lamblia*.

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Key words: *Giardia lamblia*, NADH oxidase, gene silencing

Introduction. *Giardia lamblia* is a unicellular flagellate protozoan that causes giardiasis, classified as a public health disease, this intestinal parasite is found worldwide and mainly affects children and immunocompromised patients[1]. Different pharmacotherapies have been employed against giardiasis; however, side effects in the host and reports of resistant strains clearly illustrate the need of develop new strategies to identify novel biologic targets for drug design.Instead, RNA interference (RNAi) may be a useful tool to selectively alter gene expression[1]. In this context, our objective was to design a vector to generate the gene silencing in Giardia, taking as a model system for studying the gene of the NADH Oxidase (NADHox)

Methods. We report the construction of a vector with two promoters positioned faced each and oriented at opposite direction between them (p*Tub*::p*Gdh*); such distribution allows the synthesis of dsRNAs for gene silence. We introduced by electroporation the integrative vector pTubGdh_*egfp*-RNAi to *G. lamblia*trophozoites; vectors were constructed with different fragments of the gene NADHox (100 to 500 bp), plus the puromycin resistance cassette for selection in Giardia[2]. The growth kinetics for measuring the enzymatic activity and gene expression is conducted in TYI with sampling times every 24 hours.

Results.The correct integration of the pTub-NADH fragment-pGdh silencing cassette was analyzed by PCR, the protein expression of *egfp*was also determined by qRT-PCR and confocal microscopy.
The results showed that transformed cells expressing dsRNA of 100 or 200 base-pairs reduce the RNAm of NADHox around of 30%; concomitantly, a significant decrease of enzyme activity was observed. A discrete effect in the growth of trophozoites, however, was detected under low oxygen concentrations suggesting that NADHox exhibits as not essential enzyme for *Giardia* metabolism in anaerobiosis. The study showed that is possible to commandeer the RNAi machinery of *Giardia* for performing the attenuation of specific gene expression of selected proteins.

Conclusions. The vector constructed with fragments of 100 and 200 bpNADHox caused silencing of the gene, even affecting their growth and metabolic activity of *G. lamblia*.

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"Regulatory divergence in paralogous genes *ALT1* and *ALT2* in *Saccharomyces cerevisiae*"

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Saccharomyces cerevisiae genome went through complete genome duplication known as: Whole Genome Duplication (WGD). *ALT1* and *ALT2* are two paralogous genes which arose after WGD. Previous results from our laboratory confirmed that *ALT1* encodes an alanine aminotransferase that translocates amino group from alanine to □-oxoglutarate to form glutamate and pyruvate. Surpringsinly, no function has been determined for Alt2 even when these two enzymes share 67% sequence identity. Previous results from our laboratory showed that the expression profile of *ALT1* and *ALT2* is contrasting. Northern Blots were carried out from cells grown in two different media, glucose-ammonia (biosynthetic conditions) and glucose-alanine (catabolic conditions), *ALT1* is predominantly expressed in RNA samples prepared from yeast grown on alanine as nitrogen source, and is poorly expressed in glucose-ammonia. The opposite occurs for *ALT2* expression, this gene is only expressed on biosynthetic conditions during the first hours, as culture grows, expression is decreased. On glucose-alanine *ALT2* expression is completely repressed. These results suggest alanine is co-inductor for *ALT1* and co-repressor for *ALT2.* In this work we have analyzed the transcription factors which are involved in the regulation of *ALT1* and *ALT2* expression. We found that Gcn4 participates as an activator of the two genes. Unexpectedly, the positive regulators Gln3 and Rtg3 function as *ALT2* expression repressors. Gln3 represses *ALT2* just in glucose-ammonia, but not on glucose-alanine, conversely, Rtg3 functions as repressor under both conditions: ammonia and alanine. The negative regulator Nrg1 negatively regulates both genes. Since Hda1 and Tup1 often participate as Nrg1 correpresors we analyzed whether these could participate as modulators. It was found that Hda1 negatively modulates *ALT1* and *ALT2*, while Tup1 only exerts its negative role on *ALT2* expression. Thus, our studies reveal there are five negative regulators for *ALT2* and a single positive activator. While *ALT1*expression is determined by one activator and two negative modulators. Chromatin analyses showed a contrasting reorganization in glucose-alanine and glucose-ammonium for both genes, this reorganization is in agreement with the expression profile we have observed for *ALT1* and *ALT2.* Results indicate that chromatin dynamics is not just a regulatory element pivotal in the expression of these two paralogous genes, but an element which shows an evolutionary divergence.

Functional characterization of the *Arabidopsis thaliana WIP2* gene in the meristematic regions of vegetative tissues

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Proteins that contain Zinc Finger domains can bind DNA, RNA or other proteins, among other molecules. The *WIP2* gene of the model plant *Arabidopsis thaliana* has 4 zinc finger domains, including a WIP domain that is shared by other 5 genes in the Arabidopsis thaliana genome. WIP2 is important for the normal development of fruit tissues, and also regulates and interacts with other fruit regulators. Interestingly, *WIP2* is also expressed at the shoot apical meristem (SAM) and interacts with known SAM transcription factors. Therefore, we are currently investigating the possible role of this gene at the SAM. One difficulty is that loss-of-function mutant vegetative phenotypes are not evident, probably due to the presence of the other related family members, which may have overlapping functions. To overcome the effect of redundancy, we are following different strategies to diminish the activity of the other family members. The latest results of these strategies will be presented.

Tissular localization of transcripts and proteins induced by water deficit in *Arabidopsis thaliana*: group 4 LEA proteins

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Late Embryogenesis Abundant (LEA) proteins accumulate during the last stages of seed development and in vegetative tissues in plants under water deficit. These proteins play relevant roles in dehydration tolerance in plants; however, little is known about their function and localization in plant organs and tissues. Previous work uncovered the relevance of a group 4 LEA protein in plant tolerance to water deficit. This group is formed by three members (*AtLEA4-1, AtLEA4-2 y AtLEA4-5*) and it was shown that mutant plants deficient in any of these members are more susceptible to water deficit. Of particular interest was the finding that mutant plants in the *AtLEA4-5* gene showed reduced number of floral buds, suggesting a role for this protein in the protection of these nascent organs under water deficit. Although LEA proteins may perform similar functions, the fact that the deficiency of these proteins exhibit detectable phenotype that is not compensated by the others members of the group could be explained by specific spatio-temporal distribution of each of these proteins during water deficit. To better understand the function of these proteins in the plant, it would be relevant to know the localization of their transcripts and proteins during the plant stress response. As a first approach to address this subject, in this work we focused on the localization of *AtLEA4-5* transcript and protein, the gene from group 4 that shows the highest response to water deficit conditions. By using transgenic Arabidopsis plants containing transcriptional fusions of the *AtLEA4-5* promoter with a reporter gene (*GUS*), we were able to detect expression of the reporter protein in primary and lateral roots of seedlings subjected to water deficit; whereas in the aerial part of these stressed seedlings, expression was specifically detected in leaf primordia. At protein level, it was localized by immune-localization techniques using specific antibodies. By looking at stems of adult stressed plants, protein was localized more abundantly in vascular and epidermal tissues. Protein was also detected in floral and axillar primordia, particularly in tunica cells. Interestingly, at the cellular level, this protein was found widely distributed in the cytoplasm and nucleus. Although limited at this point, this information indicates that *LEA4-5* transcript accumulates in different plant cell types and tissues suggesting that AtLEA4-5 protein may be protecting different cell functions during water deficit, including those necessary to maintain developing organs.

This work was partially supported by Consejo Nacional de Ciencia y Tecnología-Mexico (CONACyT-132258) and Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-DGAPA-UNAM; IN-208212.

Molecular characterization of TbZ5, a protein with multiple zinc-finger domains in *Trypanosoma brucei*

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Trypanosoma brucei is a flagellated protozoan parasite that causes sleeping sickness in humans. The parasite presents atypical mechanisms of genetic expression, such as polycistronic transcription and trans-splicing. Our group is interested in the study of transcription by RNA Polymerase III (Pol III), which is responsible for the synthesis of 5S rRNA and other essential RNA molecules. Transcription factor TFIIIA, required for 5S rRNA transcription, is characterized bythe presence of eight or nineC_2H_2zinc fingers. Interestingly, TFIIIA has not been identified in *T. brucei*. To try to identify the TFIIIA orthologue in *T. brucei* we performed *in silico* analysis to search for proteins with multiple C_2H_2 zinc fingers. We found a protein predicted to contain five zinc fingers, that we named TbZ5, which is a likely candidate for the *T. brucei* TFIIIA transcription factor. To analyze the function of TbZ5, RNAi knock-down stable cell lines were generated. To do so, a fragment of the TbZ5 gene was cloned into an RNAi inducible vector, which was transfected on *T. brucei* 29-13 parasites. Northern blot assays showed an 80-90% decrease of TbZ5 mRNA after RNAi induction. However, growth curves indicated that TbZ5 is not essential for cell survival. The effect of the TbZ5 knock-down on transcription by all RNA polymerases will be analyzed by nuclear run-on assays. To identify the proteins that interact with TbZ5 we are currently performing tandem affinity purifications. To do so, the C-terminal region of TbZ5 was cloned into a PTP vector to generate the plasmid pTbZ5-PTP. The linearized construct was transfected into*T. brucei*, and cellular clones that express the recombinant protein TbZ5-PTP were obtained. The complexes co-purified with TbZ5-PTP will be analyzed by mass spectrometry.

Study of Azotobacter vinelandii small non-coding RNARsmY

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The nitrogen-fixing soil bacterium *Azotobacter vinelandii* produces alginate and polyhydroxybutyrate (PHB) two polymers of industrial interest.This bacteriumhas the ability toform desiccation-resistant cysts and both polymers are involved in the encystment process. Poly-β-hydroxybutyrate is the most common bacterial storage polymer, its accumulation occurs mainly during the stationary phase. In *Azotobacter vinelandii* cysts, PHB accumulates in large granules and alkylresorcinols replace the phospholipids in the membranes and are components of the exine, the outer layer of the cyst envelope. In *A.vinelandii*thebiosynthesis of alginate, poly-β-hydroxybutyrate and alkylresorcinols are upregulated by the Gac-Rsm system. GacS/GacAtwo-component system is responsible for activate the transcription of seven small noncoding RNAs: *rsm*Z (*rsm*Z1-*rsm*Z7) and *rsm*Y (*rsm*Y1-*rsm*Y2) these regulatory RNAs influence gene expression at a posttranscriptional level, sequestering several homodimeric RNA binding potein: RsmA.

In all *rsm*sRNA genes, the GacA-binding sequence is present in its regulatory regions except for *rsm*Y2. Between Y1 and Y2 there are 88 bp and apparently there isn´t a putative promoter and GacA box. In this study we corroborate genetically if there are two genesor one, for this purpose we use two plasmids pGemrsmY1Y2::Gm and pGemrsmY1::Gm to transformed *A. vinelandii* UW136 to obtain *rsm*Y1Y2::Gm and *rsm*Y1::Gm mutants. The mutants carried the rsmY1Y2 or rsmY1 deletion. In order to carry out complementation analysis we construct the plasmids pUMATcY1Y2 and pUMATcY1, carrying wild type copies of the *rsm*Y1Y2 and *rsm*Y1 genes plus its regulatory region, respectively. The resultant plasmids, was transformed into *rsm*Y1Y2::Gm and*rsm*Y1::Gm mutants for complementation experiments. Oncedone, PHB and alkylresorcinols are quantifiedcomparing UW136 strain, both mutants (*rsm*Y1Y2::Gm and *rsm*Y1::Gm) and the complemented strains. Finally we prove the RsmAbinding ofRmA to *rsmY1Y2* by gel mobility shift assay (EMSA).

Ethylene-auxin interaction modulates primary root growth and cell division in *Arabidopsis thaliana*

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SUMMARY
The root system is essential for terrestrial plants as it provides them anchorage to the soil and plays a role in water and nutrient acquisition. Developmental processes in plants are tightly regulated via a plethora of substances known as Plant Growth Regulators. Root system development underlies mitotic cell generation at stem cells and their controlled expansion and differentiation. Because these three processes are related, the normal root growth program is called to be undetermined and ultimately forms the different structures that shape the root. Among these, the primary root has an embryonic origin, thus being the first to develop and the mean source of cells forming the root at early ages, making it a suitable parameter for studying fundamental cell processes.
In *A. thaliana,* a tight interaction between auxin and ethylene takes part regulating the primary root development. Basically, the interaction resides on each of them inducing the other´s biosynthesis, thus inducing responsiveness to both regulators and together, altering certain primary root phenotypic traits.
However, much information on the genetic basis of this interaction remains to be elucidated. Through a deep characterization of mutants in genes involved in the ethylene synthesis and response pathways; by studying the pharmacological effects of auxin and ethylene; and by introducing reporter genes for auxin response and transport in the primary root, we provide evidence that CTR1, the canonic regulator of the ethylene response pathway, functions as the main mediator of the expression of auxin response and transport elements during the *Arabidopsis* responses to ethylene, as well as that of genes necessary for the maintenance of the stem region. We further demonstrate that *ctr1* mutant have a determinate primary root growth, making it an important tool to further investigate the hormonal control of growth and the configuration of the root system.

KEY WORDS
Arabidopsis thaliana, Plant Growth Regulators, Ethylene, Auxin, Primary Root, CTR1

Intronic circles as pre-mRNA splicing products in *Entamoebahistolytica*

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Pre-mRNA splicing is a crucial step in gene expression and precise intron removal depends on the proper recognition of three main splicing signals: the 5' splice site (ss), the 3'ss and the branch point (BP) sequence.In the first step of the splicing reaction, the 2' hydroxyl of the BP adenosine attacks the phosphodiester bond at the 5'ss, yielding a lariat intermediate and a free 5' exon, which attacks the 3'ss in the second step to produce and excised lariat intron and spliced mRNA. In the protozoan parasite *Entamoebahistolytica* only the 5'ss and the 3'ss have been determined but not the amoebic BP sequence. In this work, first we focused in identifying the BP nucleotide in the amoebic introns of RabX13, ClcB (intron 2), rpL12, and rpS14 genes, by means of divergent-primers PCR. In all cases, the products amplified corresponded to 5'ss-3'ss-ligated (circular) introns and surprisingly no lariat structures were detected. After Actinomycin D RNA polymerase II inhibition and self-splicing reactions we concluded that RabX13 circularized introns are produced during pre-mRNA processing and not from self-splicing reactions. *In vivo* second step of splicing inhibition with boric acid slightly diminished both spliced variant and intronic circles formation, and we were able to identify lariat structures (canonical and alternative ones) suggesting that intronic circles arise after the lariat has been released from the lariat-3' exon intermediate in a fast circularization reaction. From the lariat structures identified, we observed that the RabX13 and rpS14 canonical BP nucleotides (nt) are adenine residues localized 6 and 11 nt upstream their respective annotated 3'ss.

To our knowledge this is the first work in which the BP in *E. histolytica* has been experimentally identified. Also this is the first report on the full-length *E. histolytica* introns are circularized during the splicing process.

EFFECT OF OVER-EXPRESSION OFTHE MEMBERS OF MIR-34 FAMILY ON SIHA CELL PROLIFERATION

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MiRNAs are small non-coding RNAs that regulate gene expression.It is well known that miRNAs alter expression favors cancer development. High-risk human papillomavirus 16 (HPV-16) are the major ethological agent of cervical cancer in México. HPV-16 E6 oncoprotein expression induces the degradation of p53 affecting apoptosis and cell cycle balance.P53 transcribe the members of miR-34 family formed by miR-34a, miR-34b and miR-34c. This family is transcriptionally regulated by p53 in response to cell damage and oncogenic stress. The ectopic expression of the miR34 family members recapitulates the biological effects of p53. In this work we over-expressed the miR-34 members to evaluated SiHa cell proliferation.Overexpression of miR34a-3p and miR34a-5p show 35% and 45%of proliferation inhibition,respectively, on the proliferation of SiHa cell. Furthermore, miR34b shows similar inhibition with 41% and 48% for the miR-34b-3p and miR-34b-5p, respectively. Importantly, miR-34c shows the higher inhibition with 68% and 74% for miR-34c-3p and miR-34c-5p, respectively, using 25000 cells. Interestingly, using 50000 cells the effect miR-34a-3p shows 50 %proliferation inhibition while miR-34a-5p shows 40%. MiR34b-3ptreatment record 21% whereas miR-34b-5p achieve 26%of proliferation inhibition. MiR34c-3pover-expression registers 65% and miR-34c-5p reach 68% of proliferation inhibition. The experiments show similar results with tendency to decrease cell proliferation in SiHa cells. Our work clearly shows that miR-34 family regulates cell proliferation and it could be used as therapeutic agent for cervical cancer.

Population density regulates *Arabidopsis thaliana* growth and development through modulating auxin transport.

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The sessile character of plants put them in the need to take environmental resources for survival, as ecosystems primary producers they play a key role in the existence of life on the earth. Their interactions with the environment are very important for sustaining agricultural productivity. When plants sense any resource as limiting, responses that allow it to adapt and /or compete for it are induced. Responses to competition depend on population density, but this has been poorly investigated at the physiological and molecular level.

The present work focuses on the characterization of the population density effects on developmental programs of *Arabidopsis thaliana* grown *in vitro* and in soil, and its interaction with the auxin signaling pathway. Our results show that the increase in population density decreases the production of chlorophyll and biomass as well as the overall size of the plant. The flowering process is accelerated at high density and the number of flowers and fruits is drastically reduced depending uponthe number of individuals grown together. Senescence occurs early in high density. Root systems decrease primary root growth and lateral root formation in a population density dependent way, whichcorrelated with decreased cell division and differentiation through auxin signaling. It is shown that the population density regulates polar auxin transport through the direct involvement of auxin efflux carrier PIN1 and PFT1 transcription factor. These results suggest that the effect of population density in the regulation of the processes described are established by plant-plant communication mediated possibly by volatile compounds and is not directly related to nutrients, CO_2 or light competition.

KEY WORDS: *Arabidopsis thaliana*, auxin, competition, populationdensity, *PFT1.*

Actin depolymerizing factor is required during the early stages of the rhizobia-legume symbiosis.

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Legumes possess the unique ability to form symbiosis with soil bacteria known as rhizobia to acquire fixed nitrogen. This interaction is preceded by a molecular dialog between the host and the bacterium. During early stages of this interaction, rhizobia secrete lipochitooligosaccharides, known as Nod Factors (NF), which trigger molecular and physiological changes in the root hair cell, such as membrane depolarization, calcium oscillations and actin cytoskeleton rearrangements, among others. Subsequently the root hair curls to form a tubular structure called the infection thread (IT) by which rhizobia enter to the root cortex where nodule development occurs (Ferguson, *et al*., 2010). It is well known that actin cytoskeleton plays a key role during the plant-microbe interactions. Previously, we reported the specific reorganization of the actin cytoskeleton in root hairs after NF treatment (Cárdenas *et al*., 1998) and recently, we found that this process requieres actin polymerization (Cárdenas *et al*, 2014). Actin polymerization is regulated by different accessory proteins, such as the Actin depolymerizing factor (ADF). ADF activity is regulated by N-terminal phosphorylation at a highly conserved serine residue (S). Studies in *Physcomitrella patens* show that N-terminal phosphorylation is important for tip growth (Augustine, *et al*., 2008). Herein, the function of one of the *Phaseolus vulgaris* ADF encoding gene (ADFE) during the progression of the infection threads and nodulation was analyzed. In this gene, two mutations in the serine 6 residue (*Pv*ADFES6A and *Pv*ADFES6D), were introduced by site-directed mutagenesis. Transgenic composite roots were generated (Estrada-Navarrete *et al*. 2007) harbouring one of the mutations. Our results show that overexpression of ADFE arrests the number and progression of the ITs within the root hair cell which resulted in the formation of fewer nodules (50% reduction) compared to control composite plants. A 60% reduction in nitrogenase activity was also observed in the few nodules formed. These data indicate that regulation at serine 6 of *Pv*ADFE is required for full *Pv*ADFE functioning. Experiments are in progress to demonstrate the active role of this protein during the rearrangement of actin microfilaments in the root hair cells from *P. vulgaris* after rhizobia of NF treatment.

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Gene expression profiling of DKK1 and patterns of methylation in osteoblast biology

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The discovery that mutations in LRP5 and sclerostin in humans alter bone mass, constituted a major breakthrough in bone research, the role of the β-catenin dependent Wnt signaling of bone implies considering how β-catenin affects bone mass and studies in mice have shown an essential role for β-catenin in osteoblast differentiation and in the regulation of bone mass and emphasized that the ultimate response to activation of the canonical Wnt signaling is cell stage-dependent. Dickkopf-1 (DKK1), a soluble inhibitor of Wnt/β-catenin signaling, antagonizes canonical Wnt signaling by inhibiting LRP5/6 interaction with Wnt and by forming a ternary complex with the transmembrane protein KREMEN that promotes internalization of LRP5/6. Studies suggest that DKK1 activation in osteoblasts is the underlying cause of glucocorticoid and estrogen deficiency mediated osteoporosis. Endogenous *Dkk1* expression was detected primarily in osteoblasts and osteocytes. Transgenic over expression resulted in distinct osteopenia degrees. On the other hand, the silencing *Dkk1* expression rescues dexamethasone-induced suppression of primary human osteoblast differentiation. Although this molecule has large implications for the development of the osteoblast is little known about their expression in different developmental stages of the cell, particularly in bone. In this study we evaluated the expression profile of *DKK1* and its correlation with the expression of Osteocalcin, a marker of bone metabolism and Osterix, a transcription factor for osteoblast differentiation. To determine the expression profile of *DKK1*, the analysis was carried out in three osteoblasts cell lines and found that *DKK1* is predominantly expressed during proliferation, this is because *DKK1* is a TCF target gene, suggesting the existence of a feedback loop of Wnt signaling. On the other hand, the expression of *DKK1* was lower during differentiation; in contrast the expression of Osterix and Osteocalcin was greater during differentiation. The increased expression of these markers correlates with a decrease in the expression of *DKK1*, which may suggest that, antagonist levels decrease in the osteoblast differentiation stage. Furthermore, in an effort to evaluate if the expression is regulated by methylation of the *DKK1* promoter we determined the patterns of methylation and the results showed that the DNA methylation status is a molecular mechanism maintained in all three osteoblasts cell lines during the proliferation and differentiation, suggesting that this mechanism regulate the expression of *DKK1*.

Merlin negative regulation by mir-146a promotes cell transformation

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Abstract

Inactivation of the tumor suppressor Merlin, by deleterious mutation or by sustained growth factors receptor signaling promoting phosphorylation-induced conformational changes and/or degradation, results in cell transformation and tumor development. Here we show that in addition to these mechanisms, miRNA-dependent negative regulation of Merlin protein levels also promotes cell transformation. We provide experimental evidence indication that miR-146a negatively regulates Merlin protein levels through its interaction with an evolutionary conserved sequence in the 3´untranslated region of the *NF2* gene. Merlin dawn-regulation by miR-146b in A549 lung epithelial cells resulted in enhanced cell proliferation, migration and tissue invasion. Accordingly, stable miR-146-transfectant cells formed tumors with metastatic capacity. Together our results uncover miRNAs as yet another negative mechanism controlling Merlin tumor suppressor functions. This work is partially supported by grants from CONACYT (155290 and 154542) and DAGPA/UNAM (IN209212 and IN227510).

Keywords: microRNAs, tumor suppressor, Merlin, cancer

In *Candida glabrata* Gln3 GATA factor is a nitrogen assimilation key regulator

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Nitrogen is an essential elementthat is contained in a wide range of compounds. It is well documented thatmany fungi as the ascomycete*Neurospora crassa* andthe hemiascomycete *Saccharomycescerevisiae*, can selectively utilize good nitrogen sources instead of bad nitrogen sources, this is achieved by two similar yet distinct mechanisms known as nitrogen metabolite repression (NMR) and nitrogen catabolite repression (NCR), respectively. When good nitrogen sources are available, NCR/NMR -sensitive genes are repressed. NCR/NMR-sensitive genes are regulated by zinc containing GATA domain factors and in the case of NCR by the Ure2 repressor also.We investigate the role, of the GATA factors and the Ure2 protein of the hemiascomycete human pathogen *Candida glabrata* when glutamine, ammonium or proline are available as sole nitrogen sources. Herewe show that the GATA factors and Ure2 protein are conserved within the hemiascomycete clade including *C glabrata*. We also position Gln3, as a key component of the nitrogen regulationbecause:a) in the absence of Gln3, the growth rate is impaired in all the nitrogen sources tested, b) Gln3 mutants are unable to efficiently transport ammonium from the media and c) transcription of the ammonium permease*MEP2* and the general amino acidpermease*GAP1* depends on Gln3. However, it is still unclear if the *C. glabrata* Gln3 GATA factor activates *MEP2* and *GAP1*transcription by directly binding to their promoters.To evaluate this, we need to perform chromatin inmunoprecipitation experiments.

Transcript profiling distinguishes complete treatment responders with locally advanced cervical cancer

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Abstract

The mortality of Cervical Cancer (CC) in less developed countries is a major public health concern since it is the second cause of death among women. Here we searched for a prognostic gene signature able to discriminate patients who do not respond to the conventional treatment employed to treat locally advanced cervical cancer (LACC). Tumor biopsies were profiled with genome-wide high-density expression microarrays. Class prediction was performed in 89 tumor tissues and the resultant gene signature was validated by qRT-PCR. A predictive gene signature —Cervical Cancer Conventional Treatment Response Profile (CC-CTRP)— was identified through its association with pathological response. The CC-CTRP was able to distinguish markedly between patients with progressive disease versus complete response. Gene expression analysis revealed two distinct groups of tumors diagnosed as LACC. Our findings could provide a strategy to select patients who would benefit from neo-adjuvant radiochemotherapy-based treatment.

Title:**Function of TrxG during*Arabidopsis* rootdevelopment.**

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Abstract: Eukaryotic genome is compacted in a dynamic structure called chromatin. The functional unit of the chromatin is the nucleosome which is a dynamic structure able to regulate accessibility of transcriptional factors to specific genome regions. The epigenetic factors play essential roles in the regulation of gene expression through their ability to promote chromatin remodeling.Polycomb group (PcG) and Trithorax group (TrxG) proteins are two antagonist complexes with histone lysine methyltransferase (HKMTase) activity that regulate chromatin structure. PcGpromotes gene repression, whereas TrxGcatalyzes the trimethylation of H3K4 to induce transcriptional activation.

In *Arabidopsis thaliana,ATX1,*a member of TrxG, is involved in the gene expression regulation of some transcriptional factorsthat act during floral organ development. *ATX1* loss-of–function mutants shown several defects in the development of different organs such as leaves and roots.Little is known about the function of *ATX1*during root development; therefore, we are interested in understandingthe function of*ATX1*with emphasis in the regulation of key homeotic genes for root development. Our data shown that *ATX1* is a promoter of root growth acting probably through the regulation of some MADS box genes.

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Characterization of transcripcional regulation of human paraoxonase 1 in human hepatoma cells

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Abstract

Human paraoxonase 1 (PON1) is an A-esterase calcium-dependentsynthesized in the liver and secreted into the plasma associated with high-density lipoproteins (HDL). PON1 acting as an antioxidant molecule in lipid metabolism preventing lipid oxidation; it also detoxifies a wide range of substrate including organophosphate compounds. The variability of serum levels and activity has been mainly attributed to polymorphisms in the gene, diet, pathological and physiological status, lifestyle and xenobiotics.However, the molecular mechanisms involved in transcriptional regulation of PON1 have been little studied. The aim of this study was to characterize the transcriptional regulation of human PON1in human hepatoma cells (HepG2). *In silico* analysis was performedon the promoter region of PON1to determineresponse elements of nuclear receptors (NR).Through Real-time PCR, was evaluated the effect of specific NR' ligands in the *in silico* analysison mRNA levels oftarget genes regulated by NR and*PON1*.The results obtained from the *in silico* analysis showed response elements of nuclear receptors to pregnenolone (PXR), glucocorticoids (GR), retinoic acid (RXR) and peroxisomes proliferator-activated receptor alpha (PPARα) with 95% homology. Treatments with dexamethasone (GR ligand), rifampicin (PXR ligand) and TCDD (AhR ligand)increased significantly mRNA levels of PON1 at 24 and 48 hours. In conclusion, PON1 is regulated positively by a mechanism that involves activation of the PXR, GR and AhR nuclear receptors.

Regulation of *WRKY* genes in 35S::*AtGRDP1* lines under ABA treatments

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AtGRDP1 is as a non-canonical glycine-rich domain protein, which contains a putative RNA binding motif and belongs to the DUF1399-GRDP family.*AtGRDP1*gene was studied in response to abiotic stress in seedlings, in agreement with the exhibit phenotypes under stress conditions the expression level analyses show that*AtGRDP1*is modulated in response to NaCl, LiCl, mannitol, sorbitol, glucose and ABA. Under ABA treatment the 35S::*AtGRDP1*lines have repressed*ABI3*and*ABI5*transcripts and shows resistance to ABA while the*Atgrdp1*-null mutant has an opposite expression pattern and phenotype.Analysis of*WRKY2*expression levels in35S::*AtGRDP1*line further indicated that ABA-induced*WRKY2*accumulationsuggesting that AtGRDP1 plays a role in the regulation ABA signalling pathway. In the same way, we analyzed other *WRKY* genes involved in ABA response such as *WRKY63* and *WRKY40* that represses expression of*ABI5*transcription factor; moreoverWRKY40proteininteract with their homologous WRKY18 and WRKY60 proteinsfor auto and cross regulation.We present evidence on the *AtGRDP1* and *WRKY* genes, showing a connection more in ABA signalling.

The expression of acidic ribosomal proteins in *Saccharomyces cerevisiae* during heat shock.

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The expression of all ribosomal proteins is highly regulated in eukaryotes such is not possible found “free” ribosomal proteins in cytoplasm. The only free ribosomal proteins that could be found in the cytoplasm are the proteins that form the ribosomal stalk. In *S. cerevisiae* four acidic ribosomal proteins (RPPs) with protein P0 form stalk. The stalk is a lateral structure in the large subunit ribosome; it is evolutionarily conserved in the 3 domains of life. RPPs P1α, P1β, P2α and P2β (10-11 kDa) non-covalently bound to P0 form a pentameric structure. The RRPs are the only ribosomal proteins that are in multi copy in the ribosome and interact with soluble factors of translation, affecting its function during the translation initiation and elongation. The RPPS form heterodimers in the stalk and the cytoplasmic pool. The P1/P2 heterodimers bind to P0 when both ribosomal subunits are joined and committed to translation, and they detached from the stalk, just after the small and large ribosomal subunits were separated from the mRNA. Cytoplasmic pool of the 4 RRPs has been described in yeast and other organisms. Additionally, there is an active refill between the cytoplasmic pool and de ribosomal stalk, the existence of a cytoplasmic pool and the presence of the RRPs in more than a copy in the ribosome, make suppose that mechanisms responsible of the expression of these proteins must be, at least in part, different from the rest of ribosomal proteins. The heat shock has a negative effect in the expression of ribosomal protein in *S. cerevisiae*. However in the case of RPPs this phenomenon isn´t studied.

Study of epigenetic changes in CCl_4 – induced cirrhosis model and the hepatoprotective effect of IFC-305

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In Mexico liver diseases have been the 5th death cause since 1998 (INEGI). One of these worldwide health problems is cirrhosis described as fibrosis with structural distortion and the liver dysfunction [Fauci et al., 2009]. We suggest IFC-305 (UNAM Patent 207422) as an alternative treatment to reverse cirrhosis because it is able restore the biochemical alterations in liver and decrease 40% of altered genes modified in CCl_4 model [Pérez-Carreón, *et al.* 2010].

We decided to investigate the epigenetic changes at the cirrhosis state and the effect of IFC-305 in the CCl_4 model. Wistar rats were treated with IFC-305 or saline (three doses per week) for 5 weeks after cirrhosis induction (CCl_4 treatment for 10 weeks); the livers were dissected and preserved at -70°C.

To know the general epigenetic state during cirrhosis and the changes involved in IFC-305 mediated reversion we evaluated by immunoassay the global DNA methylation and by western blot the global histone H4 acetylation. In the first assay, we found that IFC-305 increase DNA methylation in cirrhotic rats after 5 weeks of administration; on the other hand, there is a decrease of histone H4 acetylation at cirrhosis and the treatment with IFC-305 during 5 weeks restore the control's levels, this last result is consistent with the microarray mRNA levels of *Hdac3* [Pérez-Carreón, *et al.* 2010] that is overexpressed in cirrhosis and it tends to control levels with 5 weeks of IFC-305 treatment. Now we are evaluating 5-hydroxymethyl cytosine levels.

One of the extracellular matrix component that has an excessive synthesis and a little degradation during cirrhosis is Collagen type I, we analyzed with sodium bisulfite the methylation state of *Col1a1* gene promoter and found that 3 CpGs lose the methylation at cirrhosis, it is possible that this change is related with the increase expression of Collagen I at this state; on the other hand IFC-305 trigger to the increase of DNA methylation at CpGs around the transcription start site which possible mediate the hepatic tissue recovery decreasing *Col1a1* expression.

This results are, to our knowledge, the first description of epigenetic changes in CCl_4-induced cirrhosis model and are part of the beginning in the comprehension of hepatoprotective mechanism of IFC-305 at this level.

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Functional analysis of Bdp1, a subunit of transcription factor TFIIIB, in *Leishmania major*

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Leishmania major is a protozoan parasite that causes leishmaniasis in humans. The parasite possesses atypical mechanisms of genetic expression, such as polycistronic transcription and trans-splicing. We are interested in the study of transcription by RNA polymerase III (Pol III), which synthesizes essential RNA molecules, like transfer RNAs (tRNAs) and 5S ribosomal RNA (rRNA). To initiate transcription, Pol III needs transcription factorsTFIIIA, TFIIIB and TFIIIC. TFIIIB is composed of three subunits: Bdp1, Brf1 and TBP. Bdp1is involved in preinitiation complex formation and start-site selection, and it is characterized by the presence of a highly conserved SANT domain. Little is known about Pol III transcription in *L. major* and other trypanosomatids. An orthologue of Bdp1, which possesses a SANT domain, was localized on chromosome 36 of the parasite. Mapping of the processing signals (miniexon addition site and polyadenylation region) by RT-PCR revealed that the Bdp1 mRNA has long 3´ and 5´-UTR regions (~1100 bases).The half-life of the mRNA was estimated to be around 50 min in mid-log phase cells and 2 hours in stationary-phase cells. Thus, it is likely that sequences present in the UTRs are involved in stabilization of the mRNA in stationary-phase cells. In order to study the function of Bdp1 in *L. major* we are generating null mutants by homologous recombination. To do so, we have made*knock-out* vectors containing puromycin(pac) and hygromycin (hyg)antibiotic-resistant markers.To obtain the single knock-out cell line for Bdp1, *L. major* was transfected with the targeting cassette from the pac vector and clones were selected in the presence of puromycin. *Southern-blot* analysis confirmed the replacement of one Bdp1 allele by the pac gene. We are in the process of obtaining the double *knock-out* cell line. Pol III transcription will be evaluated in the *knock-out* cell lines by nuclear *run-on* assays.

Sucrose: sucrose 1-fructosyltransferase(1-SST) gene isolation and a method for quantifying gene expression

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Fructans are fructose polymers that derive from sucrose, and they are synthesized by the action of fructosyltransferases (FT), which are enzymes that transfer fructosyl residues to form certain types of linking. Inulin is a linear fructan with β (2-1) bonds and it's synthesized by the consecutive action of two enzymes (1-SST and 1-FFT). Up to date sucrose, light and some other abiotic stress components have been identified as inulin synthesis inductors. Little is known about its biosynthesis pathway gene regulation; nevertheless some recent research suggests mainly a transcriptional regulation. Therefore, to deepen ourunderstanding of regulation and the factors modifying it, we isolated a gene that codifies for the first enzyme (1-SST) involved in inulin synthesis and we designed a system that allows an induction of gene expression in dahlia (*Dahlia variabilis*) leafs, aninulin producing plant.A complete sequence of cDNA of 1,988 pb was isolated. This sequence was obtained byRT-PCR-based cloning method from *in-vitro* plantlets stressed by PEG-8000 in the culture media. For this, a first cDNA fragment of 700 pbwas obtained with degenerated-primersdesigned over conserved regions of dicot 1-SST reportedsequences. The 5' and 3' ends were obtained with a RACE commercial kit. Once the partial sequences were obtained, new oligonucleotides were designed to get the complete cDNA sequence. Finally, reported domains and motifs were compared and analyzed by an *in-silico* study.In order to assess mRNA expression in dahlia,a leaf immersion system was adapted from a previously published barley study. To adapt the system to dahlia leaves a series of components were employed, which allowed immersion of leaf petioles in a liquid solution, having also a rigid base to suspend leaf blades, and a dome-like structure to avoid leaf dehydration. To validate the system, a 0.5 M sucrose solution was chosen to immerse the petiolesof dahlia leaves, because of the known influence of this compound over the expression of fructan genes, so RT-PCR semi-quantitative analysis were made and differences on expression were found. With the validated system, expression of fructan-related or other genes could be explored.

Analysis of legume-miRNAs present in *Medicago truncatula* in response to water deficit

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Nowadays water deficit is considered as a severe environmental constraint to plant productivity. Legumes represent the second most-important crop family, and a key source of biological nitrogen in agriculture. Recently, microRNAs (miRNAs) have been identified as important stress-responsive regulatory molecules in plants, including their participation during responses to water deficit. MiRNAs are 21-24 nt small RNAs involved in gene regulation through base-pairing to target mRNAs, which in turn results in mRNA cleavage or inhibition of its translation, effectively repressing their expression. For this reason we are interested in the characterization of legume-specific miRNAs which might reveal novel strategies unique to this group of plants. Our group has started the characterization of novel legume-specific microRNAs, however we have been thwarted by the limited availability of genetic resources in *Phaseolus vulgaris* (common bean). To overcome this situation we have turned our attention to the analysis of drought responsive microRNAs in the model legume *Medicago truncatula*, which offers the advantages of a fully sequenced and annotated genome, availability of global expression analyses and mutant lines, as well as its susceptibility to genetic modification. We established growth conditions to study water deficit in *M. truncatula* and to address the analysis of legume-specific miRNAs, specifically the response of the legume-specific miRNA mtr-miR2199. This miRNA is accumulated in the root in an early stage of the response to the stress. Currently we are assessing the correlation of mtr-miR2199 and the expression pattern of its bioinformatically predicted target, which is a transcription factor of the bHLH family. These results will be compared to our previous results studying miR2199 in common bean, where it is also induced upon water deficit conditions in response to drought conditions.

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NF-kappaB inducing kinase (NIK) overexpression increases spheroid formation in breast cancer.

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NF-κB is a family of transcription factors that control the expression of a great number of genes related with the immune response, development, survival, proliferation, angiogenesis, invasion and metastasis. NF-kB pathway has a major role in tumor biology, in breast cancer NF-κB pathway is constitutively active promoting processes such as proliferation and survival of cancer cells. The main signalling pathways to activate NF-κB are the canonical and non-canonical NF-κB pathways. Non canonical NF-kB pathway depends on NIK activation. NIK is a kinase responsible to phosphorylate and activate IKKα. IKKα and NIK phosphorylates p100 causing its partial proteolysis and provoking the liberation and translocation of p52/RelB to the nucleus. NIK has been recently linked to cancer stem cells (CSC). CSCs are a subpopulation of cells responsible for the maintainance and progression of tumor. CSCs are characterized by their ability to form mammospheres. In order to demonstrate that NIK increased mammosphere forming ability, we overexpress NIK in a breast cancer cell line exhibiting low levels of NIK (MCF7) and we generated a stable cell line overexpressing NIK. In order to evaluate the ability of NIK-overexpressing MCF7 cells to form mammospheres, we growth MCF7 cells in non-adherent culture conditions. Our results show that NIK overexpression increase the ability to growth spheres in vitro

Polymorphisms in ABCA1, HNF1α or PPARγ genes and their participation in dyslipidemias

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Keywords: ABCA1, HNF1A, PPARG, dyslipidemia, type 2 diabetes.

Background: Changes in lifestyle and diet coupled with genetic background of each human being have a significant increase in the prevalence of dyslipidemias, they are a risk factor for mortality caused by type 2 diabetes and cardiovascular disease. Therefore, markers of dyslipidemia are essential for both early diagnostic and for personalized treatments that promotes prevention of its complications. ABCA1, PPARG and HNF1A are genes involved in regulation of lipid metabolism, so polymorphisms on these genes may predispose to dyslipidemias. Identify SNPs that correlate with dyslipidemia it will be relevant to be used as biomarkers. **Aim:** The aim of this work was identify single nucleotide polymorphisms in the ABCA1, PPARγ and HNF1α genes by PCR and sequencing in samples of blood donors, and establish its possible association with dyslipidemia. **Methods:** Buffy coat was obtained from the Blood Bank of the Hospital ISSSTE of Cuautla and State Center for Blood Transfusion, Morelos. DNA was purified of buffy coat by treatment with erythrocyte lysis buffer, then the leukocytes were isolating and exposed to proteinase K, followed by 16 hours of incubation at 65°C. Purification of DNA was performed using the phenol/chloroform method. The resulting DNA pellet was resuspended in TE solution, pH: 7.4. The quality of sample was assessed by spectrophotometry at 260/280 nm to determine the yield and purity. Likewise, to assess the integrity, electrophoresis was performed at 180 V for 45'. The amplify ability of the samples was assessed using PCR endpoint for GAPDH to 40 cycles. The design of primers was performed for the identification of polymorphisms using the Oligo 6 software according to the relevant criteria. Similarly, the standardization of PCR carried out using a curve of temperature and $MgCl_2$ to determine the optimum condition of amplification for each pair of primers. The analysis of the results will be performed using Stata software. **Results:** We have assessed the presence of the R230C/C230C SNP in 162 samples of which 14 were positive for R230C variant (8.64%) and one for C230C genotype (0.62%). The 86.67% of the population with the SNP had hypoalphalipoproteinemia. **Perspectives:** Identification of

Identification of miRNAs associated to pharmacologically induced autophagy in the colorectal cancer HCT116 cell line.

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Abstract

Colorectal cancer (CRC) has a high incidence and mortality indexes in both sexes, approximately 25,600 new diagnosed cases each year. Previous research has shown that the initiation and progression of CRC may be associated with aberrant expression or deregulation of microRNAs. Due to this fact, some drugs have been proposed whose mechanism of action could be directly related with the regulation of biological processes as apoptosis and autophagy. Autophagy is an evolutionarily conserved, multistep lysosomal degradation process in which a cell degrades long-lived proteins and damaged organelles. Therefore, our group is interested in studying the autophagy induction in the HCT116 CRC human cell line, by the patent-pending combination of three drugs: Metformin and F3, inhibitors of mTOR and glycolysis respectively, in synergy with Doxorubicin, which is a conventional chemotherapeutic. The main objective of this project was to evaluate the autophagy associated miRNAs in this cell line. When calculating the Inhibitory Concentration 50 (IC_{50}) of the three drugs (individually and in combination) by Sulforhodamine B colorimetric assay, we observed a synergistic effect in the toxicity of the drug combinations; therefore we performed assays using a suboptimal Inhibitory Concentration (Doxorubicin, Metformin, F3). Real Time RT-PCR was performed at different times (2, 4, 8, 12 and 24 h) showed considerable changes in the relative expression of autophagy-associated miRNAs such as miR-101, miR-106b and miR-183 in treated cells. A better understanding of miRNA modulation of autophagic signaling networks is likely to be crucial to the current and future cancer therapeutic strategies.

Deep Sequencing Analysis of *Ustilago maydis* Transcriptome in Response to Nitrogen Starvation.

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In the course of evolution, organisms have adapted to exploit diverse habitats, including the ability to grow and reproduce at the expense of others. Several pathogens have evolved sophisticated strategies to first attach to and subsequently infect their hosts. Yet sometimes in nature have to go through periods of lack of nutrients, some die, others adapt and still others remain dormant until environmental conditions are favorable. Discovery of the underlying molecular mechanisms of how pathogens survived extreme conditions of food scarcity is important to understanding pathogen biology and might help for the development of effective disease control strategies. Nitrogen (N) is one of the most important macronutrient essential for growth and development of living organisms. Nitrogen is required for the synthesis of proteins, nucleic acids, amino acids, and so on. To grow and develop normally, fungi must obtain sufficient carbon sources, and either inorganic or organic nitrogen from the media.

We found that *Ustilago maydis* DC, Corda, a plant parasitic basidiomycota causing corn smut disease, was able to survive and grow in nitrogen lacking media. This property was originally assumed to the remarkable capacity of some organisms (named oligonitrophylic) to scavenge traces of combined nitrogen. Now, our research group is working to elucidate how occurs the acquisition or assimilation of nitrogen in this fungus. Recent data suggest that *U. maydis* is able to fix nitrogen by an endosymbiotic association with a Gram+ bacterium (1).

Illumina RNA-Seq is a robust and reliable sequencing platform. It is now a preferred choice for global transcriptome analysis in both bacterial and eukaryotic systems. The efficiency and reliability of differential expression profiling by the illumina platform has been validated using several techniques like real-time quantitative reverse transcriptase PCR, microarray expression analysis, this powerful tool provide information on the transcript profile as well as the abundance. In the present study, Illumina RNA-Seq was used to identify the genes differentially expressed in *U. maydis* grown in the absence of a fixed nitrogen source. We found 90 genes differentially regulated, 49 up-regulated, and 41 down-regulated. Based on Functional catalogue (http://mips.helmholtz-muenchen.de/funcatDB/), we found that metabolism, cellular transport, interaction with the environment, cell rescue, defense and virulence among others were the most represented categories. Besides, we found that, oxidation-reduction processes, transmembrane transport and nitrogen compound transport were also enriched.

The nature of the regulated genes suggests that there is no difference in ammonium metabolism independently if it comes from the external medium or provided by the putative endosymbiont. Other pathways are affected by the different ammonium origin.

Reference: 1. - Una novedosa asociación endosimbiótica entre un basidiomiceto fitopatógeno y un firmicutes que fija N_2. José Ruiz-Herrera, Claudia León-Ramírez, Alejandro Sanchez-Arreguin, Holjes Salgado-Lugo, Antonio Vera y Juan J. peña-Cabriales. Congreso Nacional de Micología. Bilbao España; 18-20 junio, 2014.

MicroRNAs-mediated regulation of the tumor suppressor Merlin in response to inflammatory signals.

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Abstract

MicroRNAs are approximately 22 nucleotide single-stranded RNA that regulate protein levels by selective binding to specific sites at the 3'-untranslated regions of their target messenger RNA (mRNA). MicroRNAs activity triggers repression of mRNA translation or promote mRNA degradation. Multiple cellular pathways are regulated by specific microRNAs expression pattern and their deregulation promotes the development of different diseases and carcinogenesis. Thus, microRNAs that promote tumor formation are called onco-miRNAs and their targets are mRNAs encoding for tumor suppressors; on the other hand, microRNAs that prevent tumor formation are considered tumor suppressors and their target mRNAs encode for ongenic proteins. Merlin, the protein product of NF2 gene acts as tumor suppressor regulating cell proliferation, migration and invasion. Loss of NF2 tumor suppressor gene is involved in the development of neurofibromatosis type 2 syndrome that is characterized by the formation of several central nervous system tumors. In addition, inactivation of NF2 gene also has been associated with other tumors as malignant mesothelioma, osteosarcomas, fibrosarcoma and hepatocellular carcinoma. Furthermore, Merlin activity can be negatively regulated by posttranslational modifications. Phosphorylation on S518 by PAK or PKA switches Merlin to an inactive conformation, while phosphorylation on S10 and S315 by AKT induces Merlin degradation. Thus, constitutive AKT activation inhibits the Merlin tumor-suppressive activity resulting in tumor formation. In addition to deleterious mutations and posttranslational modification, we have recently shown that Merlin protein also can be negatively regulated by microRNAs. Downregulation of Merlin by microRNAs 146a and 7 in A549 cells enhanced proliferation, cell migration and tumor formation. Given that pro-inflammatory signals as LPS and IL-1β induce miR-146 expression we propose that microRNAs induced by proinflammatory conditions promote cell transformation by targeting Merlin. Here we will discuss the results of the experiments designed to test this hypothesis. This work is partially supported by grants from CONACYT (155290 and 154542) and DAGPA/UNAM (IN209212 and IN227510).

THE RESPONSE TO INJURY OF THE FILAMENTOUS FUNGUS *Trichoderma atroviride*IS REGULATED BY SMALL RNAs.

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The wound response of multicellular eukaryotes is essential for survival is highly conservedin plants and animals. Recently in our laboratory wediscovered that the filamentous fungus *Trichoderma atroviride*responds to injury by triggeringhyphalregeneration and the formation of asexual reproductive structures. A transcriptomic analysis showed that the mechanism of response to this stimulus is very similar to that of animals and plants, suggesting that this response is highly conserved among the three eukaryotic kingdoms. On the other hand several studies have recently reported that post-transcriptional regulation by microRNAs is involved in the response to injury in animals and plants.

Based on this background we decided to evaluate the injury response in mutants of theRNAi synthesis machinery of *T. atroviride*. In this analysis Δ*dcr2* and Δ*rdr3* presented a dramatic defect in the ability of regeneration and asexual reproduction in response to injury. To understand the molecular processes affected by the absence of the RNAi pathway, a transcriptomic analysis of the WT and Δ*dcr2* strains subjected to injury was performed, this analyses showed that signaling processes, DNA repair and cell cycle progression are essential to overcome this stress and are affected in the Δ*dcr2*mutant, explaining the phenotype of this strain. Even more interesting was the presence of a population of small RNAs of 21-22 nt in response to injury in the WT, which is absent in Δ*dcr2* mutant, suggesting that this type of small RNAs are product of the processing enzyme Dcr2 and they can be involved in theregulation ofgene expressionduring the response to injury in this fungus.

These results indicate that gene regulation by small RNAs is essential to respond to injuryin *T. atroviride* and that this mechanism is highly conserved in all three kingdoms. This phenomenon gives us the opportunity to understand in a simple biological model, the complex process of tissue repair, which can culminate in enhancing regenerative therapies in humans.

Study of common bean (*Phaseolus vulgaris*) microRNA2199 in water deficit conditions.

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Plant miRNAs are single-strand RNA molecules of 21-24nt in length that participate in various processes such as development, stress response and self-regulatory pathways in a post-transcriptional way. miRNAs recognize target transcripts by RNA:RNA base-pairing, generally a perfect match between the miRNA and its target favors cleavage of the mRNA at the position opposite to the 10nt and 11nt of the miRNA, and this is the most common mechanism reported in plants. On the other hand, an imperfect pairing favors inhibition of translation of the target mRNA, the main pathway of regulation observed for miRNAs in animals.

miRNA2199 (miR2199) is a conserved 21nt–long miRNA in the *Fabaceae* family that increases its expression in response to NaCl, drought and ABA treatment in the legume *Phaseolus vulgaris* (Arenas-Huertero, 2009). *In silico* predictions showed three target genes (Phvul.010G158400, Phvul.010G158500 and Phvul.010G158600) of miR2199, these genes are members of the bHLH (basic helix-loop-helix) transcriptional factor family. Later by degradome and 5´RACE assays the cleavage of the three target transcripts was confirmed and localized to the 3´ UTR region of the transcript (Velarde-Garduño, 2012). The cleavage site corresponds to one recognition sequence with perfect match to miR2199, immediately followed for a second sequence with an imperfect pairing, both sites conserved in the three genes mentioned above and in different legume species as well. The action mechanism of miR2199 seems to be non-canonical, because its transcript recognition site is located at the
3´ UTR and the presence of two regulation sites is typical of animal miRNA activity.

To evaluate the role of each regulation site, we will use the reporter gene GFP (green fluorescent protein) fused to the 3´UTR region of the target gene Phvul.010G158400 with the first and second recognition sites for miR2199 individually disrupted, and introduced back into common bean to study their repression by the miRNA. Additionally, we are interested in determining the expression levels of the three target genes and miR2199, in seedlings grown under optimal water conditions and water deficit with RT-PCR experiments and establish the contribution of each of these genes under stress conditions.

The aim of this work is to find which target transcripts are regulated by miR2199 under water deficit conditions and the role of the two miR2199 recognition sites in this process.

This work was supported by grants from CONACYT (CB2010-151571) and from PAPIIT-GDAPA (IN205112) to JLR.

The roleof histone acetytransferase*HAM1*during DNA damage response in *Arabidopsis thaliana.*

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*Abstract.*Plantsare sessile organisms; their inability to move toward favorable environments has led them to develop sophisticated mechanisms to overcome adverse conditions.To prevent DNA damage, plants have different mechanisms of DNA repair. In animals, DNA repairrequires of the function of DNA repair protein and the chromatin remodeling factorssuch asMYST (MOZ, YBF2, SAS2 and TIP60) histone acetyltransferases, which are involved in DNA damage response, DNA repair as well as DNA replication.HAM1 is a member of MYST histone acetyltransferasesfrom *Arabidopsis thaliana,* which interacts with DNA replication proteins and has specific activity onlysine 5 of histone H4.

To define the role of HAM1 in response to DNA damage by double-strand breaks (DSBs), we analyzed different cellular responses to DSBs in *HAM1* loss-of-function mutants (*ham1-1*),*pHAM1:HAM1:GFP*and *ham1-1*/*pHAM1:HAM1:GFP*transgenic plants.Our studiesrevealed that*HAM1*is required to prevent cell death and accumulation of CYCB1;1 protein after DSB induction. *HAM1* is not involved in the transcriptional activationof some key genes of DSB response such as *BRCA1* and *PARP1*. Together,our datasuggest that *HAM1* play a role during DSB responses, however more analysis are needed to define its function.

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NIKis co-expressed with ALDH in breast tumors and regulates the breast cancer stem cells population.

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Breast cancer stem cells (BCSCs) overexpress components of the Nuclear factor-kappa B (NF-kB) signaling cascade and consequently display high NF-kB activity levels. Breast cancer cell lines with higher proportions of cancer stem cells exhibit high NF-kB-inducing kinase (NIK) expression. NIK is required for the activation of the non-canonical NF-kB pathway, which controls the transcriptional expression of genes involved in developmental processes. The role of NIK in the phenotype of cancer stem cell regulation is poorly understood. To determine whether NIK mediates stemness in breast cancer cell lines, we analyzed its expression in BCSCs isolated from MCF7 and MDA-MB-231 cell lines and determined the effects of NIK disruption on cancer stem cell properties. BCSCs from both cell lines expressed higher levels of NIK and its inhibition through small hairpin (shRNA), reduced the expression of CSC markers and impaired clonogenicity and tumorigenesis in breast cancer cells. In addition, forced expression of NIK increased the BCSC population and enhanced breast cancer cell tumorigenicity. The in vivo relevance of these results is further supported by a tissue microarray of 191 breast cancer samples in which we observed correlated expression and physical co-localization of Aldehyde dehydrogenase (ALDH), a well known BCSC marker, and NIK protein. In conclusion, our results support the essential involvement of NIK in BCSC phenotypic regulation.

Transcriptomic Analysis of Pacific White Shrimp (*Litopenaeus vannamei*, Boone 1931) Exposed to Organic Silicon-Enriched Fertilizer

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Every year losses of up to 1 billion USD are recorded due to viral and bacterial infections in shrimp cultures, especially during their early life stages. As part of the life cycle in *L. vannamei*, 6 stages are considered where the organism increases its size and must change its exoskeleton; this process is known as 'moulting cycle' (ecdysis). The organism releases its old exoskeleton to synthesize another one according to the size acquired during the growth process. The moult cycle has 4 substages: Postmoult (A, B), Intermoult (C), Premoult (D0, D1, D2, D3) and Exuviae (E). Posmoult represents a critical susceptibility stage for pathogen infections. *L. vannamei* lacks an adaptive immune system; it is composed of two components: a barrier defense (cuticle) and a recognition system based on cellular (hemocytes) and humoral components which is why vaccines against pathogens in *L. vannamei* cannot be developed. Thus novel strategies to control viral and bacterial infections in shrimp cultures may include alternatives as the use of immunostimulatory supplements that may be able to trigger immunologic reactivity to increase shrimp's natural resistance to infection diseases and ensure sustainable shrimp aquaculture. The aim in this research is to evaluate the response of *L. vannamei* subjected to an Organic Silicon-Enriched Fertilizer (OSEF). Three shrimp groups were settled (i) a group of juvenile shrimps in Intermoult stage; (ii) a shrimp group exposed to OSEF; and (iii) a shrimp control group. Total RNA was isolated from muscle and cuticle. cDNA was synthetized using aminollyl-dUTP and labeled with alexa-555 and alexa-647 dye for further microarray hybridization analysis using a DNA chip containing 20 000 EST's and genes of *L. vannamei*. The DNA microarray data acquisition was done using a Genepix scanner, and then analyzed with the software *'Genarise'*. Data on the up and down regulated genes were analyzed with the Blast2go software using the following tools: Blast (Non-redundant, NCBI) and annotating (gene ontology). Up- regulated genes related to immune system and cuticle proteins in the DNA microarray analysis were selected to design specific primers for their validation by real-time polymerase chain reaction (qPCR). Towards this end, total RNA from hemocytes and cuticle tissue were isolated from the four shrimp groups: (1) Posmoult exposed to OSEF; (2) Posmoult control; (3) Intermoult exposed to OSEF; and (4) Intermoult control. After RNA was cleaned up with DNAse I and cDNA synthetized, three reference genes were first evaluated: beta-actin (ACTB), elongation factor 1-α (EF1A) and ribosomal protein L7 (RPL7) followed by four target genes: superoxide dismutase Mn (SODMn), ferritin (FMTM), Toll-like receptor (TLR), and Chitinase 4 (CHIA4). Standard curves of those genes were constructed to establish primer efficiency; then, the relative expressions of those genes were evaluated. We found a group of 20 Up-Regulated genes in the microarray analysis, related with the immune system and cuticle proteins. We also found that the rate expression of SODMn in hemocytes was 3 times more in Intermoult than in Posmoult; ferritin expression was not significant. In cuticle the expressions of SODMn, FMTM, TLR. and CHIA4 were significant and the results match with the microarray analysis in Intermoult and Posmoult. Our results revealed that OSEF promotes gene expression of the immune system; therefore, we may state that OSEF could be a putative immunostimulatory supplement for shrimp culture.

Transcriptional regulation of the sodium-coupled neutral amino acid transporter SNAT2 by 17β-estradiol during gestation in rat mammary gland.

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The mammary gland has a high demand for amino acids during the gestation and lactation periods. TheSNAT2amino acid transportertranslocates small neutral amino acids intothe mammary gland to promotecell proliferationduring gestation, and also provides efflux substrates for other amino acid transport systems.It is known that there is an increase in SNAT2 expression during pregnancy, previous *in vitro* studies demonstrated that this transporter is induced by 17β-estradiol. In this work, we elucidated themechanism by which17β-estradiol regulates the transcriptionofSNAT2. *In silico*analysis revealed the presence ofa potentialestrogenresponse element(ERE) inthe SNAT2 promoter.Reporter assaysshowed an increase in the activity of theSNAT2 promoter when co- transfected with estrogenreceptoralpha (ERα) after 17β-estradiol stimulation. Additionally, EMSAand ChIPassaysshowed that ERαbinds to the SNAT2 promoter *in vitro* and *in vivo* respectively. In the mammary gland the maximal binding occurred at the highest 17β-estradiolserum concentration during gestation. LC-MS^E and western blot analysisrevealed that the ERE in the SNAT2 promoter binds to a specific complex containing PARP1, Ku70 and GAPDH proteins in the presence of ERα, and that the silencing of each of these proteins nearly abolished 17β- estradiol-stimulated SNAT2 promoter activity. Nuclear levels of GAPDH increased progressively during gestation in the mammary gland, and GAPDH binding was also demonstrated in vivo and is nucleotide-specific for the SNAT2 ERE. Thus, this study provides new insights into how the mammary epithelium adapts to control amino acid uptake through the transcriptional regulation of the SNAT2 transporter via 17β-estradiol.

In vivo assessment of DNA methyltransferases inhibitor on cytochrome P4501A1 induction.

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Modulation of nerve growth factor expression (NGF) by ionizing radiation in different mouse tissues.

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Absorption of ionizing radiation by cells produces alterations in structure and function of biomolecules. Damage is dependent on the differentiation degree and the proliferation and metabolic rates of each tissue. Damage is induced by the direct (disruption of the atomic structure of macromolecules) or indirect effect (production of free radicals as a consequence of water radiolysis) of the IR. Water radiolysis generates reactive oxygen species (ROS) that can change the redox state of the cell inducing cell damage. The nerve growth factor (NGF) is a neurotrophin involved in development, survival, differentiation and plasticity of sensory and sympathetic neurons. In addition to these functions, there are evidences that show that NGF is involved in antioxidant response in the CNS and other tissues such as the liver. These responses are achieved when NGF binds to TrkA, a tropomyosin-related kinase receptor that is specific for this NT and the NGF/TrkA/PI3K/Akt signaling cascade is activated. We investigated the modulation of *ngfb* transcription, the downstream phosphorilation of TrkA and the Akt translocation to the nucleus in different male mouse tissues with distinctive radiosensibilities such as brain, spleen and heart. Animals were anesthetized and killed 1 h after whole body irradiation with 0.5, 2.5 and 4 Gy of γ radiation. Our results show that ionizing radiation up regulates the transcription of *ngfb* in the brain but not in the spleen or heart of the animals exposed to the intermediate and high doses. The treatment also modulated the phosporilation of TrkA in brain and spleen, as for Akt translocation there are no significant changes in the studied tissues. Thus, our results suggest that NGF-signalling cascade is being activated in the brain in response to the ROS generated as a consequence to the exposure to ionizing radiation, while in the spleen the radiation induces a decrease in the signaling cascade. The responses observed in this work are tissue-dependent, and are related to the radiosensibility degree previously reported for each tissue.

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Effect of airborne fine particle exposure on 5HT$_{1A}$- and D$_2$- receptor density and signaling in rat striatum, prefrontal cerebral cortex and olfactory bulb

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Exposure to fine particles (FP) is associated with the development of lung, cardiovascular and central nervous system diseases. FP produces oxidative stress and inflammation in nervous central system structures such as striatum (Str), prefrontal cerebral cortex (PFC) and olfactory bulb (OB). These molecular mechanisms have also been related to psychiatric and neurodegenerative disorders, some of which are the result of dysfunction of the serotonergic and dopaminergic systems. These neurotransmitter systems regulate mood and motor function, respectively, through the activation of their receptors, primarily 5-HT$_{1A}$ (5-HT$_{1A}$R) and D$_2$-like (D$_2$R) receptors.

In this work we explored further whether FP exposure is involved in the development of neurological impairment by analyzing glial activation and the density and function of 5- HT$_{1A}$Rs and D$_2$Rs in brain structures critical for mood and motor control.

Sprague Dawley rats were exposed for three days (acute exposure) or eight weeks (subchronic exposure) to either FP or filtered air using a particle concentrator. The FP concentration yielded ~470 µg/m^3 (19-fold the ambient concentration, ~25 µg/m^3). The density of 5-HT$_{1A}$Rs and D$_2$Rs was assessed by [^{3}H]-OH-DPAT and [^{3}H]-spiperone binding, respectively, and receptor function by [^{35}S]-GTPγS binding to membranes from Str, PFC and OB. Resident microglia and glial activation were determined by immunohistochemistry with an antibody against GFAP.

Acute exposure to FP induced a decrease in striatal 5-HT$_{1A}$R and D$_2$R expression, and an increase in 5-HT$_{1A}$R-induced [^{35}S]-GTPγS binding, with no change in D$_2$R signaling. In OB, FP exposure resulted in augmented 5-HT$_{1A}$R density with no effect on signaling. Subchronic exposure to FP resulted in increased striatal 5-HT$_{1A}$R density with no effect on signaling, and no change in D$_2$R density but decreased receptor signaling. In OB, there was no change in 5-HT$_{1A}$R density but receptor signaling decreased. No changes were detected in PFC after acute or subchronic exposure to FP. Reactive gliosis was only observed in Str following both acute and subchronic exposure to FP.

These results indicate that glial activation and altered striatal serotonergic and dopaminergic transmission may contribute to the risk of developing mood and motor disorders, associated with inflammatory and oxidative processes.

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Fluoride induced tubular injury and apoptosis in male Wistar rat subchronically exposed through drinking water and challenged with gentamicin.

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Introduction: In many countries, fluoride (F^-) is a common pollutant in drinking water. Previous studies have demonstrated that F^- may cause deleterious effects on renal function, particularly at proximal tubule level; however, the F^- levels used in these studies do not reflect those at a human population is exposed as well as, the renal function evaluation is carried out with markers that lack of sensitivity and specificity. Apoptosis has been suggested as one of the toxic effects responsible of renal alteration induced by F^- *in vitro* experiments.
Objective: The aim of this study was to evaluate the kidney injury and apoptosis induced by the F^- exposure at environmental relevant concentrations and if this event would make this organ more susceptible at the damage caused by an antibiotic with well-described nephrotoxic effect in renal cortex using an *in vivo* model.
Methods: Male rats recently weaned, were exposed through drinking water at two concentrations of F^- (15 and 50 mg/L) for 40 days with co-exposure to 40mg/kg bw per day (7days) of gentamicin (GM). At the end of the exposure period, overnight urine samples were collected and biomarker Kim-1 was measured (MagPix, Luminex). Next, animals were sacrificed and kidneys were dissected to obtain mRNA extracts and renal slices. Caspase 3 expression and localization was determined using confocal microscopy. RT-PCR was performed to quantify the mRNA expression of apoptotic regulators *Bax* and *Bcl2*.
Results: Levels of Kim-1 in urine were significantly higher in rats treated with GM compared with control (35.2 fold). In F15+GM and F50+GM groups, this increase was smaller in comparison with F0+GM (30.6 and 22.9 fold, respectively).
In the renal cortex of the F0+GM group, the ratio of *Bax* to *Bcl2* mRNA shifted in favor of Bax. However, in the co-exposed F15ppm+GM and F50ppm+GM groups, Bax to Bcl2 ratio almost returned to the control value (0.5 and 1.4, respectively). Confocal imaging showed a mitigation of Caspase-3 labeling in the presence of F^- (15 and 50 ppm) confirming the slight decrease of apoptosis.
Conclusion: Exposure to GM induced renal injury, evidenced by an increase of urinary Kim-1 and apoptosis in renal tissue. Interestingly, pre-exposure to F^- strongly significantly decreases the nephrotoxic effect induced by the antibiotic suggesting a hormetic conditioning by F^- exposure.
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Genotoxic effect of temephos in human lymphocytes

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Temephos is an organophosphorus pesticide used in control campaigns against the mosquito *Aedes aegypti*, transmitter agent of dengue fever. Despite the widespread use of temephos, few studies in the literature have examined it´s genotoxic potential. The aim of this study was to evaluate the cytotoxic, cytostatic and genotoxic effects caused by the *in vitro* exposure of human lymphocytes to temephos at 0.12-10.0 µM concentrations. The cytotoxicity was measured by simultaneous staining. The cytostatic and genotoxic effects were evaluated through the micronucleus and comet assays, as well as a DNA repair assay. The results demonstrated that temephos was not cytotoxic in the concentrations tested, in the comet assay, the outcome showed that although temephos increased DNA damage levels, at doses as low as 1 µM, the damage was repaired at 60 min post-exposure. Furthermore, temephos did not increase the micronucleus, nucleoplasmic bridge, nuclear bud or necrotic cell frequencies; however, exposure to 10 µM temephos caused a significant decrease in the percentage of binucleated cells and in the nuclear division index, as well as an increase in apoptotic cell frequency. In conclusion, temephos exposure, under this *in vitro* conditions, did not cause stable DNA damage. This study was supported by CONACyT-C. Básica, Mexico (#156673).

Chronic low level arsenic exposure induces progressive aberrant DNA methylation that correlates with cell transformation in HaCaT cell line.

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Ground water arsenic is the main source of human exposure worldwide and it represents an important health issue. Arsenic is type A carcinogen and the strongest link has been found with skin cancer. It has been proposed that epigenetic dysregulation could play a key role in the malignant cell transformation induced by arsenic, particularly alterations in DNA methylation by disrupting the pool of S-adenosylmethionine by arsenic metabolism.

To analyze the role of arsenic induced epigenetic changes in the transformation process, we evaluated genome wide DNA methylation and expression patterns in HaCaT cells chronically exposed to sodium arsenite at low doses (1 and 0.1 µM) for 25 weeks. Illumina Infinium Human Methylation 27K BeadChips were used to analyze genome wide DNA methylation status and Illumina HumanHT-12 v4 Expression BeadChip to analyze gene expression. We employed DAVID Bioinformatics Resources 6.7 for pathways functional enrichment analysis and a model-based clustering to analyze the relation between DNA methylation and gene expression.

We found progressive aberrant DNA methylation in arsenic exposed cells to 0.1 µM sodium arsenite but the cells exposed to 1 µM only showed slight modifications through the treatment. Altered DNA methylation correlates with cell transformation since only cells exposed to 0.1µM were positives to soft agar colony formation assays and produced tumors in nude mice. Pathway overrepresentation analysis showed enrichment of Wnt signaling and other associated pathways in genes related to cancer, cell differentiation and oxidative stress. Analysis of correlation between DNA methylation and gene expression showed alteration in gene clustering, specially hypermetilation and downregulation of several genes.

Proteomic characterization of venom from male and female scorpions of the species *Centruroides limpidus limpidus*

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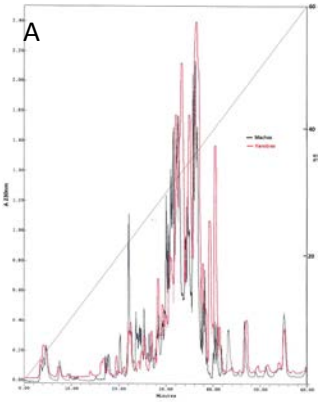
Introduction: Scorpions have prevailed in the world for million of years. These arachnids are distributed all over the world including Mexico, in which the genus *Centruroides* is abundant., Particullary, *Centruroides limpidus limpidus* have medically relevance because they cause the highest incidence of human stings in Morelos. This species shows sexual dimorphism and we want to know if the venom components are also distinct. **Material and methods**: Soluble venom from both sexes of scorpions collected in Morelos, were obtained by electrical stimulation and separated by high performance liquid chromatography (HPLC) before mass spectrometry determination. This analysis was performed separately for each sex. In addition a gel filtration separation (Sephadex G-50) was also used to select components of comparable molecular masses, before HPLC separation. **Results and discussion**: The various components of their venoms show similar masses and present some changes at sequence level (figure 1B), which could be due to the presence of different isoforms of toxins or because their abundance is not the same and therefore cannot be detected. The size exclusion separation followed by HPLC confirmed the presence of different chromatographic profile for females and males (figure 1A).

Figure 1. A) Reverse phase chromatographic profile of whole venom. B) Molecular weight ratio with the sequence of selected fractions



B

Retention time/masses/sequence; female	Retention time/masses/sequence; male
21.95/3421/ KYCYNKDDSKSECMVV // TVINVDYQKPWNCLPCCKCI	22.08/3421.47/KYCYNDDGQKSEPMVVKYCQ YGTCY// TVILVKATSPKCCCPVCKY
22.37/4243.38/ TVINVKCTSPKQCLPPCKEIYGRHAGAKCMVG KCHCSC	22.60/4242.6/ TVINVKCTSPKQCLPPCKEIYGRHAGAKKM
39.21/6780.94/ KEGYPMNSKGCKIGCVIGNT	40.07/6782.83/ RDGYALRKDGCATPCL
39.83/6574.7/ KEGYALNKDGCPIPYLLDSAYCNKEQVQP	40.42/6575.5/ KEGYPMNSEGCKIGCVIGNTFXDTE

Conclusions: We have found differences in the chromatographic profiles of *Centruroides limpidus limpidus* males and females and also differences at sequence level of some fractions (v.g.: 22.08 and 21.95). Our findings will be discussed in comparison with literature data. **Acknowlegements:** CONACYT, for financial support of the student (Exp. 512560), DGAPA-UNAM IN200113 and SEP-CONACyT 153496 for expenses support to the laboratory of LDP.

Cloning and expression of a serinoprotease from the venom of *Bothropsammodytoides*

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The bilateral collaboration between Argentina and Mexico has permitted the exploration of interesting venomous species from these two countries. *Bothropsammodytoides* is the most Southern situated viper in the world. This snake inhabits a geographical region that goes from the warm regions of the La Plata basin to the cold Patagonia of Argentina. It is a small viper that usually does not surpass 70 cm in length, but can cause the typical problems of bothropic envenoming. This snake has different feeding habits from other Bothrops species of Argentina, because its diet also includes small lizards. The venom of this snake cause hemorrhage, dermonecrosis and edema in mice, and its LD50 is 2µg/g de ratón. It is also myotoxic to mice, and shows procoagulant activity on human plasma, but it has low or absent of thrombin like activity over bovine fibrinogen. Since the serine proteases with high specificity for arginine ester hydrolysis activity are widely distributed in crotalidae and viperidae snake venoms, and they are considered to play important roles in blood-clotting disorders. Therefore, serine proteases are of potential therapeutic use for the treatment of myocardial infarctions and thrombotic diseases. In this work, we report the isolation and expression of a serine protease from *Bothropsammodytoides*.The venom of *B. ammodytoides* was separated using size-exclusion and reverse phase chromatography. Protease zymograms of the protein fractions obtained from the chromatographic steps were obtained. A protein fraction with an apparent molecular mass of 25,985 Da showed high proteolytic activity. This fraction was N-terminal sequenced and together with the reported primary structures of serinoproteases from the genus Bothrops, various oligonucleotides were designed to amplify the corresponding cDNA. A single venom gland from a specimen was obtained by surgical extraction. By using the total RNA from the venom gland and standard 3'RACE techniques using the designed oligonucleotides, the full-length of a serinoproteasecDNA that codes for a protein of 236 amino acid residues was found. The gene that codifies for the serinoprotease was cloned into the vector cloning Topo 2.1 from Invitrogen and it was cloned into the vector expression pQE-30 that introduces a 6 His-tag and aFXaproteolytic cleavage region fusion protein. The extra 6 His-tag residues improve the affinity purification of the recombinant neurotoxin, and the FXaproteolytic cleavage region allows to cleave the N-terminal fusion protein and release the correct mature protein sequence. The pQE-30 vector was transformed into *E. coli* M15 cells, and the expression was induced with 1 mM Isopropyl ß-D-1-thiogalactopyranoside (IPTG) at 37 °C for 3 h. Until now, we have obtained a recombinant serinoprotease and further details will be presented.

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Isolation and characterization of a phospholipase in *Palythoa caribaeorum.*

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Phospholipases are a group of enzymes that are widely distributed in nature and that catalyze the hydrolysis of membrane phospholipids. They are classified according to the bond cleavage site as: PLA_1 (EC 3.1.1.3), PLA_2 (EC 3.1.1.4), PLB (EC 3.1.1.5), PLC (EC 3.1.4.3) and PLD (EC 3.1.4.4) . These enzymes have been extensively studied for a long time; the first evidence of the existence of these enzymes was in pancreatic juice when studying the hydrolysis of lecithin in the glycerophosphoric acid, fatty acids and choline (Bokay A, 1877).

Structural studies have shown that these proteins contain a high degree of disulfide bridges, making them extremely stable to heat treatment and pH changes.

Our work is focused on the study of PLA_2 because these enzymes are part of the poisons produced by animals and play an important functions in it. PLA_2 have been extensively studied in snakes and bees but the study of marine organisms has been slow. In particular our organism has been little studied compared whit other marine organisms.

The aim of our work is to obtain at least a phospholipase in the venom *of Palythoa caribaeorum.*

Until now two phospholipases were obtained through chromatographic techniques, followed by MALDI-TOF and finally amino acid sequence analysis. A search of homologous proteins by sequence aligment through the BLAST tool was also performed.

Our results suggest that these two enzymes are very similar to other proteins previously reported in the literature although there are small differences in structure which is attributed to the different evolution between organisms that have been studied until now.

Further studies will be focouse on:

Evaluating the neurotoxic activity
Determining structure by circular dichroism spectroscopic technique and X-Ray.

Coadministration of High Doses of Rosuvastatin and a Cholesterol-Rich Diet Produces Premature Death in CD-1 Male Mice

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Objectives
We analyzed the side effects of high rosuvastatin (RVT) doses given to CD-1 mice along with either normal diet or 2% cholesterol diet (HD).
Methods
In experiment 1, animals (n=6) received 0, 20, 50, 100, 200 or 400 mg/Kg/day of RVT and HD. In experiment 2 animals received the same RVT doses and no cholesterol. Cumulative mortality was registered.
Results
Animals from experiment 1 began to die from day 5. After 6 weeks the survival percentages were 100%, 50% and 17% for RVT 0mg/day, 20mg/day and 50mg/day, respectively, and 0% for the higher RVT doses. All animals from experiment 2 survived, without apparent damage. Some livers were studied through optical microscopy and liver mitochondria by electronic microscopy. The animals with RVT and normal diet had higher glucose levels than the control normal group, but it was significant only for RVT 400 mg/g. Electronic microscopy showed more dense mitochondria and certain disorder, cellular detritus, matrix loss, in 0-RVT/HD; mitochondrial zones of lower density, higher matrix density, some mitochondria destroyed or deformed, in 20-RVT/HD; conglomerated, swollen, more dense mitochondria, in 50-RVT/HD.
Conclusion
In summary, coadministration of high doses of RVT, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, and HD is severely harmful for mice. The potential side effects of RVT should be considered for humans in moderate-intensive RVT therapy.

Evaluation of lipid profile and association to cardiac risk factors in rural communities of state of Chihuahua exposed to fluoride in drinking water

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In various regions in the world high fluoride (F^-) levels in drinking water represent a public health problem. Excessive chronic F^- intake results in fluorosis. Effects on lipids metabolism and decreased activity of plasmatic paraoxonase have been observed in rodents exposed to F^-. However, the adverse role of dyslipidemia in predicting cardiovascular outcomes has not been elucidated in endemic fluoride areas. A cross-sectional study was conducted with 239 adults (18-77 years old) residents in 3 communities of the State of Chihuahua with different concentrations of F^- in water, Guadalupe Victoria (3.94 ± 0.12 mg/L), Aldama (1.56 ± 0.14 mg/L) and El Sauz as control population (0.21 ± 0.08 mg/L). Lipid profile was analyzed in plasma using an automatized method. The atherogenic factor (AF) was calculated for each subject as total cholesterol/HDL. Systemic blood pressure and anthropometric parameters such as BMI, fat percentage, MME and RCC were evaluated. We evaluated concentration in water and in urine using F-ion selective and dental
fluorosis using the Dean index. The results show a positive correlation between the concentration of F^- in water and F^- in urine (r_s = 0.5904, p <0.001), suggesting that water is the main source of exposure to the contaminant. The dental fluorosis was in agreement with the levels of F^- in the localities: 89.4,
69.7 and 20% in Guadalupe Victoria, Aldama and El Sauz, respectively. Most participants were overweight and obese (>76%). They presented dyslipidemic effects reflected in hypertriglyceridemia (56%), hypercholesterolemia (42%) and HDL depletion (55%). However, we did not observe an association between AF or dyslipidemia with F^- exposure. Further studies are needed to know the participation of F^- exposure in lipid dysfunctional states in human populations.

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Genotoxic Safety of Jacareubein in Bone Marrow Cells of Female Balb/c Mice

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Because chemotherapy as a cancer treatment produce deleterious side effects, it is important to identify new compounds that are clinically effective and produce fewer side effects. Naturally occurring compounds are an important source of new drugs such as xanthones; which have shown several pharmacological activities such as inhibition of cell proliferation and activation of signal transduction pathways in tumor cell lines. Jacareubein is a xanthone isolated from *Calophyllumbrasiliense* heartwood with cytotoxic activity against human tumor cell lines inducing cell cycle arrest at the G2/M phaseand apoptosis. Due to its antineoplastic potential, and the possibility of pharmacological use, it is important to evaluate the genotoxic potential of Jacareubein in normal cells.

To this end, Jacareubein was administered intraperitoneally to female Balb/c mice (6-8 weeks of age) at 100 mg/kgevery 2 days for 7 or 14 days.Animals were killed by cervical dislocation and both femora were dissected and their marrow cells were collected with fetal bovine serum and smeared onto clean glass slides. Samples were dried over-night, fixed with methanol for 5 min and stained with Wright-Giemsa for 5 min. Three slides were prepared from each sample and 2000 polychromatic erythrocytes (PCE) were counted to determine the frequency of micronucleated(MN) PCEs. Also, 2000 normochromatic erythrocytes (NCE) were counted, as well as the frequency of PCE within the same microscope fields of the NCEs to calculate the PCE/NCE ratio and the proportion of cell maturation. The slides were analysed using an Olympus CH30 microscope (100 X).

We observed thatafter 1 week oftreatment with Jacareubein the basal frequency of MNdecreasedwhencompared with the negative control (vehicle)(2.3+0.5 and 5.3+1.3, respectively). This effect was transient because after 2 weeksofcontinuous treatmentwith Jacareubeinno differencesin the MN frequency were found among treatments. The results obtained indicate that Jacareubein shows no genotoxic effects *in vivo* in normal bone marrow cells. Thus supporting the pharmacological use of this compound as an antineoplastic agent.

Expression and folding of cysteine rich neurotoxinsfrom the venoms of spidersandcoralsnakes

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The venom of spiders and coral snakes contain neurotoxic peptides, which are of biotechnological interest because they could be used as archetypes for the development of insecticides and anti-venoms, respectively. Two interesting neurotoxic peptides were previously reported. One of them is insecticidal (Ba1) and it consists of 39 amino acids including three disulfide bridges. It is found in the venom of thetheraphosidspider *B. albiceps* from the State of Morelos, Mexico. The second one is a mammalian neurotoxin (MlatA1) and it contains 61 residues comprising four disulfide bridges.MlatA1is found in the venom of the coral snake *M. laticorallis* from the State of Guerrero. Ba1 and MlatA1 are naturally obtained in minute amounts in their respective venoms; therefore, two protein expression systems in bacteria were designed to gain higher amounts of these two interesting molecules. In this work, Ba1 and MlatA1 were cloned and expressed using the pET28a^{+} and pQE30 plasmid vectors, respectively. The two vectors encoded also for a N-terminal fusion protein having a 6His-tag and aFXaproteolytic cleavage region.*Escherichia coli*strain BL21 and strain Origami DE3 were respectively the host for the pET28a^{+}-Ba1 and the pQE30-MlatA1 expression plasmids. Protein expression was induced with 0.1 mM isopropyl thiogalactoside and further purified using Ni-NTA affinity chromatography. The protein yields were 1 and 2 mg for Ba1 and MlatA1, respectively. The folded Ba1 was enzymatically cleaved by FXa and tested for its biological activity in crickets having a lethal dose at 36.6 µg/g cricket. Regarding the recombinant peptide MlatA1, it was tested in mice showing the expected neurotoxic symptoms and median lethal dose of 19.4 µg/20 g mouse.

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Phage-displayed variants of the Css2 toxin from *Centruroidessuffusussuffusus*

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Abstract: *Centruroidessuffusussuffusus* is one of the few Mexican scorpion species that are dangerous to humans, Css2 is the most toxic neurotoxin form *C. suffusussuffusus* and is specific for mammalian voltage-gated sodium channels (Nav). Other toxins from scorpions of the same genus share a high degree of similarity with Css2. Although they share the same structural scaffold and have highly homologous sequences, they differ in their specificity and affinity towards the Nav's. Two regions of variability can be readily singled out in a sequence alignment of these toxins: the carboxyl-terminus and a discrete 3-amino acids region close to the amino-terminus. The carboxyl-terminus has already been shown to be partially responsible for the differences in activity. The effect of the changes in the amino-terminus remains unexplored and is the goal of this project. We propose to assess the effect of the variability in the 3 amino-proximal amino acids on the Css2 affinity towards its main toxin target, the Nav1.6 channel. For this matter we constructed a library of phage-displayed Css2 variants covering all possible mutations at those 3 positions. We aim to select variants with improved affinity by selecting the phage-displayed library against whole Nav1.6-expressing HEK cells.

Methodology: The gene coding for the native toxin (CSS2) was amplified using a degenerate oligonucleotide to introducethe codons for all possible variants at the three amino-proximal residues (20^3=8,000 variants).SfiI and NotIrestriction sites were also added to the sequence flanksby the PCR primers. The gene library was ligated into thepSyn2phage display phagemid and cloned into XL1-Blue *E. coli*cells. After infection with a helper phage, the Css2 variants were displayed fused to the pIII protein of the filamentous phages. HEK cells expressing the Nav1.6 channel were used for the screening rounds.

Results: The Css2 variants in the three amino-proximal residues were obtained, with an estimated mutagenesis ratio of 75% (Figure 1).The protocol for phage- displayed toxin screening on whole cell was standardized.

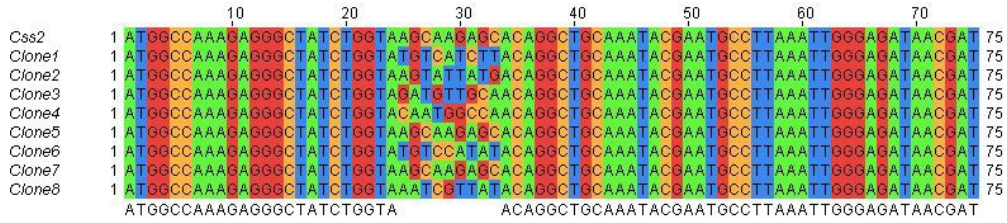

Figure 1: Sequence alignment of the native Css2 gene with several clones from the mutageniclibrary. Only the first 75 nucleotides are shown.

"PARTICIPATION OF MTOR IN THE TRANSPORT OF AMINOACIDS INVOLVED IN GLUTATHIONE (GSH) SYNTHESIS IN MOUSE STRIATUM".

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Glutathione (GSH) is the most important intracellular antioxidant system. It plays a primordial role in the protection of cells against oxidative stress, mainly in the central nervous system (CNS). The synthesis of GSH is limited by the availability of cysteine. Cysteine incorporates into the brain through the blood brain barrier (BBB) where specific transporters like LAT1, Xc- and EAAC1 are expressed.
The nerve growth factor (NGF) have shown to participate in the antioxidant response; activating its receptor TrkA and downstream PKB/AKT through PI3K. The activation of this pathway is associated with the increased transcription of *xct, lat1, eaac1.* On the other hand, TOR (target of rapamycin) a serine/threonine kinase regulates cellular growth in response to growth factors. The substrates of mTOR are the binding protein 4E-BP1 and the ribosomal kinase S6 (S6K) that modulate the CAP dependent translation and the translation of mRNAs with oligopyrimidine motives; PKB/AKT indirectly activates mTOR by inhibiting TSC2.
We investigate the participation of mTOR in the NGF modulation of cysteine/cysteine transport in the CNS.

Four week old male BALB/c mice were injected intraperitoneally with 6 mmol kg−1 of body weight of L-buthioninesulfoximine (BSO) dissolved in saline solution. Mice were sacrificed by cervical dislocation on 30 min and 2h following BSO administration. As a control of the modulation of mTOR and S6K, mice were subjected to 24h fast and re-feed (24h+1h). The phosphorylation and expression of mTOR, S6k and LAT1, xCT and EAAC1 transporters were determined by immunoblotting. Non-significant up-regulation of S6K phosphorylation was seen in the liver and olfactory bulb of the mice injected with BSO, while in the hippocampus, a down-regulation tendency of S6K phosphorylation was noted. The marginal p-values obtained in these preliminary experiments suggest more samples are needed to augment statistical significance and reach more conclusive results. The results indicate that the BSO effects appear to be tissue dependent.

Genotoxicity of Dialkilophosphates in Lymphocytes and Human Hepatic Cells.

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The most commonly used pesticides are organophosphates (OPs). OPs are phosphoric acid esters or thiophosphoric acid and are highly toxic because they inhibit the activity of acetylcholinesterase. Human exposure to OPs increases the frequency of sister chromatid exchange, chromosomal aberrations and micronuclei in peripheral blood mononuclear cells (PBMC). Previous studies demonstrate the genotoxic effect of two of the six major organophosphate metabolites; the diethythiophosphate (DETP) and diethyldithiophosphate (DEDTP) using the comet assay in liver cell lines, in conjunction with cytochrome P450 dependent biotransformation. Therefore we are interested in determining the genotoxic effect of other OPs metabolites; The diethylphosphate (DEP) and dimetiltiofosfato (DMTP), tertiary products of biotransformation metabolites of OPs.

WRL-68 cell lines (transformed embryonic liver), HepG2 (transformed adult liver), HeLa (cervical carcinoma) and PBMCwere treated with different concentrations of DEP and DMTP. The viability was determined by the MTT assay and the genotoxicity of these compounds was evaluated using the comet assay ay concentrationsthat did not reduce cell viability in more tan 20%.

Effects of temephos on DNA damage in human liver carcinoma (HepG2) cells

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Temephos is an organophosphate pesticide widely used in health campaigns and urban spraying. Even though the widespread use of this pesticide, few studies in the literature have examined it´s genotoxic potential at low concentrations. The aim of this study was to investigate whether temephos can cause mitogenic or genotoxic effects in human hepatoma HepG2 cells, which retained activities of xenobiotic-metabolizing enzymes, which make them a suitable model for the study of bioactivation processes. To evaluate the citotoxicity, the MTT colorimetric assay was used. Citostatic and genotoxic effects were determinated by the comet assay in it's alkaline version and the cytokinesis-block micronucleus assay (CBMN). The viability of HepG2 cells exposed to 1-10 µM of temephos for 72 hr was not affected (> 75%), therefore, these concentrations were used to evaluate the genotoxic effects. Furthermore not cytostatic effect was observed under these experimental conditions. With respect to genotoxicity, measured by comet assay, the treatment with 10 µM of temephos increased the parameters of tail length, tail moment and percentage of DNA, compared to control. In addition, temephos at 10 µM, did not increased the nuclear buds, nucleoplasmic bridges or apoptotic/necrotic cells, but increased the number of micronucleus. Our results did not agree with that obtained by our group in lymphocytes, which suggest that temephos biotransformation might generate genotoxic metabolites in cell HepG2. This study was supported by CONACyT-C. Básica, Mexico (#156673).

Evaluation of glomerular filtrationrate, cystatin-C, β-2-microglobulin, KIM-1 and clusterinonadultpopulationenvironmentallyexposed to fluoride

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Abstract

In many regions, the population is environment tally exposed to highfluoride (F^-) concentration in drinking water. Experimental studies had been show renal toxic effects induced by F^-exposure.However, the epidemiological information is limited, because the adequate assessment of renal function required the use of kidney injury biomarkers more sensible and specific than commonly used (glomerular filtration nrate, eGFR). Theaim of this study was to evaluate the association between the F^-exposure in drinking water and the levels of new and traditional kidney injury biomarkers. A cross-sectional study was conducted in 239 adults (18-77 years of age) who were residents of three municipalities in the state of Chihuahua, México. The study design was approved by the Institutional Review Boards of Cinvestav-IPN. We assessed in urine four novel kidney injury biomarkers: cystatin- C, β-2-microglobulin, KIM-1 and clusterin by a microsphere immunoassay (Lumin extechnology),

Thee GFR was estimate dusing the serum creatinine concentration. Concentration of F^- in water and urine were measured by potentiometry, using a F^- ion-selective electrode. Our results showed a positive correlation between water and urine F^- concentrations(r=0.5904, p<0.001)with a concentrations intervals of 0.07-4.28 mg/L and 0.34-22.7 µg/mL respectively, suggesting that water was the principal source of exposure to the contaminant in the population. In the simple linear regressions, the urinary F^- concentrations were positive and significantly associated with the urinary excretion of cystatin-C (β=22.29, p<0.001), β-2-microglobulin (β=21.99, p<0.001), KIM-1 (β=0.05, p=0.001) and clusterin (β=0.07, p=0.003). While the association with GFR was not significant(β=-0.081, p=0.785). This results indicate that early kidney injury biomarkers are more sensitive than the commonly used (eGFR) to assess the kidney functions and show the possible relation ship between the chronic exposure to F^- in drinking water and Kidney injury. Never the less is necessary major statistical evaluation, considering confounding variables, to establish this association. Acknowledgment: this research was supported by Conacyt (grant SSA/IMSS/ISSSTE 180847).

Kinetics of toxicity of aluminum 3^+ and free radicals in evaluating (ATCC ® CCL-81 ™) Vero cells

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Usually aluminum is considered as a harmless material for its low reactivity, however, there is evidence that chronic exposure is related to the development of neurodegenerative diseases such as Alzheimer's and acts as neurotoxin inhibiting nearly 200 important biological functions in plants and animals and generates membrane lipids and cholesterol peroxidation.

Aluminum was associated with Alzheimer's disease primarily in patients with renal failure with symptoms of dementia, and schizophrenic states and loss of mobility; further bone degeneration known as osteomalacia (bone disease) occurs, this because the aluminum raises levels of parathyroid hormone, which is involved in calcium metabolism, which added to the interference that has this metal in the gastrointestinal absorption of fluoride, calcium and iron, is reflected in bone formation. Is removed from the blood via the kidneys.

Because the kidney is an organ widely exposed to this contaminant, it is necessary to conduct toxicity studies of aluminum in renal epithelium to determine whether the damage is done by generating free radicals. The objective was to establish the LC_{50} of soluble aluminum in cultured Vero (ATCC ® CCL-81 ™) cell line and evaluate the free radical generation by means of Tbars.

To evaluate the kinetics cell viability in cultures exposed to varying concentrations of aluminum (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156 and 0.078 mM) for 10 h, by evaluating this property MTT test; considering the inflection point in the curve obtained, the optimal exposure time for the quantification of MDA by the TBARS test was established.

The results show that the amount of MDA produced is related to the aluminum concentration, so it is suggested that one mechanism of toxicity is oxidative stress involved.

Toxicological evaluation of ethanolic extract of *Justicia spicigera* in 30 days-treated Wistar rats

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In Mexico, there are about 6.4 million Mexicans diagnosed with Type 2 Diabetes Mellitus (T2DM). Oral hypoglycemic agents are the basis of pharmacotherapy for T2DM control, but they represent a high economic cost to the patient. This influences the patient to seek alternative therapies such as the use of medicinal plants. *Justice spicigera* (JS) is widely distributed in the Huasteca Potosina and there is scientific evidence demonstrating its hypoglycemic activity in both *in vitro* and *in vivo* models. In the present work, we evaluated the toxicity of ethanolic extract of JS (EEJS) in Wistar rats treated for 30 days. The EEJS was obtained by maceration method. For toxicological evaluation, animals (n = 6) were treated daily with single oral doses of 10, 25, 50 and 100 mg/kg of EEJS for 30 days. After treatment, the body weight, food consumption, hematological parameters (red blood cells, hemoglobin, hematocrit, and eritroctic indexes), blood glucose levels, neurological changes and behavioral abnormalities were evaluated. No significant differences between groups on body weight, food consumption, hematological parameters and blood glucose concentration were observed after 30 days of treatment. 100 mg/kg EEJS-treated group exhibited symptoms of irritability and agressivity. Our results regarding the toxicological effects of EEJS evaluated *in vivo* suggest that JS is no toxic after a subacute exposure. However, a chronic toxicity study should be carried out in order to validate EEJS safety on long term use.

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Evaluation of theEffect ofFreeze Dried*Thalassiatestudinum* in the cell ProliferationandViability ofHuman Tumor Cell Lines

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Abstract

Free radicals(RLs) have a dualrole in the bodytoexertbeneficialand harmful effectsdepending on its concentration; at low concentrationsare involved in cellsignaling, whilehigh concentrationsgenerateoxidative stress. In previous studiesit was shown thatantioxidantswithphenolic structuresinduce deathofcancer cellsto a greater degreethan in normal cells. Inthis regardit has been demonstratedthatthealgae*Thalassia testudinum*has polyphenolicstructuresthat provideantioxidant properties, suggesting that it couldhaveregulatory effects on viability cell. Therefore we evaluatedif thelyophilized of*T.testudinum*reduces viability oftransformedkeratinocytes (HaCaT), HepG2, HeLa, SW-480 and MCF-7 tumor cells lines.

The lyophilizedof*T.testudinum*reduced the viabilityofHaCaT cellsby 70%when was administered as asuspensioninwater after 24 and 48 h. Then, the lyophilizedwas fractionatedin 7solutions andthey weretestedintransformed cell linesMCF-7, HaCaT, HepG2andSW-480. FractionsAandBshowed a cytotoxic effectonsometumor cell lines, but this was lower than inHaCaT cellswithsuspension.The indexofapoptosisandnecrosiswas evaluatedinHaCaTcells, the most sensitive cells, andthe results show that*T.testudinum* induces apoptosis, this results isconsistent with theviability by MTT.CONACyTgrant 281226.

Evaluation of methyl parathion to determine LC_{50} bullfrog tadpole *Lithobates catesbeianus* (Shaw, 1802).

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Methyl parathion (MP) is a systemic insecticide and acaricide that is produced worldwide and has registered its use in many crops. It is mainly distributed in the air and soil; although it is less persistent in the environment compared to other pesticides, it does pose a risk to the deterioration of ecosystems.

Amphibian populations have been decreased worldwide by several factors, among which is the use pesticides, which are in particular one of the causes of pollution of aquatic environments, affecting the development of these organisms mainly in their larval stage. The effects of organophosphorus compounds such as MP manifested in abnormalities and low rates of development of embryos and tadpoles, leading to the decrease in the number of mature individuals capable of reproduction. In Mexico City currently uses the MP in agricultural crops; the application of this pesticide occurs during the breeding of some species of amphibians; which threatens the survival and development of these species.

The aim of this study was to determine the toxic effects of organophosphorus compound MP by determining the median lethal concentration (LC_{50}) in tadpoles of bullfrog *Lithobates catesbeianus* (Shaw, 1802).

To determine LC_{50}, 6 concentrations were tested: (0.0037, 0.00296, 0.00222, 0.00148, 0.00074, 0.00037 ppm) to 10 tadpoles by exposure for 24 hours in a static system without renewal; with light-dark cycles of 12 h. The literature has not been reported until today a related data; the nearest source consulted is for adult bullfrog: the 96 has 3.7 ppm; moreover the WHO published in 2003, the guide to the quality of drinking water in which states that the LC_{50} of MP is 0.2 ng / kg in mice. LC_{50} for 24 hours was determined 0.0018 ppm for bullfrog tadpole.

Study of the metabolism effects of arsenicby expression of arsenic (+3 oxidation state)-methyltransferase in human bladder transfected cells (HTB-1) on cell cycle regulation

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Arsenic is an ubiquitous element; epidemiological studies indicate a relationship between arsenic exposure by drinking water and increase in the incidence of bladder cancer. Inorganic arsenic can cause DNA damage and genomic instability having an effect on the development of the cell cycle. Metabolism of arsenic is catalyzed by arsenic (+3 oxidation state)-methyltransferaseexpressed in rat liver and human hepatocytes. Biomethylation is the major pathway for the metabolic conversion of inorganic arsenic into methylated products mono- and dimethylated species that are more reactive and toxic than the parent compound. Several studies haveshown that exposure to arsenic and bladder cancer development has been accidental exposure. Amodel oftransfection*in vitro* can help assess the effects of arsenic and its metabolites in the cell cycle in human bladder cells transfected. This study reports a transfection method for heterologous expression of rat As3mt in human bladder cell line by a synthetic plasmid, to test the effects of the methylated species by exposure of sodium arsenite. HTB-1 cell line is characterized by a mutant p53 a tumor suppressor which is a regulator of the G1/S transition on cell cycle in response to DNA damage. Gene alteration in p53, expression of rAs3mt and exposure to arsenic in human bladder transfected cells (HTB-1) could cause altered DNA repair, proliferation or apoptosis which may encourage carcinogenesis in human bladder cells. Manipulation of expression of this gene is an effective tool to examine the role of metabolism of inorganic arsenic in effects on key cellular processes. Inorganic arsenic and mono- and dimethylated species were quantified by hydride generationcryotrappingatomic absorption spectrometry to determine a time-action curve by exposure of 10µM of sodium arsenite during 4, 8, 12, 16, 20 and 24 hours, assessed the concentration-response [0, 1, 5 and 10 µM] of sodium arsenite and identify checkpoints cell cycle affected by the effect of arsenic and its metabolites in human bladder cells transfected.

Differential expression of drugs transporters associated with chemoresistance in childhood rhabdomyosarcoma: Comparison between tumoral and normal tissue.

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INTRODUCTION: Rhabdomyosarcoma (RMS) is the most common sarcoma of soft tissues in childhood. Although chemotherapy has resulted in an improvement in survival and quality of life of patients with RMS, most eventually developed progressive disease, metastasis and multiple drug resistance (MDR), after they initially respond to treatment. MDR is a phenomenon whereby tumor cells acquire resistance to a broad range of structurally and functionally diverse chemotherapeutic drugs. Most attention has been directed to the role played by membrane transporter proteins belonging to the ATP binding cassette family in MDR, particularly by the MDR1 Pgp drug transporter and another efflux pump as MRP. The role of membrane transporter genes has been reported to be effective in causing MDR phenotype in *in-vitro* conditions but the results on patients have been controversial. Besides, it has been shown that these transporters are markers of poor prognosis in childhood cancers and neuroblastomas, Wilms tumor, and Ewing sarcoma. Little is known about the expression in rhabdomyosarcoma of these transporters. In this study we compare MDR1, MRP1 and MRP2 genes expression in paired tumor and normal tissue of child patients with RMS. **METHODS:** We analyzed the expression level of MDR1, MRP1 and MRP2 genes by using RT-qPCR in 11 pairs of nontumoral and tumoral tissues of RMS patients. **RESULTS:** The expression of the transporter genes was normalized to the expression of the endogenous gene ß-actin. Differences were found when comparing the mRNA concentration of the transporters between neoplasic tissue and normal tissue of patients with RMS. The relative expression analysis showed that the concentrations of the genes MDR1, MRP1 and MRP2 are 7.62, 13.14 and
15.96-fold increased in tumor tissue than in normal tissue. **CONCLUSION**: The overexpression of MDR1, MRP1 and MRP2 genes in tumor tissue of RMS, possibly represents the main limiting factor in chemotherapy and is an important cause of treatment failure, especially in cases of advanced and relapsed RMS.

Effect of the biotransformation of arsenic on cell cycle regulation in human bladder cells

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The inorganic arsenic (iAs) is an ubiquitous element in the environment. This is considered an environmental pollutant. People around the world can be exposed to iAs by various means; however the main route of exposure is from ingestion through drinking water. Once that iAs enters to the body, it can be biotransformed by the liver, resulting in methylation of trivalent arsenic. This reaction is catalyzed by the enzyme Arsenic-III-methyltransferase (AS3MT), which uses the S-adenosylmethionine (SAM) as a methyl donor group. In recent years, information on the effects of trivalent methylated arsenicals [monomethyl arsenic (MMA^{III}) and dimethyl arsenic (DMA^{III})] has been reported to be much more toxic than iAs^{III}. The toxic effects of exposure to iAs^{III} are different, depending on the route and time of exposure; however exposure to this element and its compounds is considered a public health problem worldwide due to its potential for carcinogenesis in different organs in the human. Several possible mechanisms of carcinogenesis had been described; however, little is known about the role of iAs^{III} and its metabolites on cell cycle regulation. Epidemiological studies have reported that bladder cancer is one of the most prevalent diseases associated with chronic exposure to iAs^{III}. However there are very few studies on bladder cells and these only described the effects due to exposure iAs^{III} because of the cell lines do not have the ability to biotransform arsenic. Some studies have reported that exposure to iAs^{III} alters p53 levels; also, there are few studies which have determined the levels of p21 and the role of CDK-cyclin complexes in G1/S phase of the cell cycle. Therefore the objective of this study is to transfect the AS3MT enzyme in a bladder cell line (HT-B2), to assess the effects of iAs and its metabolites (MMA and DMA) on the cell cycle, with reference to expression levels of genes involved on cell cycle regulation such as p53, p21 and CDKs - cyclin D. These results will provide information to propose a possible mechanism of metabolites toxicity on a cell line of bladder and alterations on the cell cycle regulation.

Bergamottin as an inhibitor of CYP1A during embryonic development in the chicken

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Cytochromes P450 (CYP) comprises a superfamily of protein monoxygenases that play a critical role in xenobiotic metabolism and are present in several species from bacteria to humans. CYP1A1 is a member of the CYP1A subfamily which is involved in the bioactivation of polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene, and in the metabolism of some endogenous substrates. CYP1A1 metabolizes PAH into reactive intermediates that can form DNA adducts, leading to the induction of mutagenesis and carcinogenesis. Recently, several compounds isolated from natural sources that inhibit CYP1A1 activity have been reported. Bergamottin (BG), a furanocoumarin component of grapefruit juice, is a competitive inhibitor of rat CYP1A1 and is able to suppress the genotoxicity of benzo[a]pyrene in mice. As in mammals, we can found two members of the CYP1A subfamily in the chicken: *CYP1A4* and *CYP1A5*, which are orthologous to the mammalian *CYP1A1* and *CYP1A2*, respectively. It has been reported that chicken CYP1As are constitutively expressed during embryonic development in the liver and kidney. Besides the chicken, CYP1A is constitutively expressed in different stages of zebra fish development and also during mouse embryonic development. This suggests that the CYP1A subfamily are not only associated with detoxification, but may also play a role in normal development through the metabolism of endogenous susbtrates such as estrogens, bilirubin, melatonin or arachidonic acid. The aim of the present study was to evaluate the effects of CYP1A4 and CYP1A5 inhibition by BG on chicken embryonic development. The first step to reach our objective was to characterize the type of inhibition caused by BG on chicken CYP1A. Liver microsomes from adult chicken showed a Km= 3.13 µM and a Vmax= 14.65 pmol/mg protein/min when etoxyresorufin was used as substrate. BG showed a typical competitive inhibition kinetic plot with a Ki= 159.9 nM that is tenfold higher compared with that reported for rat CYP1A supersome®. This suggests that rat CYP1A is more sensitive to BG inhibition. Finally, preliminary experiments shown that BG may interfere with normal chicken hearth development.

Gestational exposure to inorganic arsenic (iAs) modulates cysteine transport in mouse brain

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Abstract

Human chronic exposure to inorganic arsenic ($_{i}$As) is associated with different types of cancer and neurotoxicity. Alterations in memory and attention processes have been observed in children environment exposed to different levels of $_{i}$As, whereas adults acutely exposed to high levels of $_{i}$As presented encephalopathies and impairments in learning, memory, and concentration. It is possible that exposure can start in gestational life, affecting neurologic development. In the presence of cellular reductants such as thioredoxin (Txn1) or glutathione (GSH), $_{i}$As is methylated in different tissues including brain. Methylated As forms have been found in maternal or cord plasma, suggesting that $_{i}$As crosses the placenta. The biomethylation of $_{i}$As in the central nervous system (CNS) is a significant process because it yields intermediate and final products that are more reactive and toxic than the parent compound. This process also requires S-adenosyl-L-methionine, Txn1 and GSH, which are important intermediate metabolites in the biochemistry of CNS neurotransmission and antioxidant responses to oxidative stress. GSH synthesis in the brain requires cysteine (L-cys), which enters through the xCT (as cystine) or EAAC1 transporters. Glutamate N-methyl-D-aspartate receptor (NMDAR) is a redox-sensitive molecule that plays an important role in brain development and plasticity as well as in spatial learning and memory. NMDAR is a heteromeric complex consisting of subunit 1 (NR1) and 2 (NR2 A, B, C and D). Decreased protein expression of NR2A in hippocampus was associated with $_{i}$As exposure in adult rats. In a mouse model, we investigated the effects of gestational exposure to $_{i}$As in drinking water us- ing biomarkers of oxidative stress and protein expression in cerebral cortex and hippocampus. At the same time, spatial memory behavioral tasks were evaluated. CD1 female and male mice were exposed to 20 mg/L of sodium arsenite in drinking water one month before mating. Females continued to be exposed during the gestational period until the end of lactation (day 15-16 after birth). Pups were killed on days 1, 15th and 90th. After killing, the brain (day1) or cortex and hippocampus were obtained. Protein expression was evaluated by western blotting. Behavioral tasks were performed in the 90 days old progeny; using a novelty-preference paradigm. We did not observe significant alterations in weight and litter size in treated animals. Water consumption was significantly lower in females during lactation only ($p<0.05$). Oxidized GSH (GSSG) was significantly elevated at day 1 in the brain of mice exposed during gestation, accompanied with increased expression of xCT. At day 15th, xCT and EAAC1 expression were also significantly up regulated in both exposed male and female cortex and hippocampus. At the same time, the expression of NMDAR NR2B subunit were significantly down regulated in exposed male and female hippocampus and male cortex. These changes correlated with alterations of the behavior in adult mice, which presented a decreased recognition index after gestational iAs exposure ($p<0.05$). These results suggest that gestational exposure is associated with increased oxidative stress at birth and with increased expression of cystine/cysteine transporters in cortex and hippocampus in both males and females. We also observed an alteration in NMDAR structure and an impairment in the spacial memory mainly in adult males.

Keywords: Arsenic, gestational exposure, neurotoxicity, EAAC1, X_c^- transporter, NMDA and GSH.

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Study of toxic effects of biotin pharmacological concentrations

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Background.Biotin is a water-soluble vitamin that acts as a covalent bound coenzyme of carboxylases. Unrelated to this role, biotin at pharmacological concentrations, 30-650 times greater than its requirement of 30 micrograms per day, modifies gene expression.Several studies in humans and animal models have found that at pharmacological concentrations biotin has favorable effects on triglycerides and glucose homeostasis. These effects indicate that biotin could be used for the treatment of dyslipidemia, glucose intolerance and diabetes. However, no studies have determined whether biotin has toxic effects at those concentrations that produce their beneficial effects on glucose and lipids homeostasis.

Objetive.To analyze various markers of toxicity in an animal model where it has been found favorable effects of biotin on triglycerides and glucose homeostasis.

Experimental procedures.Three weeks old BALB/cAnN strain male mice were divided into 2 groups of 10 mice each, one of which received a control diet containing biotin concentrations to meet daily requirements (1.76 mg of biotin/kg feed) and the other group received a supplemented diet with 56 times this amount of biotin. (23). After 8 weeks of ad libitum feeding, the mice were fasted overnight, andserum and liver samples were obtained. The enzymatic activity of aspartate aminotransferase (AST); alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum, and the activity of superoxide dismutase (SOD) in the liver were determined. Data are presented as the average (±.). Differences between groups were analyzed by student- T test using the GraphPad Prism program 6 taking $P \leq 0.05$ as significant.

Results.The data found no significant differences ($p < 0.05$) in serum activity of ALP, and ALT between the groups. A significant increase in serum AST activity were found in the biotin supplemented group (Control: 114.48 ± 24.88U/L vs supplemented 204.4 ± 42.71U/L). However, the values obtained were in the normal range, as well as the ratio ALT/AST. Taken together, these results indicate no hepatic detrimental effects. Finally, SOD activity in the liver showed no significant differences between groupscontrol 37.3±1.2; supplemented 39.2±0.8.

Conclusions.There is no detrimental effect of biotin supplementation on enzymes that are indicative of liver damage or on SOD activity in an animal model where it has been found favorable effects on triglycerides and glucose homeostasis.

Human and rat CYP1A1 enzyme inhibition by grapefruit juice compounds

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Cytochrome P450 (CYP) enzymes constitute a family of proteins involved in the metabolism of a large number of compounds. Bioactive molecules present in grapefruit juice are known to increase the bioavailability of certain medications by acting as potent CYP inhibitors.

Based on their inhibitory properties, we studied the effects of two naturally occurring constituents contained within grapefruit juice, naringenin and 6', 7'-dihydroxybergamottin (DHB), on CYP1A1 activity by the analysis of their inhibitory kinetics and computational simulations.

Kinetic analysis revealed that DHB competitively inhibited ethoxyresorufin *O*-deethylase activity of human and rat CYP1A1, whereas naringenin caused both competitive and mixed type inhibition, respectively.

In the rat enzyme, the Ki value for naringenin (Ki = 409.20 ± 97.50 µM) is higher than the Ki value for DHB (Ki= 57.79 ± 5.10 µM). In the same way, in human CYP1A1, the Ki values are different for DHB and naringenin (1.38 ± 0.26 µM and 0.18 ± 0.04 µM, respectively). This indicates that there is an interspecies difference between human and rat CYP1A1 inhibition involving different inhibitory mechanism for these molecules.

The results of computational docking showed that inhibitors interact mainly with polar and hydrophobic residues, and that they might block the oxidation of 7-ethoxyresorufin by binding to the CYP1A1 active site. In human CYP1A1, the Ser122, Phe123, Asn222, Phe224, Phe258, Gly316, Ala317, Phe319, Asp320, Ile386 and Leu496 residues participated almost in all of the inhibitory conformations, suggesting that these residues are important for the regioselectivity and positioning of the inhibitor. The same was observed for the Ile119, Thr126, Phe127, Phe228, Phe255, Gly320, Ala321, Thr389, Ile390 and Leu500 residues of rat CYP1A1 isoform. Furthermore, a second energetically favored binding mode was found for naringenin, in which the molecule interacts with Ser448, Glu449, Val451, Ile452, Leu453, Phe454, Gly455, Leu456, Gly457, Lys460 and Arg468.

In conclusion, this study describes the application of *in vitro* and *in silico* tools to study the inhibitory profiles of dietary compounds such as naringenin and DHB, aiming to investigate the ability of these compounds to interact with CYP1A1.

The CYP1 family has been shown to metabolize a wide variety of carcinogenic xenobiotics, thus, it might play a role in the carcinogenic initiation step. Therefore, that availability of selective inhibitors of CYP1A1 will facilitate the study of the metabolic role of this enzyme in cancer and toxicity, and potentially could lead to the development of CYP1A1-targeted cancer therapeutic and preventative agents.

Effect of Metformin on the metabolic regulation of carbohydrates and lipids in Wistar rats chronically administered with Cd

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Cadmium (Cd) is a non-essential element that is toxic to animals and humans. This metal is found at low levels in the environment, however the exposition to this metal either in the work place or through the ingestion of Cd-contaminated foods or water leads to the development of several alterations. Previous works have shown that intoxication with Cd produces a similar pathology to metabolic syndrome (SM). SM is currently treated with metformin, this drug have as pharmacological effect a decrease in hepatic glucose production, and promotes the bioavailability and entry of glucose to peripheral organs such as muscle and adipose tissue. Also metformin has shown that regulates lipogenesis, improves the triglycerides transport and storing. However there is little information about the effect of metformin during the SM produced by intoxication with Cd. Therefore in this work was studied the effect of metformin on the metabolism of carbohydrate and lipids in plasma, muscle, kidney, adipose tissue, heart and liver, using as experimental model, a group rats with a SM phenotype induced with Cd administration. Animals were exposed to Cd for 3 months, and later were divided in two groups. One group was administered metformin during two months, and the other was given vehicle. Other group of rats without any treatment was used as control group. To each animal was determined glucose tolerance and were measured the levels of triglycerides, total lipids and lipoprotein fractions. The concentration of triglycerides in the tissues was assayed with the aid of commercial kits, and the level of glycogen by the method described by Bennett and Keirs. Animals treated with metformin exhibited a significant improvement in lipid regulation and the level of plasma glucose, when were compared with the group of animals only exposed to Cd, however, this metabolic regulation was only showed by liver. Muscle, heart, kidney and adipose tissue showed an increase in the amount of glycogen and triglycerides stored. Results obtained in this work suggest that metformin favor the redistribution and storing of lipids and carbohydrates in peripheral tissues inducing steatosis and secondarily a loss in its functionality.

Modulation of the GSH and Trx systems by arsenic in the mouse brain.

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INTRODUCTION. Inorganic arsenic (iAs) contaminates well water in several countries, including Mexico. Toxic effects of this metalloid include not only carcinogenic but also neurochemical, memory and learning alterations. iAs is metabolized in many tissues including different brain regions which also show a distinctive accumulation. iAs metabolism generates reactive species that induce oxidative stress. Glutathione (GSH) and thioredoxin (Trx) are two central nervous System (CNS) antioxidant systems that participate in the metabolism of As. The CNS is vulnerable to oxidative stress due to their high oxygen consumption and lipid composition. There is evidence that demonstrate that GSH and Trx systems are modulated by exposure to iAs. Nrf2 is a transcription factor that is activated by oxidative stress and modulates the expression of antioxidant enzymes and proteins such as γ–glutamyl cysteine synthetase (γ-GCS) and thioredoxin reductase 1 (TrxR1) that participate in the GSH and Trx systems. Due to the differential accumulation of iAs in the CNS, it is important to document the effect of iAs in these antioxidant systems and the regulation of Nrf2, in different brain regions. **APPROACH.** We investigate the expression of Nrf2, γ-GCS and TrxR1 in several brain regions of mice exposed to As. **EXPERIMENTAL DESIGN**. CD-1 male mice (28-30g) were treated with destilled water, 2.5, and 5.0 mg/kg of $NaAsO_2$ orally and sacrificed 24 h after treatment. Immunohistochemistry, immunofluorescence and western blot techniques in sagittal cuts were used to compare the expression and cell localization of γ-GCS, TrxR1 and the transcription factor Nrf-2. **RESULTS.** Preliminary results show that iAs exposure induced changes in Nrf2, γ-GCS and TrxR1 expression in the cerebellum, striatum and hippocampus. We could also document that the basal expression of these three proteins was different in CNS regions, showing distinctive patterns in the different cell types. **CONCLUSION.** There is a distinctive modulation of γ-GCS, TrxR1 and Nrf2 expression by $NaAsO_2$ exposure in different regions of the mouse CNS**. BIBLIOGRAPHY**. 1) Rodriguez et al. 2003. Toxicol Lett. 145: 1-18. 2) Thomas D. 2007.Toxicol Appl Pharmacol. 222: 365-373. 3) Limón et al. 2007. Free Radic Biol Med 43: 1335 to 1347. 4) Pena et al. 2010. Environ Res 110: 428 - 434.

VENOM OF THE CENTIPEDE *Scolopendra polymorpha* CAUSES MUSCULAR AND NERVOUS TISSUE ALTERATIONS IN MOUSE

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Key words: centipede, venom, tissue damage

Scolopendra polymorpha (Fig. 1) is a poisonous arthropod widely distributed in Mexico and the US; its bite produces burning pain, paresthesia and edema, among other symptoms (1). Venom of centipedes (genus *Scolopendra)* has been used in traditional medicine in countries such as China, Korea, India and Mexico to treat arthritis, epilepsy, headaches and infections (2-4). It has been found that intramuscular injection of S. *polymorpha* venom produces muscular and nervous tissue damage in mouse (5), thus the aim of this investigation is the isolation and characterization of the S. *polymorpha* venom compound(s) responsible for alterations in muscular and nervous tissue, by means of venom fractioning. Whole venom was obtained by mechanical stimulation of the centipede forcipules. Then it was lyophilized and quantified by the Lowry method. Skeletal muscle samples were incubated with whole venom or fractions obtained by anionic exchange chromatography to identify the fractions causing tissue damage (Fig. 2). Alteration was assessed by H&E and enzymatic staining. SDS-PAGE (12%) was used to separate the active fractions. The purified compounds were further purified to be administered in mice and to be sequenced. This project is sponsored by the National Council for Science and Technology (CONACyT), through grant No. 267392.

Fig.1 *Scolopendra polymorpha* morphology. A: antennae; F: forcipules; CP: cephalic plate; TE: tergites; 5-20: number of walking legs; TL: terminal/sensitive leos.

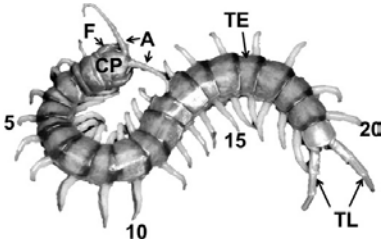
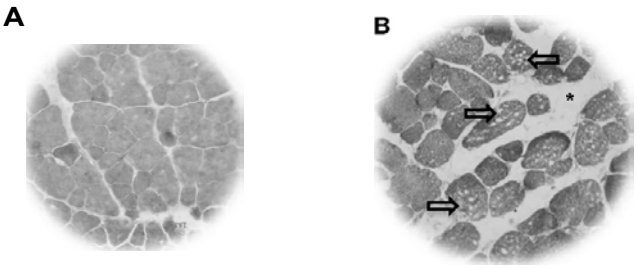


Fig. 2. Mice skeletal muscle. A: control, incubated with distilled water. B: incubated for 15 min with whole venom. Transversal slices. H&E stain. Apparent vacuolization of muscular fibers and areater soace between fibers(*) is shown in *B.*



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Evaluation of plasmatic concentrations of natriuretic peptides in children environmentally exposed to arsenic. José M. Torres Arellano[1], Guadalupe Aguilar Madrid[2], Laura Arreola-Mendoza[3], Angel Barrera Hernandez[1], Luz C. Sánchez Peña[1], Citlalli Osorio Yáñez[1], Luz M. Del Razo[1].

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Inorganic arsenic (iAs) exposure is an important public health issue worldwide, due to in several regions the drinking water exceed the international maximum permissible limit of 10 µg/l. The chronic exposure to iAs has been associated with cardiovascular disease. Recently, we have been reported increased blood pressure, increased left ventricular mass and decreased systolic function in children exposed to this metalloid. In other studies, these alterations have been associated with increased plasma concentrations of natriuretic peptides (NP) B-type (BNP), the amino terminal fragment (NT-proBNP) and atrial (ANP).

The aim of this study was to evaluate plasma concentrations of natriuretic peptides in 172 children with arsenic endemic area in Zimapán, Hidalgo. The study design was approved by the Institutional Review Boards of Cinvestav-IPN. The total concentration of arsenic species in urine (U-tAs) determined by HG-CT-AAS ranged of 5.7-370 µg/ml. In total, 79% of children had U-tAs values higher than the Biological Exposure Index of 35 µg/ml, this index is used as references in occupationally exposed population. In addition, the lifetime iAs exposure (LAsE) was calculated multiplying As concentration in drinking water by the duration of water consumption in years. The plasmatic levels of BNP, NT-proBNP and ANP, evaluated by ELISA ranged of 1.44-967.76 pg/ml, 16.80-1,404.82 pg/ml and 7.01-284.85 ng/ml, respectively.

The plasma concentration of BNP was positive and significantly associated with both markers of iAs exposure; Simple linear regression showed an increase of 0.343 pgBNP/ml per µg/ml of U-tAs ($p = 0.05$) while for LAsE an increase of 0.123 pgBNP/ml for each µg/l-year (<0.001). The association between NT-proBNP and LAsE also was positive and significative ($\beta = 0.09$, $p = 0.02$), whereas the relationship between NT-proBNP and U-tAs was positive but not significant ($p = 0.22$). For ANP, no association was found with exposure to iAs. Nevertheless, major analysis is required to establish the association between plasmatic NP and iAs exposure, considering confounding variables. Furthermore is necessary to establish NP relations with echocardiographic parameters of cardiac structure and function. Acknowledgment: This project was supported by Cinvestav fund. JMTA and COY was supported by Conacyt scholarship.

Systemic nerve growth factor modulates the transcription of amino acid transporters and glutathione synthesis in mouse striatum

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Nerve growth factor (NGF) is a member of structurally related proteins, named neurotrophins (NTs), that regulate neuronal survival, development, function, and plasticity. Moreover, NGF is an important activator of antioxidant mechanisms. These functions of NGF are mediated by the tropomyosin-related kinase receptor A (TrkA). There is evidence that NTs and their receptors are expressed also in visceral tissues. Physical exercise and stress increase levels of NGF in plasma. Using a murine model we have shown that systemic inhibition of GSH synthesis with L-buthionine-S-R-sulfoximine (BSO) increased brain GSH content and induced the transcription of *ngfb* in liver. Murine striatum cholinergic neurons express TrkA receptors thus, we investigated if an i.p. injection of BSO or of sodium arsenite (iAs) modulate the transcription of *ngfb* and *trka* as well TrkA phosphorylation in mouse striatum. Both agents induced the transcription of *ngfb* and *trka* as well TrkA phosphorylation. Also, BSO and iAs induced the activation of the NGF/TrkA pathway which correlated with increased transcription of *xCT, LAT1, EAAC1* which are amino acid transporter genes that provide L-cys/L-cys$_2$ to central nervous system and of *GCLm* which participates in the de novo synthesis of GSH. The inhibition of TrkA phosphorylation by K252a or anti-NGF neutralizing antibody abrogated the transcription of *xCT , LAT1 EAAC1* and *GCLm* induced by BSO or iAs suggesting the participation of this pathway in the *in vivo* antioxidant response at least in striatum. Furthermore, since anti-NGF neutralizing antibodies would not cross the blood-brain barrier, our results suggest that NGF functions as a systemic redox-sensor in both CNS and peripheral tissues and that the NGF/TrkA pathway plays a critical role in the antioxidant response in the striatum in our murine model.

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Cytotoxic effects and antimicrobial activity of Cuachalalate (*Amphipterygium adstringens*) extracts, prepared in a traditional way

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The bark of Cuachalalate is traditionally used for treatments of gastritis or gastrointestinal cancer (Olivera *et al*., 1999); but scientific studies on the cytotoxic properties of these extracts are still lacking. On the another side, the interest in discovering new antimicrobial agents increased, because more and more bacteria become resistant against classical antibiotics. Specially *Staphylococcus aureus*, *Enterococcus faecalis* or *Bacillus cereus* are human opportunistic pathogens (Salwiczek *et al*. 2014), which often give health problems in developing countries with substandard hygiene and unsafe water supplies. For both reasons Cuachalalate is a good candidate to investigate its toxic effects on cancer cell lines like HeLa and to detect antimicrobial activity.

The bark of Cuachalalate was obtained from a local market in Ocotlán (Jalisco, México), dried, ground (electrical grinder) and passed through a 30 mesh sieve to get a fine powder. 25 g of this powder was extracted by maceration at room temperature with 250 ml of water (briefly heated to 70 °C) or ethanol. After filtration the extracts were concentrated to 4 ml under reduced pressure (60°C for water or 50°C for EtOH). HeLa cell line was grown in DMEM supplemented with 5 % FBS to 70% confluence before different concentrations of the extracts were added for further metabolic analysis. For the Minimal Inhibitory Concentration (MIC) three bacterial strains (*S. aureus*, *E. faecalis*, *B. cereus*) were grown in Müller-Hinton broth for 24 h at 37 °C inoculated with a cell concentration equivalent to 0.5 McFarland standard (OD_{620}) and a series of microdilutions of the plant extracts in 96-well microtiter plates.

HeLa had normal metabolic activity (WST-1) and showed any cell membrane damages (LDH-release) with Cuachalalate Watery Extract (CWE) from 0.005 to 0.515 mg/ml or with Cuachalalate Ethanolic Extract (CEE) from 0.002 to 0.022 mg/ml. At concentrations of 5.15 mg/ml CWE or 0.22 mg/ml CEE necrosis could be detected, which was confirmed by propidium iodide, trypan blue and neutral red staining. The MIC for CWE and CEE are for *S. aureus* 3.25 & 1.37 mg/ml, for *E. faecalis* 1.62 & 0.68 mg/ml and for *B. cereus* 1.62 and 0.17 mg/ml, respectively.

In all cases the ethanolic extract exhibited higher activity than the watery extract; but the antimicrobial activity of these extracts was only achieved at concentrations, which are already toxic for the cell lines. The traditional form to consume the bark of Cuachalalate is a watery infusion or sometimes as alcoholic solution. The popular belief about of the infusion of Cuachalalate could be effective against gastrointestinal cancer continue to be doubtful, because any apoptotic effects were observed.

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Signal integration by P-Rex1, a multidomainRacGEF, linked to its interaction with cAMP-dependent Protein Kinase

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Signal transduction cascades critical for tumor angiogenesis and metastasis are essential for cancer progression. Their constituents are potential targets of drugs designed to treat patients suffering various malignant neoplastic diseases. Previously, we have shown that P-Rex1, a multidomain guanine nucleotide exchange factor for the RhoGTPase Rac1 (RacGEF), participates in the angiogenic signals of chemotactic CXCR4 receptors and interacts with different signal transduction proteins, including the serine/threonine kinases mTOR (mammalian target of rapamycin) and PKA (cAMP-dependent Protein Kinase). These kinases are known to be involved in fundamental cellular processes, such as growth, metabolism and cell migration, suggesting that P-Rex1 plays a role as a signaling scaffold that integrates the actions of mTOR and PKA.

In this work we have characterized novel interactions between PKA subunits and multiple domains of P-Rex1 and identified serine 436 as the main phosphorylation site by PKA. Additionally, we have been characterizing the dynamic interaction between P-Rex1 and proteins that detect the phosphorylation status of this GEF. In particular, we found that 14-3-3γ, a phospho-serine/threonine-dependent adaptor, pointed as a potential regulator of P-Rex1 activity. Ongoing experiments include the characterization of P-Rex1 mutants found in human tumors and their influence on RacGEF activity and its phosphorylation by PKA. In order to pursue a systematic evaluation of the functional effect of these structural modifications, including point mutations on the PKA consensus phosphorylation site, these mutants are being tested as fluorescent constructs constitutively attached to the plasma membrane.

Thus, P-Rex1 integrates angiogenic signals regulated by its interaction with PKA, which phosphorylates this RacGEF at S436, within the domain previously characterized as relevant for its interaction with mTOR. Accordingly, P-Rex1 might constitute a signal integration node potentially relevant in tumor angiogenesis and attractive as potential drug target.

Subchronictreatmentwithferricnitrilotriacetate (FeNTA) induces AP-1 activation and cyclin D1 overexpression: possiblemechanisms of renal carcinogenicity

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Renal cell carcinoma (RCC) representsalmost 90% of cancerarisingfromadultkidney. Thiscancerisasymptomatic, and initial diagnosis generallyoccurs in advancedoreven in metastaticstages. Earlystagesdetectionorstudies are almostimpossible in patients. Ferricnitrilotriacetate (FeNTA) induced RCC is a usefulmodeltoanalyzeearlyeventson RCC developmentforthisgoal. ChronicintraperitonealFeNTAinjectionsgiveriseto RCC in rats, and itsadministrationalong 1 or 2 months leads to renal dysplasia. Oxidative stress isinvolved in FeNTAcarcinogenicity, butthetie molecular mechanismsremainunclear. Cyclin D1 and c-Jun has beenproposedtoparticipate in RCC development, and bothmoleculesrespondtooxidative stress. In thepresentworkwefoundthatlessadvancedFeNTAinducedtumours show high nuclear p-c-Jun levels, comparedto normal tissues, and thisalterationdiminshesordisappear in more advancedtumours, suggestingthat c-Jun mayparticipate in earlydevelopmentalstages of FeNTAinduced RCC. To test thishypothesiswedesignedsubcronicFeNTAschemesduring 1 and 2 month of FeNTAtreatment. SubchorincFeNTAadministrationinduced renal overexpression and phosphorylation of c-Jun, anincrease of AP-1 activity and highlevels of cyclin D1 protein. Thesechangeswere more severe at 2 monthscomparedto 1 monthprotocol. Thesealterationswerenotinduced in liverorlungs. Weconcludedthat AP-1 hyperactivation and cyclin D1 overexpressionmay be relatedtoFeNTAcarcinogenicity.

Study of the role played by the protein p32 (gC1qR) in maintaining the malignant phenotype of colon cancer.

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P32 (gC1qR) is a ubiquitous and highly evolutionarily conserved protein that interacts with multiple cellular and viral proteins; and is localized in cytoplasm, mitochondria and nucleus. Its precise function is unknown, but its association with other proteins p32 has been involved in various biological processes. Data indicate that p32 is overexpressed in cancer cells and contributes to the malignant phenotype; however, its specific role in cancer remains unclear.

In this study, we report that in the colon cancer cell lines RKO and SW480, p32 protein is overexpressed compared to non-cancerous colon cell line 112CoN. To describe p32 function, we blocked its expression by siRNA in the cell lines RKO and SW480; checking knockdown efficiency by cytofluorometry, western blot and RT-PCR.

Our results demonstrated that p32 plays an important rol in the migration capacity, since blocking its expression reduces the ability of migrating cells. Contrary, blocking p32 expression in RKO and SW480 cell lines, has no effect on cancer metabolism and proliferation. Also, microarray analysis indicated that blocking p32 expression modifies the expression of genes associated with cancer.

Also, microarray analysis indicated that blocking p32 expression modifies the expression of genes associated with cancer; for example, HAS2 is down regulated, this is a protein highly related to the malignant phenotype. Even more important, in xenografts experiments there was a significant decrease in tumor growth when the injected cells have blocked p32 expression. This data demonstrate that p32 is directly involved in the formation of tumors.

The phosphoinositide-dependent protein kinase 1 inhibitor , UCN-01, induces fragmentation. Possible role of metalloproteinases. Rocio Alcántara-Hernández, Aurelio Hernández-Méndez and J. Adolfo García-Sáinz.
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Abstract

Phosphoinositide-dependent protein kinase 1 (PDK1) is a key enzyme, master regulator of cellular proliferation and metabolism; it is considered a key target for pharmacological intervention. Using membranes obtained from DDT1-FM2 cells, phospho-PDK1 was identified by Western blotting, as two major protein bands of Mr 58-68 kDa.

Cell incubation with the PDK1 inhibitor, UCN-01, induced a time- and concentration-dependent decrease in the amount of phospho-PDK1 with a concomitant appearance of a 42 kDa phosphorylated fragment. Knocking down diminished the amount of phospho-PDK1 detected in membranes, accompanied by similarly decreased fragment generation. UCN-01-induced fragment generation was also observed in membranes from cells stably expressing a myc-tagged PDK1 construct. Other PDK1 inhibitors were also tested: OSU-03012 induced a clear decrease in phospho-PDK1 and increased the presence of the phosphorylated fragment in membrane preparations; in contrast, GSK2334470 and staurosporine induced only marginal increases in the amount of PDK1 fragment. Galardin and batimastat, two metalloproteinase inhibitors, markedly attenuated inhibitor-induced PDK1 fragment generation. Metalloproteinases 2, 3, and 9 co-immunoprecipitated with myc-PDK1 under baseline conditions and this interaction was stimulated by UCN-01; batimastat also markedly diminished this effect of the PDK1 inhibitor.

Our results indicate that a serie of protein kinase inhibitors, namely UCN-01 and OSU-03012 and to lesser extent GSK2334470 and staurosporine induce PDK1 fragmentation and suggest that metalloproteinases could participate in this effect.

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Function/structure of the human alpha 1d adrenoceptor.

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Alpha 1D adrenoceptors (α_{1D} ARs) mediate many of the actions of adrenaline and noradrenaline and are involved in the regulation of blood pressure. α_{1D} ARs are members of the heterogeneous family of seven transmembrane receptors (7TMRs), which have been shown traditionally to exert their effects through G protein activation. Agonist stimulation of α_{1D} ARs leads to Gq activation and phosphoinositide turnover/calcium signaling. Also, it has been demonstrated that α_1 ARs activate the Raf-MEK-ERK MAP kinase (MAPK) cascade. The carboxyl terminus and the third intracelullar loop appear to play key roles in the regulation of these receptors. To study the possible roles of human α_{1D}-adrenoceptor carboxyl tail, Rat-1 fibroblasts expressing amino terminus-truncated (ΔN) or amino and carboxyl termini-truncated (ΔNΔC) α_{1D}-adrenoceptor deletion mutants were used. Changes in intracellular calcium concentration, extracellular signal-regulated kinase (ERK) phosphorylation and internalization of the receptors were studied.
Cell lines expressing these receptor mutants increase intracellular calcium concentration in response to noradrenaline, an effect that was markedly inhibited by pretreatment with phorbol esters. No difference in the maximal response to noradrenaline was observed but the decay of the response was different, reaching a higher plateau in cells expressing the ΔNΔC mutant. Noradrenaline induced a rapid and transient increase in ERK phosphorylation in cells expressing the ΔN mutant but the signal obtained in cells expressing the ΔNΔC receptor was barely detectable, and in the majority of experiments, absent. Expression of this mutant not only blocked the adrenergic effect but also those induced by lysophosphatidic acid, epidermal growth factor and phorbolmyristate acetate. Moreover, activephorbol esters elicited markedly internalization of the ΔN mutants, while the ΔNΔC receptor remained at the plasma membrane even after 10 min of stimulation with phorbolmyristate acetate.
Our data indicate that the receptor's carboxyl tail plays a role in turning-off the calcium response and suggest that different receptors domains might participate to achieve full desensitization. Furthermore, the carboxyl tail seems to participate in the internalization of the receptor. Expression of the ΔNΔC receptor alters a common downstream step in the mitogen-activated protein (MAP) kinase pathway. The possibility that receptor truncation at the carboxyl terminus could expose binding sites present in the receptor which might result in sequestration of essential components of this pathway is suggested; a function of the receptor’s carboxyl tail might be avoiding exposure of such sites.

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Regulation of the regulatory subunit of Protein Kinase A subcellular localization in *Saccharomyces cerevisiae*

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Protein Kinase A (PKA), also known as cAMP-dependent protein kinase, is a heterotetrameric complex formed by two regulatory and two catalytic subunits, which activity is regulated by intracellular levels of cyclic AMP(cAMP), and it is involved in control of glucose metabolism, protein translation, ribosome biogenesis, stress responses, autophagy and cell lifespan. However,the knowledgeabout up-regulators of PKA, being the TOR pathway one of them, is reduced.In *Saccharomyces cerevisiae,* the regulatory subunits are encoded by the gene *BCY1*, while the catalytic subunits are encoded by three different genes: *TPK1*, *TPK2* and *TPK3*.In our laboratory, we replicated other groupsresults (Tudisca, *et al)* about PKA subcellular localization, focusing on Bcy1p in response to the carbon source and growth phase, using Yellow Fluorescent Protein (YFP) and Green Fluorescent Protein (GFP) fusions as reporters. We confirmed that growing on glucose and during exponential growth phase PKA has mainlya nuclear localization, whereas in a non- fermentable carbon source and during stationary growth phase, the Bcy1p shows redistribution towards the cytoplasmic compartment.

In a second stage of our work, we evaluated the effect of a mutant in a process regulatedby PKA (autophagy) and a mutant of a PKA regulator (TOR pathway)have on Bcy1p subcellular localization. These genes are *CCZ1* and *TCO89,*Ccz1p is a protein taking part in the autophagy machinery and Tco89p is involved in the TOR pathway which activates the PKA preventing the phosphorylation and subsequent activation of Byc1p through Mpk1.

This work allowed the study of regulatory subunit of PKA dynamics in response to different conditions and genetic backgrounds.

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The effects of Hydrogen Sulfide on Protein Kinase A activation in the yeast *Saccharomyces cerevisiae*.

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Recently, the gasotransmitter Hydrogen sulfide (H_2S) has gained relevance as a signaling molecule after being studied only as a poisonous molecule for many years. The H_2S is produced naturally not only in bacteria, but also in eukaryotic cells, however its regulation and targets are not yet understood. Recent studies performed in rats indicated that H_2S mediates cAMP elevation and PKA activation but the molecular mechanism has not yet been described.

PKA is a component of signaling pathways controlling a number of cellular processes. In *Saccharomyces cerevisiae,* PKA is a tetramer composed of two regulatory subunits (encoded by *BCY1* gene) and two catalytic subunits (encoded by either *TPK1* or *TPK2* or *TPK3*). When the cAMP levels are low the PKA complex is formed, when the cAMP levels increase the catalytic subunits are released and can phosphorylate their targets.

The role of H_2S as a signaling molecule has never been studied in yeast. In this work we studied the physiology of H_2S in yeast and explored its effect in PKA activation.

We determined the endogenous sources of H_2S in yeast, as well as the toxic concentrations of H_2S. We measured the PKA complex formation using a DHFR Protein Complementation Assay (PCA) in order to assess the effect of H_2S in the interaction between Bcy1p and Tpk2.

To the best of our knowledge, this is the first report describing the potential of H_2S as a signaling molecule in a unicellular eukaryotic organism.

Akt degradation by direct interaction with Bag5

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The serine threonine kinase AKT/PKB regulates cell survival, metabolism, proliferation and migration. Thus, AKT activity and stability must be tightly regulated to keep balanced these fundamental processes. However, despite their broad impact, the mechanisms that target AKT to degradation during critical starvation remain elusive. Here, we demonstrate that BAG5, a chaperone regulator and a novel AKT-interactor, attenuate AKT stability. This effect is exacerbated by inhibition of Hsp90 and protein synthesis. BAG5 is more effective in promoting AKT degradation in the presence of 17AAG, an anti-cancer agent being assessed in clinical trials, whereas BAG5 knockdown attenuates AKT degradation under these stressful AKT-destabilizing conditions. BAG5 in coordination with Hsp70 and Parkin targets AKT to ubiquitination-dependent degradation. In contrast, BAG5 knockdown attenuates Hsp70-dependent AKT-degradation. AKT and BAG5 are reciprocally regulated, under serum starved conditions BAG5 interacts with AKT, whereas in HGF stimulated cells BAG5 is phosphorylated and dissociates from AKT. Functional assays indicate that BAG5 knockdown increases AKT phosphorylation at Ser473 whereas BAG5 overexpression reduces AKT activation and cell migration. Our model suggests that AKT signaling and stability is attenuated under starvation via BAG5, which targets AKT for degradation, while reduction of BAG5 protein levels increases AKT expression and signaling.

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MOLECULAR FEATURES OF RACK1 FROM THE SYMBIOTIC DINOFLAGELLATE *Symbiodinium microadriaticum*

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We have used the photosynthetic dinoflagellate *Symbiodinium microadriaticum* in culture due to its ease of manipulation under controlled laboratory conditions, as a model for the characterization of signal-transduction components, especially those involving the Receptor for Activated C Kinase (RACK1) and its interactive ligands. The cDNA that encodes RACK1, was PCR-amplified and when fully sequenced from this organism and has been termed SymRACK1. The sequence presented an open reading frame of 942 bp's. The *in silico* translated sequence presented the characteristic seven WD-40 domains that form the β-propeller structure and three conserved PKC-binding sequences. The cDNA encoded a protein of 312 amino acids with a theoretical molecular mass of 34,200 kDa and a pI of 5.9. The identity was highest with phylogenetically related organisms at both, the amino acid and nucleotide levels and was confirmed by phylogenetic tree analysis. We are currently studying the protein accumulation and mRNA expression patterns during the growth and circadian cycles of this microorganism. It will be interesting to study if during these phenomena there are also interactions with the putative SymRACK1 binding partners that we are characterizing using the yeast two-hybrid system (see abstract by Islas Flores and Villanueva Méndez) based on libraries constructed from this dinoflagellate.

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Effect of KPNA2 downregulation on the nuclearlocalization of small GTPase Rac1 in HaCaT cells.

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Rac1 belongs to the Rho family of small GTPases and plays diverse roles in cellular function including formationof lamellipodia, cell proliferation, cell division, migration, and invasion and plays animportant role in tumourigenesis. Recently we shown that Rac1 is overexpressed in cervicalpremalignant lesions and cervical cáncercells, in addition have demonstrated in experiments using non-tumorigenicHaCaT cell culturethat estrogen lead nuclear accumulation and this localization may contribute to cancer progression, but the mechanismby which Rac1 istranslocated into the nucleus has not been published.

Recently has shown a direct interaction between Rac1 and KPNA2 for a KPNA2-dependent nuclear import of Rac1 in HeLa and HEK293 cells. In order to explore the role of KPNA2 in the translocation of Rac1 in HaCaT cells, the present study used RNA interference technology to knock down KPNA2 expression in HaCaT cells and then determine the effects on mRNA and protein levels in response to estrogen.Interestly, the reduction of KPNA2 expression inhibits the nuclear import of Rac1, showen a direct interaction between Rac1 and KPNA2, in addition the Erk inhibition suggest that the activation of Erk regulate nuclear translocation of Rac1.

The global regulator CsrA is required for proper BarA activation and *UvrY* translation.

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In prokaryotic cells, the sensing and processing of environmental signals depends mainly on two-component signal transduction system (TCS). These systems consist of a membrane-bound histidine kinase (HK) and a cytosolic response regulator (RR). The BarA/UvrY TCS of *Escherichia coli* consist of BarA as the HK, and UvrY as its cognate RR. BarA has been shown to autophosphorylate in response to extracellular acetate or other aliphatic carboxylic acids, and transphosphorylate UvrY. UvrY-P, in turn, acts as a transcriptional regulator that directly activates the expression of the noncoding RNAs (sRNAs) CsrB and CsrC. These sRNAs act as antagonists of CsrA, an RNA binding protein with profound effects on many physiological processes, such as central carbon metabolism, virulence, motility and biofilm formation.

Curiously, *csrB* transcription, which directly depends upon UvrY, is not activated in a *csrA* mutant strain, suggesting that CsrA may affect directly or indirectly UvrY expression. Here, we present the results of experiments aiming at elucidating the regulatory interactions of BarA/UvrY and CsrA/CsrB. A simple model for this regulatory circuitry is presented and discussed.

The sustained activation of AT1R prevents the Akt/PKB desensitization in hepatic C-9 cells.

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Ang II is a pleiotropic hormone with effects in the short and long term. In the rat heart, ang II produces a positive inotropic effect (PIE)-dependent activation of receptors for Ang II subtype 1 (AT1R) expressed in the luminal wall of the coronary endothelium[1](LWCE). To show that, joined covalently to the hormone to a base of inert dextran of high molecular weight (> 2,000 kDa) to form a complex called Ang II-POL, and so we confined the hormone into the intravascular space and thus activate exclusively ATRs expressed on LWCE.Intracoronary administration of Ang II in the isolated perfused heart by Langgendorf method produces a transient PIE plus tachyphylaxis.Conversely intracoronary AngII-POL produced a sustained effect also induces no taquifilaxis[2]. Such differences could be explained becauseang II induces desensitization of responses mainly through the internalization phenomenon of AT1R expressed on LWCE while AngII-POL does not.AT1R belongs to the family of G protein coupled receptors (Gq/11), short-term activation leads to the production of DAG and PKC activation, further increase in intracellular Ca^{2+}, hence phosphorylation of tyrosine and serine / threonine protein kinases (e.g. ERK and Akt). In the present study we evaluated whether Ang II-POL triggers intracellular signaling mechanisms in like manner to angII; particularly the activation of two major pathways for cell proliferation and survival: ERK and Akt.Using an endogenous expression model as the C-9 cells (liver rat origin), we show that both Ang II and Ang II-POL induced ERK phosphorylation via AT1R, with a similar pattern. On the other hand AngII induces phosphorylation of Akt transiently; contrary to this AngII-POL does it in sustained manner. This suggests that: 1) Sustained AT1R activation does not impact ERK-phosphorylation desensitization. 2). Sustained AT1R activation has effect on desensitization of Aktphosphorylation and 3) speculatively manner likely the AT1R internalization phenomenon plays a key role in the desensitization only in several intracellular signals induced by AngII but not all.

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BIOCHEMICAL AND MOLECULAR FEATURES OF A HSP90-LIKE PROTEIN FROM *Symbiodinium microadriaticum* THAT UNDERGOES PHOSPHORYLATION CHANGES IN RESPONSE TO LIGHT.

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Symbiodinium is a genus of photosynthetic dinoflagellates that live frequently as endosymbionts inside many marine invertebrates such as anemones, jellyfish and reef-building stony corals. *Symbiodinium* cells however, are also capable to live outside their hosts, and as free-living microorganisms, they can thus be grown in culture. Due to the photosynthetic nature of these organisms, either within their symbiotic relationship, or as free-living *Symbiodinium*, they are susceptible to environmental changes arising from the prevailing light conditions. Thus, *Symbiodinium* must have finely regulated sensing mechanisms to respond to these variations. There is evidence that both partners of these symbioses have a considerable capacity to tolerate a wide variety of stress conditions using protective mechanisms. These include an increased expression of antioxidant enzymes, protective pigments, and the increased expression of heat shock proteins (HSP's). Partial sequencing of peptides revealed that a *Symbiodinium* spp. 75 kDa protein (SymHSP75), whose phosphorylation level changes in response to light, had a high identity with HSP90 family proteins. The complete sequence of this protein was detected in an EST database (Mónica Medina, http://sequoia.ucmerced.edu/SymBioSys/) and *in silico* translation of the full sequence identified a signal peptide typical of chloroplast-targeted proteins. At present, there are no reports linking light perception and the direct involvement of HSP90-like proteins, and much less of a phosphorylation response ocurring in these proteins as part of the light perception mechanism. We are carrying out a detailed characterization of this protein to determine its functional role in the physiology of *Symbiodinium*.

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Constitutively active RhoGEFs,representing the most abundantly expressed branch of endothelial RhoGEFs, including RGS-RhoGEFs, Intersectins,PLEKHG5 and NGEF, reveal a strong angiogenic signal transduction potential.

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Generation of new blood vessels during angiogenesis involves a highly coordinated array of multiple steps such as cell polarization, membrane protrusion, contraction andassemblyof cell-cell and cell-extracellular matrix adhesions. This process involves a large number of proteins,includingRho family GTPaseswhichare particularly relevant because of their participation in each step that leads to morphological changes. As molecular switches, Rho GTPaseshave to be efficientlyregulated in time andspaceduring an angiogenic response. Thus,RhoGEFs,the group of guaninenucleotide exchange factorsthat activate Rho GTPases, are potential integrators of angiogenic signaling cascades.

This work provides the initial approximation towards the systematic characterization of RhoGEFs in terms of their angiogenic potential. Based on the endothelialexpression of RhoGEFs and theircomplex and interesting structures, we decided to study the signal transduction potential a group of 20 RhoGEFs, including PLEKHG5, RGS-RhoGEFs, Intersectins and NGEF. We hypothesized that their catalytic domains engineered with an isoprenylation signal would function as constitutively active variants. Our strategy consisted in the amplification of cDNA encoding the catalytic domains of each DH-PH RhoGEF and cloning them into the pCEFL-EGFP-CAAX vector, resulting in expression of fluorescent-tagged DH-PH constructs, anchored to the plasma membrane. Twelve RhoGEFsconstructs were obtained, whose functional characterization supported the hypothesis that their DH-PH domains anchored to the plasma membrane constitutively activate various Rho GTPases. We demonstrated their ability to activate different Rho GTPases, and using nucleotide free GTPases, performed capture assays selective for active GEFs. Besides, we characterized the cytoskeletal effects of these constitutively active RhoGEF constructs. We found that different constitutivelyactive RhoGEFvariants, showeddifferential activity towards Cdc42, RhoA and Rac1. Each catalytically active RhoGEFconstruct affected the phenotype of cells in which it was expressed and some exerted paracrine effects on non-transfected cells. Our findings encourage further exploration on the role of various RhoGEFs in endothelial cell migration and angiogenesis, and their potential role in pathological events such as tumor angiogenesis and metastasis, and extend their potential as novel therapeutic targets.

Detectingphosphorylations in Efl1 withMassSpectometry

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The ribosomopathies are hereditary diseases caused by deficiencies or defects in the regulation of the ribosome biogenesis. The mutations in the SBDS gene (*Shwachman-Bodian-Diamond Syndrome*) are responsible of the development of Shwachman-Diamond syndrome characterized by short height of those who suffer it and the development of leukemia in advanced stages.

The SBDS protein, Sdo1 in S. cerevisiae, is needed to release the inhibition factor Tif6 of the 60S ribosomal subunit and to allow the formation of a functional 80S particle. To liberate Tif6, SBDS/Sdo1 works together with the GTPase Efl1 by an unknown mechanism. During our research we overexpressed, purified and characterized the proteins involved in this mechanism to understand how Tif6 is released and what does Efl1 do.Thus, in the characterization of the GTPase Efl1 we identified undescribedphosphorylations suggesting the participation of other cellular proteins (kinases) in its regulation. These phosphorylations appear to be on serine, threonine and tyrosine residues. By mass spectrometry MALDI-TOF we identified some phosphorylations although the biological function of this modification remains unknown. To know the function of these phosphorylations we will be doing complementation tests and mutations in serine, threonine and tyrosine residues.

Prostaglandin E_2 differentially alters protein expressionin female *Anopheles albimanus* mosquito midguts

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Malaria is the most important parasitic disease in public health worldwide. It is caused by protozoans of*Plasmodium genus*and,they are transmitted by female anopheline mosquitoes during bloodfeeding. In Mexico, malaria caused by *P. vivax* persists, is a potential risk and one of the major vectors is *Anopheles albimanus*.When the mosquito obtains infected blood, the cells of the midgut epithelium are activated beginning the digestion and, in addition, the interaction with the parasite induces in the epithelium innate immune cellular responses. In this work,a comprehensive analysis of protein expression in female *An. albimanus*midguts,cultured *ex vivo*and under the effect of the hormone prostaglandin E2 (PGE_2) was performed by two-dimensional electrophoresis (2DE)and mass spectrometry (MS). Between others changes, mitochondrial ATPase and calreticulin decreased their concentration and three actin isoforms and a serine proteaseincreased. Actin, ATPase and calreticulin modifications were validated by WB and PCR. There are reports indicating the role of thesemolecules in energy metabolism, cell signaling, cytoskeleton and activation of humoral immune response setting a scenary where midgut should coordinate digestive, hormonal and immune functions in order to face the huge blood quantity ingested, the possible invasion by pathogens and initiate reproductive conducts.

Contribution of bone marrow-derived cells to tumor growth: Potential role of RhoGEFs

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Cancer progression and metastases is facilitated by blood vessels formed by ramification of existing capillaries and bone marrow-derived precursor cells recruited in response to chemotactic factors secreted by growing tumors. Signaling pathways activated by G protein-coupled receptors (GPCRs) and kinase-coupled receptors promote migration of these bone marrow-resident cells towards tumors and metastatic niches. This process involves the participation of Rho GTPases, represented by Rho, Rac and Cdc42. These molecular switches are activated by RhoGEFs, which are modular proteins that, in this situation, putatively integrate the signaling pathways activated by tumor-derived angiogenic and chemotactic factors. We hypothesized that RhoGEFs activated by chemotactic GPCRs are critical in the response of the bone marrow to growing tumors and can be considered potential therapeutic targets. Thus, we tested the contribution of bone marrow derived cells to the growth of LLC and LAP0297 carcinoma cells in C57 and FVB mice, respectively, and investigated, in Tie-2 positive bone marrow derived cells, the expression of all the RhoGEFs encoded in the mouse genome (~60 RhoGEFs). We also tested the signaling responsiveness of bone marrow derived cells to known angiogenic stimuli (ej., VEGF, SDF-1, LPA, S1P, FGF, EGF, HGF) and cancer cell-derived conditioned media. We found that 46 RhoGEFs are expressed in Tie2-GFP+ bone marrow derived cells, being Intersectin2, Sos1, Bcr, Abr, and PDZ-RhoGEF, the most abundant. Bone marrow-derived cells co-injected with carcinoma cells contributed to tumor growth. Furthermore, cancer cell-derived conditioned media and defined angiogenic factors activated protein phosphorylation and classical signal transduction pathways, such Akt and Erk, in bone marrow cells. The influence of bone marrow-derived conditioned media on cancer cell signaling pathways is also being tested. In conclusion, Tie-2 positive bone marrow cells are activated by angiogenic stimuli, contribute to tumor growth, and express a complex array of RhoGEFs that might contribute to its positive effect on cancer progression. Ongoing experiments are assessing this possibility, with the ultimate goal to understand the molecular basis of bone marrow contribution to cancer progression, with emphasis on the participation of specific RhoGEFs whose inhibition might interfere with tumoral growth and metastatic events.

LPS of *Helicobacter pylori* regulates claudins expression via ERK1/2.

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Helicobacter pylori is associated with the development of gastroduodenal diseases and gastric adenocarcinoma. The endotoxin lipopolysaccharide (LPS) of *H. pylori* is recognized by immune cells and gastric epithelial cells through Toll-like receptors (TLRs), activating the synthesis and secretion of inflammatory cytokines and interferons type I. *H. pylori* adheres to epithelial cells and changes the structure and function of claudins, essential constituents of the tight junctions (UE).

H. pylori disrupts epithelial barrier function through the relocation of claudins 4 and 5. Furthermore it has been reported the increase in the claudins 6, 7 and 9 expression in stomach biopsies of *H. pylori* positive patients. However, the process mediator of these alterations and their relationship with *H. pylori* is unknown.

In this work, an *in vitro* model was used with human gastric adenocarcinoma cells (AGS) to assess whether exposure to *H. pylori* LPS alters the expression and localization of claudins 4, 6, 7 and 9 through the participation of TLR2 and TLR4. The results showed that LPS increased the TLR2 and claudins and decreased TLR4 expression.

Because ERK 1/2 proteins are involved in the claudins phosphorylation and localization we examined if the increase of claudins expression by TLR2 was regulated by ERK1/2 activation. The results showed that LPS treatment increased expression of ERK 1/2 through the time of incubation. Increased ERK 1/2 expression in the nucleus suggests that the observed changes in the expression of claudins are very likely induced by proinflammatory cytokine synthesis.

Our findings indicate that an inflammatory reaction caused by the LPS of *H. pylori*, increaseTLR2 activation through ERK 1/2 pathway increasing claudins 4, 6, 7 and 9 expression.

Regulating of the activity of NF-κB by action of glycine dare channel Ca^{+2} in adipocytes.

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Abstract

Glycine, a nonessential amino acid and simple structure; has been shown to be protective against cellular injury, providing cytoprotection. It also has been associated with systemic inflammatory process block originating from a wide variety of disease states. In these processes potent inflammatory mediators such as cytokines are involved. The effects of glycine are regulated by a specific receptor (RGly) that is anchored to the plasma membrane and Cl^- associated channels. When the glycine binding to its receptor creates the hyperpolarization of the plasma membrane, generating an increase in permeability to Cl^- and inhibition of channel opening of voltage-dependent calcium blocking the production of intracellular signals and presents production of cytokines that are dependent on intracellular calcium increase. However the action of glycine can be selectively blocked by the antagonist strychnine. So far in vitro and in vivo have demonstrated that glycine reduces the expression and secretion of pro-inflammatory cytokines while increasing anti-inflammatory. The transcription factor NF-κB by multiple stimuli regulates transcription of genes encoding cytokines. It has been shown that pretreatment with glycine abolishes the activation of this transcription factor and the activity of the IKK inhibitor and the resulting complex a decrease in the synthesis of pro-inflammatory cytokines and inflammatory improved profile. So the objective of this research was to determine whether glycine modify the $[Ca^{2+}]_i$ by hyperpolarizing effect of adipocyte plasma membrane. The results show that glycine significantly decreases $[Ca^{+2}]_i$ mRNA expression promotes the RGly. These results support the idea that the glycine exerts its action by binding to a specific receptor to cause hyperpolarization of the plasma membrane and generate adipocyte blocking voltage-dependent channels such as Ca^{+2}, regulating the inflammatory process characteristic of obesity.

The effect of multiple carboxylase deficiency indecrease the mitochondrial mass is mediated via STAT3-HIF1a-BNIP3 in brain.

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Biotin is a water soluble vitamin that is part of the complex B. His most well-known role is to be the prosthetic group of four carboxylases: pyruvate carboxylase, propionyl coenzyme A carboxylase, methyl crotonyl coenzyme A carboxylase and acetyl coenzyme A carboxylase. Biotinidase deficiency disease causes an inability of the cells to recycle the vitamin Biotin. Individuals with this diseaseshow neurological abnormalities, such as ataxia and developmental delay, among others. Neurodegenerative diseases share three characteristics: inflammation, pseudohypoxia and alterations in mitochondrial mass and function. This work proposes that the activation of STAT3 leads to increased expression and stability of HIF-1a, which in turn activates mitophagy pathways, and results in the decrease in mitochondrial mass. Biotin deficient rats, biotinidase KO mice, and a cell culture of glioblastoma U373 line grown in biotin free medium, were used as experimental model. The results indicate that in multiple carboxylase deficiency STAT3 activation and concentrations of HIF are increased,the latter correlates with increased markers of mitophagy such as BNIP3 and PINK and decreased mitochondrial mass as evidenced by activity citrate synthase, and expression of the mitochondrial transcription factor (TFAM). To test the hypothesis, biotin deficient U373 cell culture was treated with inhibitors for nf-kb (curcumin) and STAT3 (III inhibitor). The results showed that both treatments were effective in inhibiting a STA3. In these cells the activation of HIF, PINK BNIP3 and were restored to control levels, in the same way, it was not observed a decrease in the citrate synthase activity and TFAM expression, which remained in the control levels. In conclusion, the activation of STAT3 decreasesmitochondrial mass through pathways that activatesmitophagy such as HIF, BNIP3 and PINK. This regulatory mechanism may have implications for other neurodegenerative diseases such as Parkinson character or Alzheimer.

Key words: Mitophagy, STAT3, HIF-1a, BNIP3, PINK, Biotin.

Mechanism for the specific targeting of methyltransferases to chemoreceptors in *Pseudmonas aeruginosa* PAO1

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Bacteria constantly sense and adapt to changing environmental conditions to assure survival. This important function is primarily mediated by one-component systems, two-component systems, and chemosensory pathways. Core proteins of chemosensory pathways are the CheA sensor kinase, CheW coupling protein, CheY response regulator, CheR methyltransferase, CheB methylesterase, and chemoreceptors. Methyltransferases of the CheR family and methylesterases of the CheB family control chemoreceptor methylation, and this dynamic posttranslational modification is necessary for proper chemotaxis of bacteria. Studies with enterobacteria that contain a single CheR and CheB show that, in addition to binding at the methylation site, some chemoreceptors bind CheR or CheB through additional high-affinity sites at distinct pentapeptide sequences in the chemoreceptors. *Pseudomonas aeruginosa* PAO1 has five gene clusters encoding chemosensory signaling proteins that assemble into four chemosensory pathways, termed Che, Che2, Wsp, and Chp. Apart from the 26 chemoreceptor *genes of P. aeruginosa* this strain has four CheR paralogous methyltransferases (CheR1, CheR2, CheR3, and WspC). Three of these chemoreceptors have C-terminal extensions with terminaal pentapeptides of sequence GWEEF, EVELF or GVEQA.

We produced the four CheR methyltransferases of *P. aeruginosa* as purified recombinant proteins, and their functionality was validated using microcalorimetric titrations with the methylation susbrate SAM and the methylation product SAH (Garcia- Fontana, Corral Lugo, & Krell, 2014). The titration of the four CheR paralogues with any of the three above mentioned pentapeptides revealed a single interaction, namely that between CheR2 and the GWEEF pentapeptide, which was derived from the McpB chemoreceptor. Interestingly, the genes encoding CheR2 and McpB are vicinal on the genome and form both part of the che2 chemosensory pathway. This interaction was also detected for the titration of full-length McpB with CheR2. *In vitro* assay shows that McpB is exclusively methylated by CheR2. Deletion of the terminal pentapeptide from McpB abolished both, the interaction and methylation of McpB. When clustered according to protein sequence, bacterial CheR proteins form two distinct families-those that bind pentapeptide: containing chemoreceptors and those that do not. These two families are distinguished by an insertion of three amino acids in the β-subdomain of CheR. Deletion of this insertion in CheR2 prevented its interaction with and methylation of McpB. Data suggest that the CheR2-McpB interaction is a strict requirement for any methylation activity.

Pentapeptide-containing chemoreceptors are common to many bacteria species; thus, these short, distinct motifs may enable the specific assembly of signaling complexes that mediate different responses.

Garcia-Fontana, C., Corral Lugo, A., & Krell, T. (2014). Specificity of the CheR2 methyltransferase in Pseudomonas aeruginosa is directed by a C-terminal pentapeptide in the McpB chemoreceptor. *Sci Signal*, *7*(320), ra34. doi: 10.1126/scisignal.2004849

The transcriptional regulator Atf1 has key roles in cellular stress and asexual reproduction in *Trichoderma atroviride*

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In fungi, the transcriptionfactorAtf1, homologous to Atf2 in humans, is a basic-leucine zipper (bZIP) protein that is the target of Stress-Activated Protein Kinase (SAPK)which together act forregulating gene expression.Atf1 plays an important role due to itscapacity to regulate stress response genes, and this is carried out once this protein is activated in the nucleus by phosphorylationthroughSAPK (Sty1/SakA/p38). In *T. atroviride*, light regulates expression of SAPK Tmk3 homologous to Sty1/SakA/p38.Tmk3 protects the cell of different kinds of stress related to sunlight, in the same wayit regulates some light responses in *T. atroviride*. To better understand this pathway and its relationship with light, in this work we obtained*T. atroviride* mutants lacking*atf1* gene(Δ*atf1*). The Δ*atf1*strains were subjected to different kinds of stress, such as osmotic, oxidative, heavy metals (Cadmium).Also,we analyzed integrity of cell wall, growth and sporulation, always comparing with the behavior of wild-type strain (WT) and strains lacking *tmk3* gene (Δ*tmk3*). When Δ*atf1* strains were exposed to stressors agents, they hadshown different degree oftolerance between mycelium and conidia, indicating that the function of Atf1 might be related withthe development stage of*T. atroviride*. All Δ*atf1* strains grew slower than*Δtmk3* and WT, and asexual reproduction was seriously affected in Δ*atf1*, indicating additional roles of this transcription factor probably independent of Tmk3.According to our results, evidence indicates that the transcription factor Atf1 function is both dependent and independent of Tmk3. Besides, Atf1 plays important roles on key processes such as conidiation, growth and survival in this mycoparasitic fungus.We conclude that the transcriptional regulator Atf1 is a point of convergence of multiple cellular stress signals and a key regulator on asexual reproduction in *T. atroviride*.

Identification of DNA binding proteins to the regulatory region implicated in the *STP1* (*SUGAR TRANSPORTER PROTEIN 1*) gene sugar regulation in *Arabidopsis thaliana*.

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Thecarbon sources preferred by many organisms are the sugars, from which the energy is obtained. In addition, these molecules have a great importance due to the fact they act as signaling molecules that modulated many processes during thelife cycle of organisms. Many studies have shown that sugars in plants, regulatevital processes such as: germination, growth, embryogenesis, reproduction, pathogenic responses,senescence, flowering,metabolic regulation, etc. Global analyses by microarrays have demonstrated that the presence or absence of sugars, suchas glucose, affects the expression of certain genes. Inthe particular case of the gene *STP1,* which encodes for a high affinity hexose transporter, has been demonstrated that its expression is repressed in the presence of glucose. This response is independent of the HXK1 (Hexokinase 1), that acts as a sensor in one of the three major pathways known that participate in the sugar signaling in *Arabidopsis thaliana*. Also, it has been identified the existence of *cis* elements, which regulate gene expression in response to sugars. Some of these *cis* elements are found in a region of 310 bp in the promoter of the*STP1*gene.In this work, we present the identification of proteins that bind to this regulatory region. Through the isolation of the nuclear proteins by DNA pull-down, which bind to this regulatory region in response to sugars, was possible to identifyby sequencing22 DNA binding proteins of different types. By analyzing the nature of these proteins, someof these could be putative candidates that participate in response to sugars.Several of these binding proteins are known and characterized,while others are not, therefore, it requiresfurther studies.In general, these results willallow us to place new possible components that participate in an HXK1 independent pathway.

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Role of the redundant proteins Ssk2p and Ssk22p in the endoplasmic reticulum stress response in the yeast *Saccharomyces cerevisiae*.

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The gene duplication processes allow organismsto increase their genetic variability, due to the drop in selective pressure among pairs of paralogous genes. Although many pairs of paralogous proteins may conserve their function and become redundant, the presence of an additional copy makes possibleto arise new functions.

The paralogous genes *SSK2* and *SSK22* (MAPKKK's) in*Saccharomyces cerevisiae*, code for two proteins involved in the High Osmolarity Glycerol (HOG) pathway, required for osmotic stress response. These proteins werepreviously described as having redundant functions in the HOG pathway; nevertheless some differences have been observed regarding differential signaling activity anddifferent strength interaction with Pbs2p (the downstream MAPKK).

In this work, we describe phenotypic differencesbetween*Δssk2* and *Δssk22* mutants when they are grown in medium containing tunicamycin (Tn), a drug that inhibits protein glycosylation and inducesEndoplasmic Reticulum Stress (ERS). Here we describe that Ssk2p has a more prominent role than Ssk22p to cope withTn.

In order to monitor possible protein interactions of Ssk2p and Ssk22p during the ERS induced by Tn, we performed a Protein-Fragments Complementation Assay (PCA) using the enzyme dihydrofolatereductase (DHFR). We found significant differences in the protein interaction profiles between Ssk2p and Ssk22p when cells are treated with Tn. Finally we selected some interaction partners of both Ssk2p and Ssk22p and tested their involvement in the Tn response. We found that null mutants of some Ssk2p interactors showed sensitivity to Tn, suggesting a concerted role in the Tn response.

Our results suggest that Ssk2p and Ssk22p are not redundant for the response to endoplasmic reticulum stress induced by tunicamycin.

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Regulation of voltage-dependent calcium channelsby G proteins in rat β-pancreatic cells

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Ca^{2+}influx through voltage-dependent Ca^{2+} channels (VDCC) plays a central role in the control of insulin release in β-pancreatic cells (Hiriart and Aguilar-Bryan, 2008). VDCC are regulated by both, Gβγ subunits of $G_{i/o}$ proteins andby a second messenger pathway likely involving$G_{q/11}$(Hille, 1994; Zamponi and Currie, 2013). However,questions arising from regulation of VDCC by G protein coupled receptors (GPCR) in rat β-pancreatic cells begin to be studied. Therefore, the presentstudyexamines the G protein-dependent regulation of VDCC by oxotremorine (Oxo-M), a muscarinic agonist.By using patch-clamp methods and biochemical reagents we examined bariumcurrents in whole-cell configuration and found thatβ pancreatic cells show a variety of barium current amplitudes while the capacitance among a large number of cells (n=60) remains unchanged. Barium currents are inhibited by nifedipine(52 ± 3%) and by ω-GVIA conotoxin (49 ±9 %). Treatment of the cells with 10 and 15 mMOxo-M decreasesbarium current 27± 4 and 33± 8%, respectively.Dialysis of the cells with GDPβS(2 mM) abolishes inhibition by Oxo-M, according to a G protein mediated response. These results support the hypothesis that insulin release in β-pancreatic cells of the rat ismodulated by VDCC linked to GPCR.

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Effects of endotoxin tolerance on the cytokine production and the secretory machinery of mas cells activated through the high affinity IgE receptor (FcεRI).

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Introduction.Mast cells (MC) are specialized secretory cells that have been studied because their participation on allergies. When they are activated through the high affinity IgE receptor (FcεRI), MC release large quantities of inflamatory mediators. This secretion needs the participation and translocation to lipid rafts, of different specialized proteins known as SNAREs.

Because of their anatomic localization, MC are constantly exposed to microorganisms that provoke different immunological states, however the effects of those states on the function, diferentiation and cytokine release of MC are poorly understood. In large cities and places with poor hygiene, individuals are constantly exposed to bacterial products (such as lipopolysaccharide, LPS). This constant exposure develops a condition known as endotoxin tolerance.

In this study we were interested in investigating the influence of endotoxin tolerance on the expression and localization of different SNAREs (SNAP-23, Stx4, VAMP8 and VAMP3), the secretion of distinct proinflamatory cytokines (TNF, CCL-2 and VEGF) and the expression of cytokines mRNA in MC activated through FcεRI receptor.

Results.Endotoxin tolerance did not induce changes in the expression of secretory machinery proteins, that include Stx4, SNAP-23, VAMP-8 and VAMP-3 on MC stimulated with IgE/antigen clomplexes but it inhibits the translocation of SNAP-23 to lipid rafts and changes the solubility of Stx4 in NP-40 buffer.With respect to cytokine secretion, endotoxin tolerance diminished *de novo-synthesized* TNF release, but didn´t change the release of preformed TNF niether CCL-2 secretion. Although we were unable to find changes in secretion, endotoxin tolerance diminished the mRNA expression of VEGF.

Conclusions.Endotoxin tolerance induces modifications to the secretory machinery and toFcεRI signaling pathway, suggesting that the cross-talk between that receptor and Toll-like receptor 4 (TLR4) is not limited to the activation of common elements, but it also includes the machinery leading to desensitization and has effects not only at transcriptional levels, but also on the proteins that regulate MC secretion.

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The SAPK signaling pathway regulates cellular stress and responses to light in *Trichoderma atroviride.*

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Light is an environmental factor that regulates many cellular processes such as metabolism, growth, development, reproduction and circadian clock. In *T. atroviride*, the BLR1/BLR2 proteins, counterparts of the *Neurospora crassa* photoreceptor complex WC1/WC2, regulate responses to blue light. A large number of light regulated genes have been identified, making evident that light regulates genes encoding DNA-repair enzymes, oxidative stress tolerance proteins, and enzymes implicated in the synthesis of photoprotective pigments, suggesting that light is seen as stress signal in living forms. Here, we show that the stress-activated protein kinase (SAPK) pathway is activated by light in *T. atroviride*. Expression of genes encoding the MAPKK Pbs2 and the MAPK Tmk3 are regulated by blue-light through the photoreceptor complex BLR1/BLR2. There are five Light Response Eelements in the *tmk*3 promoter region of which LRE2 is highly conserved among all *Trichoderma* species. Mutants lacking the *pbs*2 (Δpbs2) or the *tmk*3 (Δtmk3) gene were obtained by double-join PCR and their roles in the response to different kinds of stresses were analyzed. This MAP-Kinase pathway is indispensable for tolerance to osmotic stress, heavy metals (Cadmium), heat shock and UV irradiation, supporting its role in stress tolerance of this SAPK pathway. Noticeably, SAPK shows specific roles depending on the development stage in *T. atroviride*. Conidia of the Δpbs2 and Δtmk3 strains were highly sensitive to oxidative stress whereas their tolerance to this stress was not affected in mycelia. Conidiation stimulated by light was reduced 80% in SAPK pathway mutants, suggesting that light regulates asexual reproduction through this signaling pathway.

Given the essential role in osmotic stress of the SAPK pathway, transcriptome regulated by light was compared with that regulated by osmotic stress previously described in *T. harzianum*. Five genes encoding glucose-repressive proteins (*blu*-1 and *grg*-2), glucoamylase, fatty acid elongase (Gig30) and small heat-shock protein appear to be regulated by both stimuli suggesting that the SAPK pathway has also roles in regulating responses to light at the molecular level. In fact, expression of *blu*-1 induced by light was drastically reduced in Δtmk3 strains, indicating key roles for this signaling pathway in gene expression regulated by light. The fact that *blu*-1 gene expression can be detected in the mutants at a very low level indicates that at least two pathways are operating in its regulation by light. Taken together, light regulates several stress response, asexual reproduction and light-induced genes through the SAPK signaling pathway.

Significant suppression of colitis-related colorectal cancer in a murinemodel

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In Mexico, colorectal cancer (CRC)has high incidence and mortality rates among the population. Chronic inflammation, as in Crohn's disease and ulcerative colitis, is the most important risk factor for developing CRC, as it favors neoplastic transformation by enhancing epithelial cell turnover in the colonicmucosa. Treatments for CRC need to be improved;currently they are not specific and have several secondary effects in patients. The main objective of this work is to evaluate a combinatorial therapy proposed as a better and more efficient antitumoral treatment against a colitis-related colorectal cancer *in vitro* and*in vivo*. Regarding the mechanisms of carcinogenesis, the proposed drugs work by inhibiting the mTOR signaling pathway and glycolysis related with autophagy and deregulation of cellular energetics, respectively. CRC was induced pharmacologically in Balb/c mice using azoxymethane (AOM) and dextran sulfate sodium (DSS). After the development of tumors, the pharmacological evaluations initiated. Treatment consisted inthe intraperitonealadministration of the drugs in combination (Metformin, F3– a clinically-approved LDHA inhibitor–and Doxorubicin) during a forty days period. This pharmacological combination is under patent application. After treatment, the mice large bowels were extracted and inspected macroscopically, then were cut into small pieces and divided for further histological, immunohistochemical, protein,and cytokine profile experiments.Primary cultures were also derived for western blot and flow cytometry analyses. Results showed that targeted inhibition of the mTOR and glycolysis leads to growth inhibition and autophagy induction, as well as considerable changes in cytokine inflammation-related profiles after the treatment. Our results represent a novel and potential therapeutic strategy for overcoming CRC.

Extracellular ATP Induces Apoptosis through P2X7 Receptor activation in MDA-MB 231 cells.

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Extracellular ATP and other nucleotides act through specific cell surface receptors and regulate a wide variety of cellular responses in many cell types and tissues. The present study aims to explore the effect of ATP on the apoptosis of MDA-MB 231 cells and the underlying mechanism. We demonstrate that MDA-MB 231 cells express several P2Y and P2X receptor subtypes including P2X7, and describe functional responses of these cells to extracellular ATP.

Given that the P2X7 receptor is involved apoptosis, necrosis, cytokine secretion but also cell proliferation and survival. We found that extracellular ATP inhibited the proliferation and induces apoptosis in MDA-MB 231 cells. The apoptosis of MDA-MB 231 cells induced by 1 mM ATP treatment by 72 hours was confirmed by: a) cell counting (Handheld automated cell counter, Scepter 2.0); b) protein concentration, and c) propidium iodide staining. The effects of ATP were mimicked by micromolarconcentrations of 3-O-(4′-benzoyl)-benzoyl-benzoyl-ATP, (Bz-ATP, specific agonist of P2X7 receptor) and were inhibited by pretreatment of MDA-MB 231 cells with a selective blocker of human P2X7 receptor BBG, as well as oxidized ATP.

The nucleotide selectivity and pharmacological profile data support the role for P2X7 receptor as the mediator of the ATP-induced responses. Given the importance of breast cancer in health public, the ability of extracellular ATP to induce the P2X7-mediated apoptosis in breast cancer cell lines may facilitate the development of new strategies to modulate proliferation, invasiveness of breast cancer.

Cancer metabolic hallmarks blockade induces death by autophagy in triple negative breast cancer cell line

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Breast Cancer is an important health issue worldwide. The most aggressive phenotype is triple negative (TN) (negative for either hormonal receptor or HER-2). Currently, this phenotype has no specific treatment and an important number of TN patients tend to show recurrent disease. Here we aimed to analyze the participation of three cancer cell metabolic inhibitors: metformin (mTOR inhibitor), doxorubicin (DNA intercalating agent) and a clinically-approved LDHA inhibitor that, for patent reasons, will only be called F3 in*in vitro* and *in vivo* models.

To validate *in vivo* that the pharmacological strategy could suppress tumor growth, we used an ectopic breast cancer mouse model and monitored tumor growth kinetics using microPET. MDA-MB-231 cells were xenografted into the right flank of female nude mice. Once the tumors reached a size of 3 mm^3, animals were treated for 15 days with doxorubicin, metformin, or F3, alone; or one of the following drug combinations: doxorubicin/metformin, doxorubicin/F3, metformin/F3 or doxorubicin/metformin/F3. Image acquisition was performed at Medicine Faculty, UNAM using a Concorde Microsystems Focus F120 Micro PET scanner equipment and the SiemensOSEM 3D software. We observed complete tumor remission from day 10 of doxorubicin/metformin/F3 treatment, while, the control group treated with doxorubicin, presented recurrent disease after 9 days of treatment.

To gain a better understanding of the molecular mechanisms of the type of death induced by the doxorubicin/metformin/F3 treatment in MDA-MB-231 cells, we performed a Western Blot analysis of apoptosis pathway components PARP-1 and caspase-3, autophagy marker LC3, and both total and phosphorylated mTOR. A synergic behavior of the three combined drugs was observed, improving significantly the autophagy process.Moreover, our data indicates the accumulation of tumor cells in G1 phase and the proapoptotic effect and increase autophagy processes caused by the synergy of the three drugs. Our proposed coadjuvant drug therapy inhibited cell growth both *in vivo* and *in vitro*; this result suggests that the combination of mTOR inhibitors and glycolysis inhibitors is a promising therapeutic target, presumably regulating cell death mechanisms such as apoptosis and autophagy.

ZmPDK1, a new component of the *Zea mays* TOR pathway.

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Abstract

The TOR-S6K signal transduction pathway is known to regulate cell growth by inducing ribosome biogenesis, as well as cell division, in most eukaryotes. Recently this pathway has been studied in plants and several of its components, downstream the TOR kinase have been described (John et al., 2012). However, the knowledge about upstream regulation of the TORpathway is still limited.In maize,an activator of this pathway named ZmIGF, was described and its functional equivalence to insulinin mammals was demonstrated by our group (Garrocho-Villegas et al., 2013). However, reports ofthe protein kinases that transmit the phosphorylation signal from the receptor to the TOR kinase, are scarce in plants. In the present research, ZmPDK1, the maize ortholog of the mammalian PDK1, was foundand characterized in active growing cells of germinating seeds. Further,*in vivo* phosphorylation assays showed an increment in the phosphorylation of ZmPDK1, ZmTOR and ZmS6K kinases,by growth factors induction, such as ZmIGF or insulin, thus indicating TOR pathway activation. Moreover,rapamycin, an inhibitor of TOR kinase, showed no effect on the phosphorylation status of cytosolic ZmPDK1,strongly suggesting that it is located upstream of the TOR kinase in this pathway. Immunolocalization analysis showed the presence of ZmPDK1 in the cytoplasm and in the nucleus of 48 h germinated maize axes. This research provides evidence that PDK1 is a component of TOR pathway in maize.

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Endogenous Ribosomal Heterogeneity in Maize Tissues: Possible Biological Relevance.

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Abstract

Recent research suggest that in eukaryotes exist heterogeneity in protein ribosomal composition (Gilbert, W.V., 2011). Particularly in maize,stimulation of ribosome biogenesis is controlled by the TOR-S6K pathway.Indeed, selective mobilization of mRNAs that codify for ribosomal proteins to polysomes has been documented (Jimenez-Lopez et al., 2011).The objective of the present research is to analyze if there are changes in the protein composition of ribosomes of contrasting maize tissues, that could imply selective speed of mRNA translation. In order to prove this hypothesis, a unique set of mRNAs was analyzedusing ribosomes from slow and fast growing tissues *by in vitro*translation systems. Results showed different patterns of synthesized proteins due to the set of ribosomes used. Further, proteomic 2D (Acid-Urea) maps of ribosomal proteins from contrasting maize tissues were obtained. Results showed protein heterogeneity between ribosomes from control versus ZmIGF induced tissues, trough the TOR pathway. These heterogeneity could be characteristic of a particular state of germination. On the overall, these results suggest that protein heterogeneity in ribosome's composition from different tissuesat different developmental states would have selectivity for mRNA translation.

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In vitro and in vivo endothelial activation mediated by tumor soluble factors secreted by different breast cancer cell lines

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Introduction: An increase in endothelial permeability is considered to contribute to the metastatic process, thus facilitating the transmigration of cancer cells. Since endothelial cells regulate both vascular permeability and the transendothelial migration processes, it has been postulated that, during the extravasation process, the tumor cells must interact with them.

Methods and results: Breast cancer cell lines with low metastatic potential (MCF7 and T47D), and with high metastatic potential (ZR-75-30) were cultivated. The culture media was collected and used to obtain the tumor soluble factors (TSFs).Primary human umbilical-vein endothelial cells (HUVEC) were stimulated with TSFs, afterwards U-937 cells were added, so that they could adhere only to the activated HUVEC. Only the TSFssecreted by ZR75-30 (10 µg/ml) activated the endothelium to a similar level to the activation produced by 10 ng/ml of human recombinant tumor necrosis factor (TNFα).

Vascular permeability assays (modified Miles assays) were done on Swiss Webster mice. Evans Blue dye was administered intravenously. Afterwards, TSFs were administeredintradermally on the dorsal area. The zones of activated endothelium increased their vascular permeability, allowing for the extravasation of the dye and thus the stained skin.The TSFssecreted by ZR75-
30(16 µg/ml) activated the endothelium in vivo to a similar level to the activation caused by 625 ng/ml of murine recombinant vascular endothelial growth factor.

Endothelial activation mediated by the TSFs is independent of the TNF signal transduction pathway sincenone of the TSFs induced cell death on L929 cells.

Conclusions: The TSFssecreted by the cell lines with low metastatic potential (MCF7 and T47D) do not activate the endothelium; whilst the TSFssecreted bythe cell line with high metastatic potential ZR-75-30 activate the endothelium in vitro and in vivo. The endothelium activation appears to be independent of the TNF signal transduction pathway.

Scaffold activity of the MAPKK Pbs2p during the endoplasmic reticulum stress response in *Saccharomyces cerevisiae*.

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Several environmental stimuli or changes in cellular processes can lead to deficiencies in protein folding,inducing their accumulation, whichgeneratesa condition known as endoplasmic reticulum stress (ERS). There are ER systemsable to sense misfolding of newly synthesized proteins, that trigger a transcriptional program known as Unfolded Protein Response (UPR), dedicated to restore cellular homeostasis. In the yeast *Saccharomyces cerevisiae,* the HOG pathway (High Osmolarity Glycerol), a MAPK transduction system thatparticipates in the osmotic stress response, has been also involved in the response to agents that induce endoplasmic reticulum stress. However, it appears that the role of the HOG components in the ERS follows a different architecture andmechanismcompared to their role in the high osmolarity pathway. For example, it has been seen that different to hyperosmotic response, the Hog1p phosphorylation and Pbs2p kinase activity may be dispensable to generate an adequate ERS response.

In this work we propose that the scaffold domains of MAPKK Pbs2p are essential and sufficient to establish a response to the antibiotic tunicamycin (Tm), anN-glycosylation inhibitor,which generates ERS. For this, we generated deletions on the kinase and scaffold domains in Pbs2p, and we tested the ability of these constructs to reverse the sensitivitythat the Δ*pbs2* mutant shows to Tm. It was observed that the presence of the Hog1p and Ssk2/22p scaffold domains is sufficient to allow growth in Tm, while the Sho1p scaffold domain and the kinase domain were dispensable. We also found that a Hog1p mutant lacking its Pbs2p binding domain dost not rescue the Tmsensitivity of a Δ*hog1* strain.

Finally, we were interested in determining the subcellular localization of Pbs2p under Tm exposure. By using a GFP-tagged version of Pbs2p, we found that it forms extranuclear aggregates that in some cases co-localize with an ER Tracker dye.

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Physical interaction between the GTPases Gpn1 and Gpn3 takes place in the cytoplasm.

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The GPN protein family comprises three members: Gpn1**,** Gpn2 and Gpn3. These proteins contain the canonical GTPase domain in their sequence and their name derives from a GPN (glycine-proline-asparagine) structural loop inserted onto the GTPase core-fold, although an actual GTPase activity has been demonstrated only for Gpn1. Gpn1 is essential for RNA polymerase II (RNAPII) nuclear import (Forget *et al*., 2010; Starensincic, 2011; Carré *et al*., 2011). Gpn3 protein was originally described as an Apaf1-interacting protein (Sánchez-Olea *et al*., 2008), and it was later shown also to be involved in RNAPII nuclear import (Calera et al., 2011; Carré et al., 2011). There is evidence that Gpn1 and Gpn3 interact with each other (Grass *et al*., 2007; Carré *et al*., 2011). In this study we co-transfected YFP-Gpn1 and Gpn3-CFP constructs in HEK293 cells to assess a direct interaction by spectral FRET imaging. We obtained a FRET efficiency of 24% from Gpn1-Gpn3 in the cytoplasm. There was no interaction between these two proteins at the nucleus. We used the combination YFP-Gpn1/CFP or Gpn3-CFP/YFP as a negative FRET control. As expected, there was a FRET efficiency of 0% from negative controls. We are presently evaluating the effect of several point mutants on the Gpn1-Gpn3 interaction.

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FGF-2 induces epithelial to mesenchymal transition in MDA-MB-231 cell line

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Epithelial-Mesenchymal Transition (EMT) is a cellular process that leads to organ and tissue morphogenesis during embryonic developmentof vertebrates. Although is also involved in two other processes, wound healing and tumor cell metastasis.

EMT is regulated by several extracellular messengers such as growth factors, including the Epidermal Growth Factor (EGF) and Basic Fibroblast Growth Factor (bFGF or FGF-2), unknown until now the molecular mechanism involved.

The main purpose of this study is to establish the molecular mechanism by which FGF-2 stimulates migratory and invasive capacities in human breast cancer cellsMDA-MB-231.

The chronic presence of FGF-2 (600 pM) induces an increase in the rate of wound closure (healing wound assay), suggesting that FGF-2 stimulates the migratory capacity of MDA-MB-231 cells.

Subsequently, it was evaluated by RT-PCR the expression levels of mRNA for the different transcription factors associated with EMT (*Snail-1, Snail-2, Twist and ZEB1*) in the presence of FGF-2 (600 pM) by different incubation times (1, 2, 4, 6 and 8 hours). FGF-2 induces a significant increase in the expression levels of mRNA that encodes for Snail-2.

The progression of any epithelial tumor requires the expression of angiogenic factors, such as Vascular Endothelial Growth Factor A (VEGF-A). The presence of FGF-2 (600 pM) for 8 hours, leads to an increase in the expression levels of mRNA that encodes for this factor, which ensure their metastatic capacity.

On the basis in the results obtained until now, we proposed that the mechanism by FGF-2 regulates the migratory and invasive capacities in MDA-MB-231 cells is through up-regulation of transcription factor associated to EMT Snail-2 andVEGF-A, which can act to promote the development of a favorable microenvironment for cell survival.

YMCA: Yeast MultiColor Assay

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Since the beginning of the study of cellular structure and functions, the protein analysishas been difficultbecauseproteins activity and functionsare regulated by theirsub-cellular localization.

The most common methods used to determinate the protein localization consist in adding tags and using of fluorescence microscopy, however, the *in vivo* visualization of protein dynamics and the accurateprotein localization monitoring within the differentcellular compartmentshas been proved difficult when chemical compounds forthe staining of each organelle are not available. To address this issue, we developed a tool to study *in vivo*protein localization dynamics in the yeast *Saccharomyces cerevisiae*.

This tool consists inlabelling proteins from different cellular compartmentswith fluorescent dyes in order to stain different organelles (nucleus, mitochondria, vacuole, endoplasmic reticulum, Golgi apparatus and plasma membrane). Thus, we have a reference to co-localize the protein of interest in a systematic way.

This tool could be associated with the strains from the collection of proteins tagged with the green fluorescent protein (GFP), as a result, the determination of the precise localization of a protein in different conditions would be easier and no further chemical staining would be needed.

TEM5/GPR124, a tumor endothelial marker orphan GPCR, modulates the signaling effects of cancer cell-secreted factors

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Tumor angiogenesis contributes to cancer progression. Hence, the proteins differentially expressed in tumor blood vessels are considered potential therapeutic targets. GPR124, also known as TEM5, was identified as a tumor endothelial marker overexpressed in tumor vessels from human colorectal cancer.Additionally, GPR124 knockout mice die during development due to reduced angiogenesis in the brain.Thus, TEM5/GPR124 regulates developmental angiogenesis and might be important in tumor-induced angiogenesis, potentially regulating endothelial cell migration. However, the signaling properties of TEM5 and its response to tumor-secreted factors have not been explored.We hypothesize that this orphan GPCR induces signaling cascades,involving Rho GTPases and PI3K/AKT/mTOR pathway, related to actin cytoskeletal remodeling and cell migration either by overexpression or in response to factors secreted by the human colon cancer cell line (HT-29).

We found that TEM5 induces filopodia formation in porcine aortic endothelial (PAE) cells. This morphological effect is linked to dynamic adjustments of the actincytoskeleton and correlates with the ability of TEM5 to activate Rac1 and Cdc42 but not RhoAGTPases in HEK 293 cells. Besides, TEM5promotes phosphorylation of AKT atS473, suggesting the intervention of PI3K, mTORC2 and guanine nucleotide exchange factors (GEFs) as potential effectors of TEM5 signaling. In response to HT-29 colon cancer cell-secreted factors TEM5 promotes AKT and GSK3β phosphorylation, attenuates the time course of ERK activation and localizes at cell projections. The mechanistic basis of these responses are potentially attributable to TEM5 carboxyl-terminus which interacts with RhoGEFs, including ITSN2L, a GEF for Cdc42, and Elmo1, an adaptor of an atypical GEF known to activate Rac. Interestingly, the interaction between TEM5 and Elmo1 increases in response to HT-29 cancer cell-conditioned media.Our results suggest that TEM5 modulates the agonistic properties of cancer derived factors that control activation of Rho GTPases and the PI3K/AKT/mTORC2 signaling pathway, promoting cytoskeletal changes and cell migration. This mechanism might be involved in TEM5-dependent angiogenic responses in cancer.

Hyperactivation rescue in spermatozoa from potassium channel *Slo3*$^{(-/-)}$ knockout mice.

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Fertilization is the most important event among sexually reproductive organisms. Before fertilization occurs the spermatozoa acquire fertilization capability through its journey towards the oocyte in the female tract in a complex process named capacitation. Among other changes that take place during capacitation motility,hyperactivation is required for fertilization. Hyperactivation is characterized by high-amplitude and asymmetrical flagellar beating, *(Chang & Suarez, 2010)*. Hyperactivation plays different roles, such as effective swimming through the viscoelastic substances present in the oviduct, to detach from the oviductal epithelium, to penetrate the external matrix of the oocyte, to reach and fuse with the oocyte plasma membrane. Fertilization encompasses a dialogue between sperm and egg which requires the regulation of ion transporters and channels, from the large repertoire of ion channels that participate in cell function only two so far appear to be uniquely expressed in sperm. CatSper channels (1-4), a new family of Ca^{2+} permeable channels and Slo3 channels, a member of the Slo family of K^+ channels, *(Martinez-López, et.al. 2009)*. Spermatozoa from CatSper and Slo3 null mice cannot hyperactivate and are sterile. Since hyperactivation is a Ca^{2+}-dependent process the role of CatSper in this event is clear. In this work we hypothesized that Slo3 activity during hyperactivation is required to hyperpolarize the sperm plasma membrane and increase the driving force for Ca^{2+} ions to permeate through Catsper and therefore provoke hyperactivation. To test this idea we used several pharmacological agents to increase Ca^{2+} either through Catsper or independently of Catsper in wild type and Slo3 knockout mice and measured motility hyperactivation under these experimental conditions.

Role of protein kinase Fyn on the regulation of TNF secretion stimulated by LPS in mast cells

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Mast cells (MC) are responsible for allergic reactions but they are also relevant to the innate immune response against Gram-negative bacteria. This derives of their capacity to secrete pre-formed inflammatory mediators that are stored in specialized secretory granules but also on the production of cytokines that are synthesized *de novo* after the stimulation of the high affinity IgE receptor (FcεRI) and the Toll-like (TLR-4) receptor.

Despite the large amount of information about the mechanism of cytokine production after FcεRI receptor triggering, the molecules participating on the coupling of TLR-4 receptor to the secretion of pre-formed and newly synthesized mediators in MC is far from being complete. Our previous work has shown that a Src-family kinase (Lyn) couples TLR-4 signaling with full activation of IKK and NFκB transcription factor. Now, in this work we asked if another member of that family of kinases (Fyn) could participate in some TLR-4-mediated responses in MC.

Bone marrow-derived mast cells (BMMCs) from WT and Fyn KO mice were utilizing to analyze some of the most important events on the signaling cascade of TLR-4 receptor, TNF secretion after lipopolysaccharide addition was higher in Fyn -/- than in WT BMMCs. This was correlated with higher PKC and IKK phosphorylation in the absence of Fyn. Our results strongly suggest that Fyn kinase controls a negative regulatory loop on the signaling cascade of TLR-4 receptor in mast cells.

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"G-protein receptor magnitude of signaling depends on the glycocalyx and flow".

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ABSTACT:

Blood pressure is a stimulus whose mechanism is still under discussion, and acting on the luminal endothelial wall generates multiple signals, so that modulate important paracrine functions specifically into metabolisms adjacent the parenchymal cells. Our laboratory has shown that luminal endothelial wall are transmembrane proteins such as G protein coupled receptors (GPCRs, **L**) like lectins, those have affinity for sugars. Since the endothelium the glycocalyx (contains glycosaminoglycans**O**) is that the GPCRs luminal and is superimposed on these lectins have proposed the following hypothesis: sign <- OL <- flow -> O + L. O = hyaluronidate or heparinate. L = GPCRs. This hypothesis would explain the molecular mechanism of action of the flow and also explain why the flow is multifunctional. This hypothesis has been tested in a system isolated perfused vessels (carotid arteries), a controlled stream to show that flow alters sensitivity to the hormone and that this effect depends on the flow or composition of the glycocalyx.Isolated and dissected carotid arteries from male guinea pigs of 500 g was implemented. Cannulated on both ends of the vessel, with a small catheter and perfused with Krebs-Henseleit solution (oxygenated maintained at 37 ° C, pH 7.4), by Langendorff perfusion method. The preparation was fixed and kept at 37 ° C.Two parameters were continuously recorded; pressure within it and perfusion flow. These state parameters measured arterial smooth muscle contraction.Therefore, it is proved that the receptor to bradykinin B2R, acts as a receptor membrane luminal endothelial sensitive to flow and that the magnitude of the response depends on the perfusion pressure.

"Novel estrogen signaling pathway in breast cancer cells migration"

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The Epithelial-Mesenchymal Transition (EMT) is increasingly viewed as a significant clinical problem in cancer, as EMT is thought to initiate metastasis. It has been shown that estradiol (E2) induces breast cancer cell progression and can enhance EMT; however, the detailed mechanisms remain unclear. We investigated the role of E2 on the expression and Estrogen promotes breast cancer proliferation through a number of established pathways however; the effects of E2 on breast tumor cell motility or invasion are poorly understood. In many types of epithelial cancers, the ability to undergo metastasis has been associated with a loss of epithelial features and acquisition of mesenchymal properties leading to migration of individual cells, a process known as epithelial-to-mesenchymal transition (EMT). The EMT is a process leading to metastasis controlled by multiprotein complexes as Zonula Occludens1 (ZO-1), transcription factor associated with ZO-1 (ZONAB) and protein kinase Rous Sarcoma Virus (Src). We demonstrated that estradiol (10-9M) induced Src -Tyr-416 phosphorylation and subsequent p-Src/ZO-1complex formation, leading to ZO-1 and of ZONAB disruption and nuclear translocation and generated changes in the HER-2 mRNA expression because is known that ZO-1 and ZONAB act as transcriptional regulators, in the promoter of the HER-2 gene. These changes correlated with the expression decrease in the epithelial markers CRB3, occludin and increased in the N-cadherin mesenchymal marker which leads to the increase in permeability and migration induced by E2. When we used an estrogen receptor antagonist (ICI 182,780), Src-Tyr-416 phosphorylation, p-Src/ZO-1 complex formation, ZO-1 and ZONAB nuclear translocation, permeability and cell migration were reversed in breast carcinoma cells MCF-7.

These studies suggest that during tumor progression, E2 promote TJ disruption, nuclear translocation, gene expression regulation, increase in permeability and invasion of breast cancer ER-positive, so our results have identified a novel pathway in which estrogens promote EMT as a process leading to metastasis.

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Leptin induces FAK activation and cell migration in a Src-dependent pathway in breast cancer cells

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Abstract

Several experimental and epidemiological studies have linked obesity andbreast cancer. Leptin is a hormone secreted by adipocytes, which experimentally has been shown to promote breast cancer progression. Cell migration is associated with different steps of metastasis;one of the most important proteins associated with cell migration is focal adhesion kinase (FAK). Several clinical studies suggest an important role of FAK kinase in the development of breast and previous studiesin breast cancer cell lines have demonstrated that many factors promote FAK activation and cell migration. However,there are no studies about the role of Src on FAK activation and cell migration in breast cancer cells stimulated with leptin. **Objective**: To determine the role of Src in FAK kinase activation and cell migration ofbreast cancer cell lines stimulated with leptin. **Materials and methods**: Breast cancer cell lines MDA-MB-231 and MCF7 were used. Cell migration was assessed by Scratch-wound assays. FAK kinase activation was evaluated by measuring the phosphorylation of P-Tyr^{397}by Western blot. FAK inhibitor (PF-573228) and Src inhibitor (PP2) were used to evaluate cell migration and FAK activation. **Results**: We determinate that leptin induces FAK activation in a time-specific manner and cell migration in a dose-specific fashion in both breast cancer cell lines. Furthermore, our results demonstrate that leptin induces FAK activation and cell migration in a FAK/Src-dependent pathway. **Conclusions**: Leptin induces FAK kinase activation and cell migration through a Src-dependent pathway.

High doses of IL-2 inhibit cell proliferation by inducing an arrest in the G1 phase of the cell cycle in cervical cancer cell lines CALO and INBL

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Cervical Cancer is the second cause of death in women worldwide according to WHO. It is one of the major causes of cancer death in low resource countries. In Mexico the highest incidence in gynecological tumors is breast cancer and secondly cervical cancer. Several therapies have been used for the treatment of cervical cancer, such as immunotherapy, gene therapy and some other based on cytokines. The IFN-γ, IL-2, IL-12 and GM-CSF are excellent candidates as activators of the antitumor immune response and they have been used in various preclinical models with high effectiveness.

Research conducted at the Cell Differentiation and Cancer Research Unit at FES-ZARAGOZA UNAM, have demonstrated that cervical cancer cell lines CALO and INBL bear a functional receptor for IL-2, so this can be considered a good biological model to elucidate the role of this interleukin in cervical cancer.

It is known that the effect of IL-2 on non-hematopoietic cells appears to be differential since higher concentrations inhibit proliferation by 50% of squamous carcinoma head and neck cell lines, either in vitro or in vivo. It has been shown that treatment with 100 IU of IL-2 induces activation and proliferation of peripheral blood lymphocytes. Therefore, we decided to treat cervical cancer cells with high concentrations of IL-2 to determine its effect on the proliferation of the cell lines CALO and INBL and upon phosphorylation of JAK3, STAT3 and STAT5 proteins.

Results from this study show that cell proliferation in cell lines CALO and INBL when treated with 100 UI/ml of IL-2 is inhibited. A decrease was observed in the phosphorylation of JAK3 kinase but not the phosphorylation of STAT3 or STAT5 when treated with IL-2. To determine if the inhibition of proliferation was related to the cell cycle deregulation, we decided to analyze the cell cycle phases and our results show that in both cell lines the treatment whit 100UI/ml of IL-2 induces an arrest of cells in the G1 phase of the cell cycle.

In conclusion, modulation of the phosphorylation affects the function of the JAK-STAT proteins probably intervening in the correct transduction and transcription of

Innate and adaptive IL-9 orchestratetype 2 immunity

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Type 2 responses induced during helminth infections and allergies are controlled by a variety of cytokines including IL-4, IL-5, IL-9 and IL-13. Previous studies, from our group, using transgenic mice that constitutively express IL-9 selectively in lung epithelial cells supported an important role for IL-9 in the pathogenesis of asthma, however its contribution in host protection during parasitic infections is still controversial. To definitively determine the role of IL-9, itscellular sources and targets*in vivo,* we generatedtwo different genetic tools, an IL-9 fluorescent reporter knock-in miceand an IL-9 deficient mice. Using a hookworm infection model, we found that IL-9 expressionis induced in T cells (Th9) and innate lymphoid cells(ILC2) in lung and mesenteric lymph nodes of infected mice; importantly, IL-9 expression preceded the expression of other Type 2 cytokines in all organs analyzed. Furthermore our data suggest that IL-9 amplify Type 2 responses *in vivo* not only inducing activation of mast cells and goblet cells as previously reported, but also by promoting IL-5 and IL-13 expression as well as basophilia and eosinophilia in mice infected with *Nippostrongylusbrasiliensis*. Interestingly, transfer of Th9 cells but not Th2 cells into Rag deficient mice, promotedbasophil recruitment leading to increased worm clearance. In conclusion, innate and adaptive IL-9 orchestrate anti-helminth responses *in vivo* throughactivation of different cellular subsets and cytokine secretion that ultimatelypromote an effective helminth expulsion.A better understanding of IL-9 biology and physiology including the molecular signals driving its expression *in vivo*will be key to the development of alternative therapeutic strategies for more effective treatments controlled by IL-9 includingallergic asthma, nematode infections, autoimmune and inflammatory responses.

Tissue inhibitor of metalloproteinase-4 regulates stemnessand apoptosis in cervical cancer cells

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The tissue inhibitors of metalloproteinases (TIMPs) comprise a multifunctional protein family that regulates the activity of matrix metalloproteinases after their secretion into the extracellular environment. TIMPs have divergent roles on cellular processes such as cell differentiation, proliferation and apoptosis, and are overexpressed in several cancers. However, the role of TIMP-4 during carcinogenesis is poorly understood. In the present report, we show that, in a nude mouse model, cervical cancer cells that overexpress TIMP-4 formed tumors faster than control cell-derived tumors, even when displaying higher cell death rates. *In vitro*, flow cytometry and quantitative gene expression analyses demonstrated that TIMP-4 overexpression resulted in an enrichment of the tumor progenitor cell (TPC) population. Additionally, this protein also demonstrated apoptosis-sensitizing effects toward TNFα, TNF-related apoptosis-inducing ligand (TRAIL) and serum starvation. Consistent with these findings, genome-wide expression and signaling pathway analyses showed that human recombinant TIMP-4 (hrTIMP-4) modulated cell death, cell survival, cell proliferation, inflammation and epithelial-mesenchymal transition signaling networks. Notably, the NFκB signaling pathway appeared to be globally activated upon hrTIMP-4 treatment and was required, at least partially, for the stemness-promoting effect. Overall, this report shows that TIMP-4 regulates carcinogenesis through enriching the TPC population and probably by triggering apoptosis in cervical cancer cells. Understanding coordinated TIMP-4 functions on tumorigenesis may provide clues for the design of future therapies.

Electrophysiological and pharmacological characterization of *Slo3* channels in mouse spermatogenic cells

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Abstract

Fertilization is the process in which the female (egg) and male (sperm) gametes fuse. To perform this event successfully sperm require a particular set of ion channels. *Slo3* channels have been recorded in mouse sperm and heterologously expressed using different techniques.The absence of this K^+ selective, voltage and intracellular pH (pHi) dependent channel produces infertility in *knock out Slo3* mice (*Slo3*$^{-/-}$*).* With this background, we asked if the *Slo3* channel is functionally present in mouse spermatogenic cells and if it has the same electrophysiological and pharmacological characteristics as those determined in more mature epididymal sperm. To answer this question, experiments were performed with the *patch-clamp* technique in the *voltage-clamp-whole-cell* configuration. In this manner, K^+ currents were recorded in round spermatogenic cells of wild type and *Slo3*$^{-/-}$ mice under the following conditions: alkalinizing pHi with 40 mM NH_4Cl and blocking the current with 50 µM clofilium. We found that *Slo3* channels expressed in 59.6% of the recorded wild type mice spermatogenic cells and not in those from *Slo3*$^{-/-}$ mice. The *Slo3* K^+ current is activated at depolarizing potentials, at alkaline pHi and blocked by clofilium. Considering the relatively large size of spermatogenic cells, they represent an excellent model to study *Slo3* channels possibly even allowing pharmacological studies using automated *patch-clamp* techniques.

Keywords: *Slo3* channel, spermatogenic cells, pHi, clofilium, *patch-clamp*.

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"The function of the Syk and STAT3 in the regulation of Interleukin 10 expression in human macrophages infected with *Mycobacterium bovis*"

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Its has been 132 years since Roberto Koch discovered the bacillus causing tuberculosis, *Mycobacterium tuberculosis* (Mtb). Mtbis aGram-positive bacterium, strictlyaerobic, slow growing, able to survivewithin macrophages. This pathogen is still a world-wide serious health problem, because its increased propagation among individual with acquired immunodeficiency syndrome and the appearance of drug resistant strains. It is well know that over one third of the global population is estimated to be infected with Mtb,causing almost two million deaths annually (according to theWorld Health Organization). In most cases, the immune responseagainst Mtb is sufficient to avoid developing active disease; however, complete destruction of the pathogen is frequently not achieved. Macrophages contribute to the elimination of bacilli via numerous mechanisms, including the successful acidification and maturation of phagosomes. However, Mtb ensures its survival within host macrophages by arresting the maturation pathway that leads to phagosome–lysosome fusion. Althought it is well known that INF-γ produced by T lymphocytes plays a key role against Mtb infection, Mtb promotes the expression of cytokines IL-10 and TGF-β that antagonize INF-γ production and functions, thus altering the normal immune response allowing successful infection. The signal transduction pathways activated by Mtb leading to IL-10 and TGF-β expression in macrophages, are poorly understood. However, recentlyit has been shown that Dectin-1 activates a signaling pathway involving tyrosine kinaseSyk leading to IL-10 expression in macrophages;in addition, it has been reported that Syk phosphorylates the transcription factor STAT3 promoting its transcriptional activity. Because of these data and the fact that the IL-10 promoter has functional STAT3 binding siteswhich mediate IL-10 expression in response to LPS stimulation, we predicted that Mtb-induced IL-10 expression involves the phosphorylation and activation of STAT3 by Syk in macrophages. Here we will discuss our data indicating that macrophages infection with *Mycobacterium bovis* promotes STAT3 tyrosine phosphorylation in a Syk-dependent manner. Current experiments are underway to show that IL-10 expression is regulated by STAT3 in response to *M. bovis* infection.

This work is partially supported by grants from ICEGEB (CRP/MEX11-01) and CONACYT (155290 and 154542).

Key words: Tuberculosis, STAT3, Syk, IL-10

The identification of kinases, cyclases and phosphatases in mitochondria from the human placenta.

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The human placenta produce progesterone without a hormonal stimulation identified until now. In addition, the mitochondria from the placenta haveparticularprocesses, like the cholesterol transport mechanism and its conversion to progesterone orthe protein phosphorylation and dephosphorylation. This last process apparently plays an important role in the regulation of steroidogenesis. In this work, we identified several mitochondrial cyclases, phosphodiesterases, kinases and phosphatases suggesting that these proteins are part of the signaling transduction phathway, associated to progesterone synthesis.
Mitochondria were purified of syncytiotrophoblast from human placenta andsubmitochondrial fractions obtained by differential centrifugation.Specific marker proteins were identified to evaluate the purity offractions.The activity of phosphatases and phosphodiesterases was determined in JEG-3cells (Human placental choriocarcinoma cell line), in the presence or absence of specific inhibitors.The following enzymes wereidentified:isoforms of adenylate cyclase enzymes (AC),kinase proteins (PINK, MEK, Akt 1/2/3), phosphodiesterases (PDE) and serine/threonine (PP2Cɣ) or tyrosine phosphatase (PTPMT1).
The adenylate cyclaseisoformswas distributed differentially in the submitochondrial fractions. AC9 was immunodetected in cytosol, mitochondrial membranes and matrix. AC3 and AC9 showed a lower molecular weight, suggest that AC's are susceptible to proteolysis process during purification procedure or, likes sAC, exists a truncated and dominant isoform that is more active than the full-length in the human placenta.
PTPMT1 and PP2Cɣ, were identified in the inner membrane. Since the system of phosphorylation/dephosphorylation of proteins is an important regulatory mechanism in any cellular process, the PP2C identification was important as a negative modulator of the MAPK pathway, dephosphorylating and inactivating kinase proteins at different levels in the signaling pathway. The adenylatecyclasesAC5/6 were identified in the trophoblast cytosolic fraction; this location makes sense because PKA, PKC and PP2B proteins are anchored into the plasma membrane, by the AKAP79/150 protein, closer to machinery components of the cAMP synthesis.
This project allows the study of the specific association between AC's proteins and kinase proteins to activate the cAMP-PKA pathway, and their association with the progesterone synthesis suggesting the involvement of this process in the steroidogenic.

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Importance of the nucleocytoplasmic transport cycle of the GTPase Gpn1 on the subcellular localization of RNAPII and RNAPII.

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RNA polymerase II (RNAPII) is a multi-enzymatic complex of 12 core subunits that synthesizes capped noncoding RNAs as well as all mRNAs in eukaryotic cells. Although multiple proteins are known to regulate the activity of RNAPII during transcription, little is known about the machinery that controls its assembly and nuclear import. Among the RNAPII associated proteins is the GTPase Gpn1 (Jeronimo et al., 2007, Mol Cell 27:262). Although the molecular mechanism is unknown, this GTPase is somehow necessary for RNAPII nuclear import (Forget et al., 2010, Mol Cell 9:2827). Our laboratory recently described a nuclear export sequence (NES) in Gpn1 that is both necessary and sufficient for nuclear export (Reyes-Pardo et al., 2012, BBA 1823: 1756). The physiological relevance of this NES for the function of endogenous Gpn1 is unknown. RNAPII-associated protein 2 (RPAP2) is a phosphatase that specifically removes the phosphate at Ser5 of the CTD in the largest RNAP II subunit. RPAP2 silencing provokes abnormal accumulation of RNAP II in the cytoplasmic space. RPAP2 is a mainly cytoplasmic protein that shuttles between the cytoplasm and the nucleus and physically associate with Gpn1 (Forget et al., 2013, NAR 10:1093).

The overall block of protein nuclear export by inhibiting the export factor Crm1 with leptomycin B (LMB) results in the retention of RNAPII in the cytoplasm (Forget et al., 2010), suggesting that a protein essential for RNAPII nuclear accumulation is trapped in the nucleus in the presence of LMB. However, since Crm1 is involved in the nuclear export of hundreds of substrates, the identity of this protein remains unknown. In this work we investigated the hypothesis that the critical protein for RNAPII nuclear import retained in the nucleus by LMB is the GTPase Gpn1. To test this hypothesis we evaluated the effect of replacing endogenous Gpn1 by a nuclear export deficient Gpn1 mutant form on the localization of RPAP2 and RNAPII. This molecular replacement strategy involves the sequential use of two retroviral vectors that confer resistance to two different antibiotics to first overexpress stably the wt o NES mutant form of Gpn1, followed by the suppression of endogenous Gpn1 with an shRNA. We will present and discuss the progress made in the development of this project.

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Injury signal molecules activate MAPK pathways to trigger asexual reproduction in *Trichoderma atroviride*

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Abstract

The response to mechanical damage is a crucial process in the survival of multicellular organisms and facilitates their adaptation to hostile environments. *Trichoderma atroviride*, a fungus of great importance in biological control, responds to mechanical damage with the activation of regenerative processes and asexual reproduction (conidiation). In this response, the production of reactive oxygen species (ROS) by a NADPH oxidase (Nox1/NoxR) complex is involved. To understand the downstream molecular mechanism of this response, we evaluated molecules such as extracellular ATP (eATP) and Ca^{+2} that could trigger wound-induced conidiation and investigated the activation of mitogen-activated protein kinase (MAPK) pathways induced by eATP, Ca^{+2} and ROS. We propose that molecules that trigger wound-induced conidiation such as eATP and Ca^{+2} are likely to represent damage-associated molecular patterns (DAMPs), which are released from the damaged hyphae. Here we show that eATP can promote Nox1-dependent production of ROS, and induce conidiation and the activation of a MAPK pathway. Mutants of the MAP Kinases Tmk1 and Tmk3 are affected in wound-induced conidiation. Phosphorylation of both Tmk1 and Tmk3 was triggered by eATP, in contrast Ca^{+2} signaling appears to participate downstream in an independent pathway. Interestingly, the fungus seems to sense eATP through an ATP specific receptor. Our data suggest that there are at least two DAMPs acting in injury-induced conidiation, namely, Ca^{+2} and eATP, which activate different pathways that converge in the regulation of asexual reproduction genes. *T. atroviride* exhibits a response to mechanical damage mechanism that in its early steps appears to contain conserved elements as they are also known from plants and animals.

Keywords: injury response, extracellular ATP (eATP), conidiation, Reactive oxygen species (ROS), Mitogen-activated protein kinase (MAPK), Calcium.

Disection of the protein-protein interaction between Sdo1 and Efl1 using yeast two-hybrid.

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Molecular recognition is a vital process in living organisms that comprises the specific interaccion of biological molecules through weak interactions. Processess such as cell signaling, synapsis, cell differentiation and even enzyme catalysis are mediated through molecular recognition. An example of the molecular recognition complexity is the synthesis of ribosomes. Ribosomes are the specialized molecular machines that decode the genetic information contained in the messenger RNA into proteins. In *S. cerevisiae* each ribosome is composed of two subunits; the small subunit (40S) contains 33 proteins and the 18S rRNA while the large subunit (60S) consists of 46 proteins and three rRNAs (5S, 5.8S and 25S).

Ribosome biognesis requieres the synthesis and modification of the 4 rRNA and the 79 structural proteins. This process is orchestrated by more than 200 accesory proteins that coordinate itsunidirectionality and correct assembly without being part of the mature particle. The proteins Sdo1 and Efl1 are two of such accesory proteins that participate in the final steps of the 60S subunit maturation occurring in the cytoplasm. Together these proteins promote the release of Tif6 from thesuface of the pre-60S subunit and its recicle to the nucleous. The presence of Tif6 on the ribosomal large subunit prevents its association with the small subunit and thus the formation of the functional ribosome. Sdo1 is a 250 amino acid protein with three domains; while domain I mediates the interaction with the ribosomal subunit the function of the other two is still largely unknown. On the other hand, Efl1 is homologous to the elongation factor 2 (EF-2) with a domain I responsible for nucleotide binding and four other domains.

In this work we studied the interaction between the proteins Sdo1 and Efl1 by yeast two-hybrid. We made several constructs of the different domains that comprise Sdo1 and Efl1, and combinations thereof, aided by the crystal structures of the archaeal Sdo1 and EF-2 respectively. The Sdo1 constructs were cloned into the pEG202 vector while those of Efl1 were introduced into the prey vector pACT2. Protein interactions were asessed by measuring the β-galactosidase activity. The results show that domains 2 and 3 of Sdo1 mediate the interaction with domains 1-2 of Efl1. Interestingly these findings are in agreement with Sdo1 modulating the affinity of Efl1 for guanine nucleotides since domain 1 of Efl1 contains the guanine nucleotide binding domain.

Gpn1 and Gpn3, GTPases involved in nuclear import of RNA polymerase II, mutually determine their subcellular distribution and protein levels.

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Gpn1 and Gpn3 constitute, together with Gpn2, a group of essential GTPasescalled simply Gpn, due to the universal presence of a glycine-proline-asparagine motif. These GTPases were identified by mass spectrometry as proteins that associate stably with RNA polymerase II (RNAPII). Interestingly, the effect of suppressing Gpn1/NPA3 on the gene expression pattern in yeast was practically identical to suppressing Gpn3/YLR243W. This similarity in the phenotype after inhibition of Gpn1 or Gpn3 suggests that these two GTPases function in the same pathway. In mammalian cells both, Gpn1 and Gpn3, are required for the nuclear accumulation of RNAPII. This function is conserved in the yeast *Saccharomyces cerevisae*, where nuclear targeting of RNAPII is also a process that depends on the yeast orthologs of Gpn1, NPA3, and Gpn3, YLR243W. Gpn1 and Gpn3 may play independent roles in the nuclear import of RNAPII or, alternatively, these two GTPases may somehow collaborate to perform a single function. Based on previous reports that Gpn1 and Gpn3 are able to interact *invitro* and in yeast two-hybrid screens, we explored here the extent of Gpn1-Gpn3 interaction in mammalian cells. We show by a subcellular relocalizationassay and by immunoprecipitation experiments, that transfected and endogenousGpn1 and Gpn3 interact strongly and stably. In addition, we demonstrate that the protein levels of either GTPase depend on the presence of the other. These resultsare consistent with the proposal that Gpn1 and Gpn3 are part of a single protein complex *in vivo,* and explain why interfering with the function of either protein produces the same phenotypic consequences in both, yeast and mammalian cells, i.e., cytoplasmic retention of RNAPII, transcription inhibition and cell growth arrest.

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Transcriptional profiling of pirfenidone reveals immunomodulatory pathways associated to Nrf2 activation in primary culture of human Hepatic Stellate Cells

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Background. Hepatic stellate cells (HSC) profibrogenic cytokines are key targets of anti-fibrotic therapies. 5-methyl-1-phenyl-2-(1H)-pyridone or pirfenidone (PFD) is a small molecule indicated for treatment of chronic inflammation and fibrogenesis. Oxidative stress is directly involved in the onset of hepatic fibrosis by HSC activation. **Aim.** In order to identify whether anti-inflammatory and anti-fibrogenic effects of PFD are related to activation of the endogenous antioxidant system, HSC were incubated with PDGF or 2-methyl-1,4-naphthoquinone (MEN) a ROS-inducer. **Methods and Results.** PFD was able to inhibit PDGF or MEN-induced pro-fibrogenic actions, including cell proliferation, cell motility and *de novo* synthesis of Collagen type I, TGFβ, TIMP-1, IL-1 and TNFα. These effects were associated with an increase of nuclear Nrf2 assessed by western blotting and confocal microscopy. Because PFD activates JNK, which stimulates Nrf2 transcriptional factor, through siRNA-mediated silencing we examined downstream antioxidant targets as antioxidant enzymes. JNK blockade by siRNA and SP600125 down-regulate Nrf2 activation. Also PFD induced a dose-and time-dependent activation of several antioxidant genes, (Glutamyl cysteine synthetase catalytic subunit, Glutamyl cysteine synthetase regulatory subunit and Heme oxygenase 1) and increase glutathione content, whose activity may contribute to the down-regulation of ROS-induced pro-fibrogenic and pro-inflammatory effects. **Conclusion.** These results provide molecular insights in anti-fibrogenic immunomodulatory action of PFD by counteracting ROS-induced pro-fibrogenic signalling, and by regulation of the biosynthesis of antioxidant proteins. This study indicates that activation of the antioxidant system plays an essential role in the modulation of inflammatory and fibrogenic cytokines in HSC. **Key words:** Hepatic stellate cells, pirfenidone, fibrosis, TGFβ, TNFα.

Analysisof the effect of*G.duodenalis*trophozoitestreated withTPCKon epithelialhomeostasisandonsignaling pathwaysusingamodel ofduodenal loopingerbils(*Merionesunguiculatus*)

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Giardiasis is an intestinal parasitic disease worldwide distributed whichis caused by *Giardia duodenalis.*The virulence factors of this parasite and the damage they may cause to the host have not yet been fully elucidated. We have previously reported thatTPCK-treated trophozoitessecrete a cathepsinlike B protease, inhibits adhesion of trophozoites to IEC-6 cell monolayers, affects the distribution pattern of claudin-1 and occludin and induce apoptosis in these cells. Recently we have found that inoculation of these trophozoitesin a duodenal loop in gerbils induced damage on intestinal epithelium. Thus in this work, we have analyzed the damage induced by *G. duodenalis*trophozoitespre-treated with TPCK on intestinal homeostasis This was approached by analyzingduodenal loop samples obtained by surgical intervention at 30 minutes, 1 h and 3 h post- inoculation of treated trophozoites for:a) intestinal epithelial cell damage by H&E staining of histological sections b) the expression of β-catenin (adherensjunction protein),p-H3 ser
28(proliferation marker) and MUC2 (a major glycoprotein in mucus)by indirect immunofluorescence and confocal microscopy and c) changes in the mTOR signaling pathway in the duodenal loop of the inoculated gerbils and by the use of temsirolimus(rapamycin analogue) prior to trophozoite inoculation.

The results showed that a significant damage was observed in sections stained with H & E at 3 h post-inoculationthe damage included, widespread areas of ulceration, presence of inflammatory infiltration in the lamina propria and mucus production. Furthermore, the expression of MUC2 at 30 min post inoculation was higher as compared to controlanimals and at 3 h pi the mucus was released covering the villi. At 30 min pi a disruption on the localization of β-catenin was observed and this protein was found in the cytoplasm. At 3 h. pi complete relocation of this protein to the lateral membrane was detected. Likewise, cell proliferation assessed by p-H3ser 28 was not altered. Finally, activation of the mTOR pathway by trophozoites treated with TPCK was observedin the experimental animals specifically mTORC1 (Ractor) complex, which is involved in the synthesis of proteins and suppression of autophagy. The activation of this complex was inhibited by temsirolimusand less damage on the dudodenal loop epithelial cells was observed when the loops were inoculated with the inhibitor prior to the inoculation of treated trophozoites. Also there were changes on the expression of β-catenin and MUC2 in animals which received the inhibitor and then inoculated with treated trophozoites as compared with control animals. Similarly the use of temsirolimusdid not alter cell proliferation in experimental animals.

All together theseresults showed that inoculation of TPCK pretreated trophozoites, which produce a cathepsin like B proteaseintothe duodenal loop of gerbils induces damage in duodenal epithelium,activates mTORC1 signaling pathway and alters the expression of β-catenin and MUC2. All these events affect the intestinal epithelium homeostasis and suggest that the cathepsin B like protease is a virulent factor in *Giardia.*

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Expression profile of Hsp90α and Hsp90β, and their involvement in signalling mediated by β-catenin on cervical cancer cell lines.

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The 90-kDa heat shock protein (Hsp90) is a major molecular chaperone of the cell. Their principal function is the maintenance of homeostasis, through promote the stability and activation of a specific group target proteins also known as “client” proteins.

Expression and location profile between two major cytoplasmic isoforms of Hsp90, Hsp90α and Hsp90β, suggest a differential functional involvement in several cellular processes. However, the precise function of these isoformsremains poorly understood, particularly their involvement in several diseases such as cancer.

Recently, we demonstrate that Hsp90α and Hsp90β protein have a differential role on the activity ofprotein kinase B or Akt. Aktis a major positive regulator of β-catenin signaling pathway by promoting the β-catenin stabilization, which plays a key role in the transcription of genes involved in cellular proliferation and migration in both normal and cancerous cells.

This work describe for the first time, the role of Hsp90α and Hsp90βon the regulation and activation of β-catenin, in cervical cancer cells. We identified two different patterns of expression of Hsp90α and Hsp90βprotein in cell lines of a same type of cancer, by Western blot. C33a (mutant-p53) and SiHacell lines (wild-type p53) shown Hsp90β over-expression, while HeLa(wild-type p53) and CaSki (wild-type p53) cell lines shown Hsp90α over-expression.

We also seen over-expression of activated oncoproteins “clients” of Hsp90:Akt and β-catenin.The activation mediated by phosphorylation of β-catenin and Akt was associated to the different patterns of Hsp90α and Hsp90βover-expressed on the cervical cancer cell lines. In a novel way, we found association between Hsp90β over-expression and the activation of Akt by phosphorylation on Ser473 residue, contrary to previous reports under physiological conditions.

Subcellular localization on nucleus of activated β-catenin, in three of the cell lines in this study, indicate the activation of signaling mediated by β-catenin.However, beside the presence of Hsp90α over-expression, the localization on HeLa and Caski was different, showing a more invasive phenotype on the HeLa cells where b-catenin was exclusively on the nucleus, possibly associated to Hsp90a also in nucleus.
Finally, the changes on the expression of Hsp90α and Hsp90β showed an important role on the migration capacity of the cervical cancer cells under treatment with Hsp90 inhibitor, evaluated by cell migration scratch assay.

The AMP-activated protein kinase (AMPK) is involved in the regulation of lipid metabolism in *Caenorhabditis elegans*

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INTRODUCTION: Deregulation of energy homeostasis is implicated in the development of several common human chronic diseases such as obesity, diabetes and metabolic syndrome. The components of signaling pathways that regulates lipid metabolism are complex and many of them are evolutionarily conserved among mammals and model organisms such as the nematode *Caenorhabditis elegans*. In mammals the AMP-activated protein (AMPK; AAK in *C. elegans*),regulates energy utilization by inhibiting key enzymes of fatty acids (FA) synthesis and activatingthoseinvolved in their oxidation.In *C. elegans*FA metabolism is regulated by transcription factors such as SBP-1, NHR-49, and MDT-15, whose orthologs in mammals are SREBP, PPARa, and PGC-1a, which modulate the activity and/or expression of key components as transporters, elongases and desaturases of FA.Because of their evolutionary conservation, it is plausible to suggest that SBP-1, NHR-49, and MDT-15 contribute to the regulation of FA synthesis by AAK activation in *C. elegans*.**AIM**: To evaluate the participation of SBP-1, NHR-49, and MDT-15 in modulating fatty acid synthesis by pharmacological AAK activation in *C. elegans*. **METHODS**: Briefly, wild-typeN2 nematodes were treated with AICAR (1mM) or metformin (50mM) and AAK activation was assessed by Western Blot.Total FA (TFA) were extracted from treated nematodes with chloroform-methanol (2:1) to obtain FA profiles by gas chromatography. Also, gene expression of *sbp-1*, *nhr-49*, *mdt-5* and some of their targets was achieved by qRT- PCR.**RESULTS**: We found that both AICAR and metformin activated AAK generated changes in the composition of TFA with a marked decrease in polyunsaturated FA.We obtained the elongases and desaturases indirect activities from the substrate/product ratios of FAprofilesand found the elongaseactivity target of SBP-1(ELO-1) diminished, as well as the desaturase activity target of MDT-15 (FAT-2), while the desaturase targets of NHR-49 linked to FA oxidation (FAT-5, 6, and 7) were increased. AAK activation also diminishedthe expression of *mdt*-15 and fasn-*1* (gene target of *sbp-1*), while the expression of *nhr-49* was increased. **CONCLUSIONS**: Altogether, these alterations suggestthat AKK activation, as it occurs in mammals, is linked to a decrease in the FA biosynthetic pathway mediated by SBP-1, NHR-49 and MDT-15 and their targets.This evidence supports that *C. elegans* could be used as tool to study metabolic regulation at molecular level *in vivo* to bring out new insights in treatment of human diseases such as obesity and metabolic syndrome.

Phospholipases C and D modulate vanillin production elicited by salicylic acid in *Capsicum chinense*Jacq.

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The signal transduction through the phospholipids is mediated by phospholipases, which catalyze the hydrolysis of structural phospholipids of the plasma membrane to produce second messengers, such as phosphatidylinositol 4, 5 bisphosphate (PIP_2). The phospholipase C (PLC) reaction generates two products, the inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG), meanwhile phospholipase D (PLD) hydrolyzes phosphatidylcholine to produce phosphatidic acid (PA), which have second messenger activity. Phospholipid signaling is also involved in plant responses to phytohormones such as salicylic acid (SA). The goal of this work is to understand whether there is a correspondence between phospholipid signaling and the production of vanillin through the regulation of phenylalanine ammonia lyase (PAL) in suspension cells of *Capsicumchinense* through the application of inhibitors of PLC (neomycin, U73122) and PLD signaling (1-butanol). Salicylic acid was found to elicit PAL activity, and consequently, vanillinproduction, which was diminished upon exposure to the PLC inhibitors (neomycin and U73122) and PLD inhibitor (1-Butanol). Our results suggest that PLC and PLD-generatedsecond messengers may be modulating SA-induced vanillin production through the activation of PAL. SA levels were evaluated in cells treated with the inhibitors and it was found that basal SA levels remained unchanged in cells treated with the inhibitor alone.Finally, the effect of SA in the expression levels of PAL was evaluated. Our results showed that regulation could be occurring at the transcriptional level in response to SA, and therefore, causing an increase in PAL enzymatic activity and thus an increase in vanillin levels.This work is funded by the project No. 98352 and 126769 Conacyt scholarship for BARJ.

The mating heterotrimeric G protein participates in the yeast endoplasmic reticulum stress response.

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Endoplasmic Reticulum Stress (ERS) occurs when misfolded proteins accumulate in the ER lumen. This triggers the Unfolded Protein Response (UPR) pathway which is mediated by Ire1p in *Saccharomyces cerevisiae*. Ire1p is a protein kinase that has riboendonuclease activity. As a result of misfolded proteins accumulation, Ire1p auto-transphosphorylates and activates its RNAse domain, which in turn, splices the *HAC1* mRNA. Hac1p is a transcription factor that activates transcription of various genes (including chaperones) in order to restore reticulum homeostasis. In case the UPR fails to restore normal ER activity, some other pathways are activated, including programmed cell death.

S. cerevisiae has one heterotrimeric G protein involved in the pheromone response pathway. It consists of Gα (Gpa1p), Gβ (Ste4p) and Gγ (Ste18p) subunits. In response to mating factor, Gα subunit dissociates from the Gβγ dimer, which in turn activates a downstream MAPK cascade that leads to haploid cell mating.

Here we present evidence that the yeast heterotrimeric G protein is involved in the ER stress response program. A cell lacking the Gγ subunit is remarkably more resistant to ERS inductors such as tunicamycin and 2- Deoxy-*D*-glucose than the wild type strain. Compared to wild type cells, a Δ*ste18* mutant has a more active unfolded protein response, accumulates less ROS and has a delayed apoptosis response when treated with ERS inductors. Finally, we found that treatment with ERS inductors does not allow yeast cells to respond properly to mating pheromone.

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The role of protein E6 and its small isoform E6*I of HPV-18 in the Wnt signaling pathway

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Cervical cancer is the second cause in cancer deaths among Mexican women. Persistent infection with high risk Human Papillomavirus (HPV) is the main risk factor associated to cervical cancer development, and the oncogenic process is mainly attributed to the expression of E6 and E7 oncoproteins. Within other mechanisms that have been proposed contributing to cervical tumorigenesis is the activation of Wnt signaling pathway, where E6 oncoprotein has been recently linked, but its participation remains unclear. There is evidence that E6*I, an isoform of HPV-18 E6 protein generated by alternative splicing, could be regulating pathways involved in cell proliferation in an E6 independent manner, affecting the biological behavior and oncogenic capacity. Moreover, the involvement of E6*I in the modulation of Wnt signaling pathway has not been studied.

HeK293 Cells were transiently transfected with expression plasmids containing HPV18-E6WT (Wild Type), E6SM (Splice Mutant) and E6*I gene fragments. Total proteins lysates and mRNA were collected and detection of targets of the Wnt pathway where performed by immunoblot and RT-PCR assays. To observe the effect of E6 and E6*I in the modulation of Wnt signaling pathway, the protein levels and transcript expression of target proteins such as c-Jun and β-catenin, were measured. The levels of c-Jun protein increased in presence of E6WT and E6SM, however E6*I did not modulate the expression of these proteins. Surprisingly β-catenin was up-regulated by E6WT and E6SM as well as by E6*Iin protein levels.

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Biotin deficiency induces a decrease in mitochondrial mass mediated by the activation of Akt in liver

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Biotin is a water-soluble vitamin that functions as the prosthetic group of all the carboxylating enzymes in the cell. The inability of an organism to recycle biotin leads to the depletion of this cofactor and is the lying cause of a metabolic disorder termed Multiple Carboxylase Deficiency (MCD). Previous data from our research group discovered that experimentally induced biotin deficiency causes an alteration in energy metabolism in rat hepatocytes. In order to investigate the underlying mechanism of this phenomenon we evaluated mitochondrial function after biotin starvation. Our results show that oxidative phosphorylation is down-regulated in isolated mitochondria and intact hepatocytes concomitantly with carboxylation enzyme depletion. In the latter conditions, a reduction in mitochondrial mass was confirmed through cytochrome quantification andthe evaluation of both biochemical (citrate synthase activity) and molecular markers (PGC-1α and TFAM). Upon biotin deficiency we observed the activation of the PI3K pathway through the phosphorylation of Akt in its Thr308 residue. A time-course analysis after biotin starvation in a hepatoma cell line revealed that Akt activation precedes the decrease of mitochondrial mass. Furthermore,the inhibition of Akt delays the down-regulation of oxidative phsophorylation and the decreased levels of PGC-1α, TFAM and citrate synthase induced by biotin deficiency. We also analyzed the mitochondrial mass markers in the livers of Biotinidase knock-out mice, an experimental model for MCD, and we confirmed that Akt is also activated concomitantly with a diminished mitochondrial content. Altogether, our results demonstrate a direct role of the Akt kinase in the modulation of energy metabolism, particularly in the down-regulation of mitochondrial mass.These findings are relevant, not only in the context of multiple carboxylase deficiency, but also they might be significant in other metabolic inborn error in which mitochondrial metabolism is impaired.

Key Words: Multiple carboxylase deficiency, biotin deficiency, AKT, mitochondrial biogenesis.

The yeast two-component response regulator Skn7 participates in the response to tunicamycin induced stress.

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Skn7p is a response regulator of a phosphorelay system present in *Saccharomyces cerevisiae*. Being an Hsf1-like transcription factor, its functions range from controlling cell wall homeostasis to oxidative stress survival. Skn7p activation is regulated by phosphorylation of its Aspartate-427 (D^{427}), although has been reported also that phosphorylation in various Threonine residues at its receiver domain is essential for its activity in response to oxidants.

The *S. cerevisiae* two-component system belongs to the Sln1p branch of the High Osmolarity Glycerol (HOG) pathway. Various components of this pathway had been shown to be important in the resistance to endoplasmic reticulum stress (ERS) inducers such as tunicamycin (Tn) and 2-Deoxy-D-glucose (2-DO).

ERS is originated when misfolded proteins accumulate in the organelle lumen, leading to activation of the Unfolded Protein Response (UPR), which activates transcription of several genes, including those encoding chaperones. This allows the cell to modulate the ER folding capacity in order to relieve the stress.

Here we present evidence that the yeast Skn7 protein is involved in the cell's ability to survive stress induced by Tn and 2-DO. A null mutant lacking *SKN7* shows a decreased capacity to grow in media supplemented with either Tn or 2-DO. The putative role of Skn7p in the resistance to ERS inducers seems to be independent of the HOG pathway. Finally we mutated the D^{427} residue to Alanine in the Skn7 protein, and introduced it into the Δ*skn7* mutant. We found that this residue is not required for Tn and 2-DO response, suggesting that survival to ERS inducers does not need an active phosphorelay system.

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Interaction of Gonadotropin-releasing hormone receptor with protein calnexin depends of the species.

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Proteins are synthesized and processed in the reticulum endoplasmic (ER) and Golgi apparatus. Quality control system (QCS), check the integrity and correct folding of new proteins in the ER. The calnexin is among the chaperone proteins of the QCS. The G-protein coupled receptors (GPCR) are synthesized in the ER like others proteins. Human gonadotropin hormone receptor (GnRHR) is part of the family of GPCR. The GnRHR is stabilizated by Cys^{14}-Cys^{200} bridge required by the cellular QCS. Previous studies showed that there are differences in the GnRHR amino acid sequence among species. One of the most important differences is the presence of Lys^{191} in the human, which is absence in mouse and rat. Another important difference between GnRHR species is the interaction with calnexin. There are 4 amino no-contiguos “motif” (Leu^{112}, Gln^{208}, Leu^{300}, Asp^{302}), that participate in the stabilization of Cys^{14}-Cys^{200} bridge. The results shows that the deletion of Lys^{191} in the human GnRHR (hGnRHR) increased the inositol phosphate (IP) production compare with WT hGnRHR. When rat GnRHR contains Lys^{191} the IP production decreased considerably compare with WT rat GnRHR. The expression and the IP production of hGnRHR decreased ~50% when is co-expressed with calnexin. When rat GnRHR is co-expressed with calnexin the IP production decreased only ~10%. When the human sequence contains the rat motif, IP production is closer to that of rat GnRHR when is co-expressed with calnexin. Here we showed the participation of the Cys^{14}-Cys^{200} bridge over the expression and IP production of GnRHR. The motif sequence appears to be a determinant of calnexin recognition.

Interferon Stimulated Gene 12 is a negative regulator of the Estrogen Receptor Alpha/Estradiol signaling

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The estrogen receptor alpha (ERα) is a transcription factor that mediates the effects of 17β- estradiol (E2) in normal mammary gland and tumorigenic breast tissue. ERα transcriptional activity is regulated by the interaction with coactivator and corepressor proteins. However, the expression, stability and subcellular localization of ERα have also consequences on its transcriptional function. We searched for proteins that interacts with ERα by yeast two hybrid screen. In this study, we identified Interferon Stimulated Gene 12 (ISG12) as a novel ERα associated protein. We demonstrated that ISG12 interacts with ERα *in vivo*. Overexpression of ISG12 in breast cancer MCF7 cells produced a decrease in ERαtransactivation. Suppression of ISG12 expression in MCF7 cells by siRNA resulted in up-regulation of ERα-mediated transcription compared to control MCF7 cells. We show that ISG12 function as a repressor of ERα is not mediated by the enzymatic activity of histone deacetylases. In contrast, we found that ISG12 inhibits the transcriptional activity of ERα by mediating its nuclear export. Interestingly, ERαbound to the promoter for ISG12and increased its expression in breast cancer cells.Thus, we identified that E2 signaling induces the ISG12 expression as part of feedback loop to control the transcriptional activity of ERα by a mechanism that involve the nuclear exclusion of ERαand subsequently the inhibition of the expression of E2 target genes in breast cancer cells.

Akt behavior in different tissues at early stages of an experimental model of renal carcinogenesis and effect of a tamarind seed extract

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Renal cell carcinoma (RCC) is the most common neoplasm of theadult kidney,it is asymptomatic even at advanced stages, hence initial diagnosis frequently occurs when metastasis is present and studies at initial developmental phases become almost impossible; therefore, an experimental model may be useful for this purpose such as that *N*-diethylnitrosamine (DEN)-initiated and ferric nitrilotriacetate (FeNTA)-promoted. The tumors obtained after 4 months of FeNTA exposition are histologically similar to the human RCC, and no primary tumors are induced in other tissues (*e.g.*liver and lung). Throughout carcinogenesis protocol, different preneoplastic lesions and pro-carcinogenic alterations were observed at 1 and 2 months of FeNTA treatment, suggesting these timesas early stages of the malignant transformation process. On the other hand, oxidative stress participation is well established in this model as well as in the three major risk factors for developing human RCC (smoke, obesity and hypertension). In fact, we have previously demonstrated that the administration of a phenols rich tamarind seed extract (TSE) as antioxidant, delayed RCC progress and decreased tumor incidence.

Akt, also known as PKB, has a key role in cellular survival, growth and proliferation. An increase intotal protein levels (t-Akt) and in its phosphorylated form in serine 473 (p-Akt), hasbeen reported in RCC tumors, as in many other types of cancer, but its response throughoutoncogenesis remains unknown.

In the present study, we characterized the behavior of Aktat early stages of FeNTA- induced RCC model and evaluated the effect of TSE administration. For this, male Wistar rats were distributed in Control (C), and treated with: TSE (T), DEN (D), FeNTA (F), DEN+FeNTA (DF)and TSE+DEN+FeNTA(TDF) groups.TSE was given orally two weeks prior to DEN administration and throughout the experiment. Fourteen days after, DEN initiated animals were treated with FeNTA twice a week during 1 and 2 months. Tissues (kidney, liver and lung) were obtained 48 hours after last FeNTA injection.

Kidney levels of t-Akt and p-Akt increased by 6 times after 1month of FeNTA treatment*vs*. control groups, and this increase persists after 2 months. Very interestingly, this behavior observed in kidney, was not found in liver or lung.

On the other hand, kidney t-Akt and p-Akt levels were similar in DF and TDF groups, which mean that TSE did not prevent the increase inAkt renal levels induced by FeNTA at the studies times.

This is the first report that describes the behavior of Aktat early stages of renal carcinogenesis induced by FeNTA, and it is consistent with that observed in human RCC tumors. This, together with the fact that no alterations in Akt levels were present in liver or lungs, where no primary tumors are induced with the followed FeNTA treatment, strongly support the probable participation of this kinase in kidney carcinogenesis in this experimental model.Also, our results suggest that Akt alterations seem not to bepart of the mechanisms whereby the extract protects against RCC development; however, it cannot be ruled out that the TSE may have an effect on Akt at other stages.

The other side of the moon: H_20_2 as a second messenger for adrenaline

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NADPH oxidase (Nox) 2 in hepatocytes catalyzes the electron transport of cytosolic NADPH to molecular oxygen from the extracelular space and generates $O_2^{\cdot -}$, which dismutates to H_2O_2 recovered in the extracelular space. Cellular uptake of this H_2O_2 in liver is mediated by aquaporine (AQ) 8. Previous studies in the laboratory showed that adrenaline modulates Nox2 in liver cells, α1 adrenergic receptors (ARs) activation increses H_2O_2 synthesis, βARs activation decreases H_2O_2 pools, and stimulates the rates of the 3 metabolic routes (gluconeogenesis, ureogenesis, and glycogenolysis), an action impaired by added H_20_2 (Ki 0.1 uM). This work expands such information and search to integrate most results. Thus 1) αARs activate the three metabolic routes, only if H_2O_2 is freely incorporated into the cell. 2) H_2O_2-mediated inhibitory action on the βARs-mediated activation of the three metabolic routes was blocked with AQ8 antibodies, but not with AQ3 antibodies. It is concluded that H_2O_2 is an additional second messenger for adrenaline actions, at least in hepatocytes. After αARs activation, H_2O_2 is formed and it is absolutely required to obtain the well-known increase in the three metabolic routes, additionally H_2O_2 prevents βARs responses. After βARs activation, a lower H_2O_2 pool is generated, which favored metabolic routes ligthed on by βARs activation, and prevents αARs responses.

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Analysis of the damage to intestinal epithelial cells by purified cathepsin B like from *Giardia duodenalis* and its effect on signaling pathways that modulate intestinal epithelial homeostasis using the duodenal loop ligation model in gerbils (*Merionesunguiculatus*)

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Giardia duodenalis is a parasite with high prevalence worldwide.This parasite colonizes the small intestine of humans and other mammals. Upon attachment of *Giardia*to the intestinal epitheliumthe parasite secretes various molecules which may play an important role in host-parasite interactions. In our groupa cathepsin B like from *G. duodenalis* protein was characterized, this protease induces*in vitro* alterationsin the distribution of proteins of intercellular junctionsand cell apoptosis when added to epithelial cell monolayer. In this work the effect of the purified cathepsin B like form *Giardia* was analyzed using a duodenal loop in gerbils as experimental model.

To approach this, a defined amount of the purified protein was inoculated into the duodenal loop, as control DMEM medium was inoculated. After 5 and 15 minutes of interaction theduodenal loops were removed and divided into three fragments. Each fragment was separately analyzed for: a)damage caused by this protease as assessed by staining histologic sections with hematoxylin and eosin; b), detection of protein such as p-histone ser 28 related with proliferation using immunofluorescence techniques and confocal microscopy and c) detection of changes in the mTOR signaling pathway by Western blot and the inhibitor temsirolimus.

RESULTS. CathepsinB like from *Giardia*induced alteration of the duodenal epithelium, assessed bythe alteration of the architecture of the villi, cellular infiltration of immune cells into the lamina propria, edema and hemorrhagic zones. Also it was determined that the cathepsin B like induced alteration of the adherent junction protein β-catenin within the first 5 minutes, but after 15 minutes of interaction the distribution of this protein was restored. Interestingly, an increased secretion of MUC2 was detected, and no marked changes in cell proliferation in the intestinal crypts were observed in cathepsin treated duodenal loops. Finally,Western blot assays showed that cathepsin B-like induces expression of proteins the mTORC1 pathway which is involved in protein synthesis survival within the intestinal epithelium.Activation of this complex is indeed induced by the cathepsin B like protein since this complex was not detected when the inhibitor temsirolimus was inoculated in duodenal loopsprior to the inoculation of cathepsin.B like protease.

All together these results showed that the cathepsin B like from Giardia, causes severe damage to the intestinal epihteliumby altering the intestinal homeostasisin the duodenal loop ligation model and under the conditions used in this study. Thus the cathepsin B like may be considered a virulence factor from *Giardia duodenalis.*

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Identification of an arginine-phospho tyrosine based endoplasmic retention motif in a G-protein activated potassium channel

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G-protein regulated inwardly-rectifier potassium channels (GIRK) are activated by heterotrimeric G_i/G_o coupled receptors. The Gβγ dimers bind directly and open the GIRK channels. To date four GIRK (Kir3 family) subunits, designated GIRK1 to GIRK4, have been identified in mammals, and GIRK5 has been found in *Xenopus laevis* oocytes. In contrast to its mammalian homologues, GIRK5 contains a longer NH2 terminal. GIRK channels are expressed in heart and brain and contribute to the maintenance of the resting membrane potential. GIRK channels have different trafficking motifs that direct them into the biosynthetic pathway as well as between the endosomal compartments and the plasma membrane. Previously, we found that phosphorylation of a tyrosine (Y16) at the N-term of GIRK5, determines its functional expression (Mora and Escobar, 2005). Furthermore, wt-GIRK5 channels are retained in the endoplasmic reticulum (ER) and a di-leucine motif targets the phospho-null Y16A mutant to the vegetal pole of the oocytes (Diaz- Bello and col. 2013). Two ER retention motifs have been characterized: a di-lysine-based (KKXX or XKXK) and a di-arginine-based (RRXX or XRRX), located near the C- term and the N-term; respectively. The sequence $K^{13}R^{14}X$ is localized downstream of Y16. Therefore, we aim to explore the role of this lysine-arginine sequence in the GIRK5 functional expression in oocytes. We performed an alanine scanning mutagenesis and whole cell voltage-clamp recordings. The single mutant K13A did not produce functional channels. In contrast, R14A and R14A/Y16A displayed inwardly rectifying currents. In conclusion, our results suggest that the sequence $R^{14}XY^{16}$ acts as an “arginine-phospho-tyrosine” retention motif.

The proto-oncoprotein SnoN is downregulated by the antibiotics anisomycin and puromicyn via proteasome

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SnoN is an important negative regulator of TGF-β pathway and is involved in several cellular processes such as proliferation, differentiation, aging, and cancer. SnoN protein has dual role acting as an oncoprotein or as a tumor supressor.

TGF-β pathway acts through the activation of Smad 2/3 transcriptional factors, and can regulates the SnoN protein stability by inducing their degradation via proteasome with the participation of phosphorylated Smad2/3 proteins that act as adaptadors for different E3 ligases. Intriguingly, the antibiotics anisomycin (ANS) and puromycin (PURO) are also able to downregulate to SnoN protein via proteasome.

In this work, we explored the effects of ANS and PURO on SnoN protein downregulation, when the activity of TGF-β signaling was inhibited by using different pharmacological and non-pharmacological approaches, either by using specific TβRI inhibitors, overexpressing the inhibitory Smad7 protein, or knocking-down TβRI receptor or Smad2 by specific shRNAs.

The results show that SnoN protein downregulation induced by ANS and PURO did not involve the induction of R-Smad phosphorylation but it was abrogated after TGF-β signaling inhibition; this effect occurred in a cell type-specific manner and independently of protein synthesis inhibition or any other ribotoxic effect. However, the antibiotics ANS and PURO require TGF-β/Smad pathway to induce SnoN protein downregulation independently of inducing R-Smad2 phosphorylation, and their effect facilitate gene transcription induced by TGF-β signaling.

Our conclusions indicate that antibiotic analogs lacking ribotoxic effects can be used as pharmacological tools to study TGF-β signaling by controlling SnoN protein levels.

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Study of the histidine protein kinase RpfC2 in *Azotobacter vinelandii*

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Azotobacter vinelandii is a nitrogen-fixing soil bacterium that produces an extracellular polysaccharide (EPS) called alginate. The polymer production is controlled by the two-component signal transduction system GacS/GacA (1).

In *Pseudomonas aeruginosa*, GacS/GacA, RetS and LadS network, regulates expression of virulence genes associated with acute or chronic infection (2).

Searching for RetS and LadS homologues in *A. vinelandii*, the group of work found one homologue to RetS, two for GacS and three for LadS histidine kinases, which were called LadS1, LadS2 and LadS3. Posterior bioinformatic analysis revealed a higher homology between LadS2 and RpfC (histidine kinase HK from the *Xanthomonas campestris* complex RpfC/G) which was confirmed by analyzing the genomic context, reason why we renamed the ladS2 HK to RpfC2 in *A. vinelandii*. In *Xanthomonas campestris* (Xcc), EPS are regulated by the RpfC/RpfG system, whose control is also seen in the production of virulence factors and biofilm formation (3).

In this work, we have generated polar and non polar mutants for the RpfC2 HK and its response regulator RpfL. This *A. vinelandii* mutants were employed to figured out if RpfC2 is involved in alginate production, motility and biofilm formation.

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HER3/HER2 heterodimer activates PI3K but not the Akt signaling pathway in cervical cancer cells

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The epidermal growth factor receptor family ErbB/HER, is involved in cell proliferation, differentiation, cell survival, and apoptosis. All members integrate an especial system of homodimer and heterodimer formation, activated by numerous and specific ligands. The ligands for ErbB receptor binds to the extracellular domain, promote a conformational switch that positions the C-terminal cytoplasmic tail of one receptor near the activation loop of the other, thereby facilitating phosphorylation in trans resulting in receptor activation by homodimers or heterodimers formation. Phosphorylation of tyrosine residues serves as docking sites for recruitment of diverse adaptor proteins containing SH2 and PTB domains. All these adaptors are often themselves targets of ErbB receptors or by citoplasmic kinases activating downstream signaling pathways including Ras-MAPK and PI3K/Akt, whose aberrant activation plays an important role in progression to tumorigenesis. Our work group has demonstrated the presence of a constitutive active form of HER2 in cervical cancer cell lines, which is important for their proliferation. In the present work we aimed to detect the presence of HER3/HER2 dimers and to evaluate its participation in the activation of the PI3K/Akt signalling pathway. To determine the presence of the heterodimer we used confocal microscopy and co-precipitation assays. Our results show the presence and co-localization of both receptors HER3/HER2. Moreover, using co-precipitation assays the interaction between both membrane receptors is shown suggesting the formation of the HER2/HER3 heterodimer. To determine the participation of HER3/HER2 heterodimer in the activation of the PI3/Akt signaling pathway, cervical cancer cell lines CALO and INBL were treated with NRG1b, a natural ligand of HER3. The results show that HER3 is phosphorylated as well as PI3K. Interestingly, Akt is constitutively active in both cervical cancer cell lines. To determine if PI3K participates in the activation of Akt, cervical cancer cells were incubated in the presence of wortmannine and results show that the constitutive phosphorylation of Akt does not change. Therefore, our results suggest that the PI3K/Akt signaling pathway is active, but PI3K does not participate in the activation of Akt which implies the possibility that other kinases, like mTOR, could activate downstream of PI3K the Akt signaling pathway in cervical cancer cells.

Phosphorylation and internalization of short splicing variant of the omega 3 fatty acid sensor, GPR120

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Abstract: GPR120, also known as Free Fatty Acid Receptor 4, is a recently deorphanized G protein-coupled receptor that seems to play cardinal roles in the regulation of metabolism and in the pathophysiology of inflammatory and metabolic disorders. In the present work a GPR120-Venus fusion protein was expressed in HEK293 Flp-In T-REx cells and its function (increase in intracellular calcium) and phosphorylation were studied. It was observed that the fusion protein migrated in sodium dodecyl sulfate-polyacrylamide gels as a band with a mass of ≈ 70-75 kDa, although other bands of higher apparent weight (> 130 kDa) were also detected. Cell stimulation with docosahexaenoic acid or α-linolenic acid induced concentration-dependent increases in intracellular calcium and GPR120 phosphorylation. Activation of protein kinase C with phorbol esters also induced marked receptor phosphorylation but did not alter the ability of 1 μM docosahexaenoic acid to increase the intracellular calcium concentration. Phorbol ester-induced GPR120 phosphorylation, but not that induced with docosahexaenoic acid, was blocked by protein kinase C inhibitors (bis-indolyl-maleimide I and Gö 6976) suggesting that conventional kinase isoforms mediate this action. The absence of effect of protein kinase C inhibitors on agonist-induced GPR120 phosphorylation indicates that this kinase does not play a major role in agonist-induced receptor phosphorylation. Docosahexaenoic acid action was associated with marked GPR120 internalization whereas that induced with phorbol esters was small.
A GPR120-Venus cell line also expressing α_{1B}-DsRed receptor construct was generated in order to depict receptor internalization as evidence of receptor crosstalk. Preliminary results show that after stimulation with noradrenaline, both receptors internalize, whereas after DHA stimulation only GPR120 internalize. PMA also induced both receptors to internalize. These results hint PKC as a kinase participating in heterologous regulation of GPR120S.

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ADIPOCYTES SHOW ALTERATIONS IN THE SIGNALING PATHWAYS THAT CONTROL GLUCOSE TRANSPORT IN A MODEL OF METABOLIC SYNDROME

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BACKGROUND: Metabolic syndrome (MS) is a group of risk factors that increase the possibility of developing Diabetes Mellitus type II and cardiovascular diseases. (1) Within these risk factors, insulin resistance stands out since it had been considered a key element in the pathophysiology of MS and has been postulated as its underlying cause.

The adipocyte is one of the principal sites of postprandial glucose capture, expressing high levels of Glut4. (2) Glut4 plays a key role in glucose homeostasis by facilitating its entry into the adipocyte.There are two mechanisms that induce Glut4 translocation to the cell membrane, the insulin and AMPK signaling cascades. Insulin and metformin, by activating the AKT and AMPK pathways respectively, modulate glucose transport in the adipocyte by controlling the subcellular location of Glut4 and contribute to the reduction of postprandial plasma glucose (3). **OBJECTIVE:** Evaluate the possible alterations in the insulin and AMPK signaling cascades and their impact on glucose capture by transport mediated by Glut4 in a model of MS.

METHODS: MS was induced in a group of Wistar rats by adding 30% sucrose in their drinking water during 16 weeks. Evaluation of insulin and AMPK signaling pathways was performed by the Western Blot in isolated adipocytes stimulated with insulin and metformin, respectively from MS rats and matched control animals receiving only tap water. Glucose uptake was studied using a fluorescent glucose analog (2-NBDG) by measuring intracellular fluorescence.

RESULTS: Activation of the insulin cascade, evaluated by the phosphorylation state of AKT was reduced in adipocytes from rats with MS. In the same manner, the activation of the AMPK pathway by its phosphorylation state was diminished in adipocytes from MS rats. 2-NBDG uptake was less efficient to the stimulus with insulin in adipocytes from rats with MS compared to the control condition.

CONCLUSIONS: The insulin and AMPK cascades are altered in adipocytes from Wistar rats with MS and these alterations reduce glucose transport mediated by the Glut4 transporter, contributing to the glucose impaired homeostasis.

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Toll-like receptor 4 activation promotes epithelial-mesenchymal transition and progression in breast cancer

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Breast cancer is a common malignancy in women and is also a leading cause of cancer death in the less development countries of the world; metastasis is the most critical related aspect. Several basic experimental, clinical and epidemiologic studies show inflammation participation on tumorigenic cells conversion to aggressiveness and malignant phenotype. There isevidence that describe the epithelial-mesenchymal transition (EMT) induction in cancer cells by inflammatory microenvironment; EMT is a critical step in a metastasis process. Toll-like receptors expression, TLR-4 specially, plays important roles in migration and metastasis of breast cancer, although the exact molecular mechanism remains elusive.

In this study, we evaluated the LPS effect, an agonist exogenous TLR4 receptor, on epithelial- mesenchymal transition and migratory capacity in MDA-MB-231 brest cancer cell line. Results to date prove an increase in *Snail-1* and Vimentin mRNA expression levels, both EMT markers, due to LPS-dependent TLR4 activation. We furthersight Nav 1.5 sodium cannel α subunit increasein expression levels; this is membrane protein associated with the migratory capacity.

These results support the hypothesis that inflammatory condition is capable factor of development and cancer progression exacerbation.

Characterization of signaling pathways that allow to mast cells to respond against bacterial compounds.

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Mast cells (MC) play an important role in allergies and other acute inflammatory conditions. Binding of monomeric IgE to its high affinity receptor (Fc RI) leads to cell maturation and proliferation. Crosslinking of Fc RI with IgE/Antigen complexes induces the rapid secretion (degranulation) of a number of pro-inflammatory mediators, such as histamine, -hexosaminidase, serotonin and tumor necrosis factor (TNF), which are stored in specialized secretory granules. Few hours after Fc RI crosslinking, *de novo* synthesis of cytokine mRNA leads to the production of diverse cytokines that regulate the late phase of allergic reactions. Recent evidence shows that MC are also able to initiate protective immune reactions against distinct pathogens, such as virus, parasites and Gram-negative bacteria. Stimulation of distinct Toll-like (TLR) receptors on MC plasma membrane leads to cytokine release without degranulation. Despite those observations, the molecular mechanisms behind the activation of MC by non-allergic stimuli remain poorly described.

In this work we utilized the rat MC line RBL-2H3 and murine bone marrow-derived mast cells (BMMCs) to analyze the signaling mechanisms connecting TLR-4 activation to the secretion of preformed and neo-formed mediators. Using RT-PCR and flow cytometry it was observed that expression of TLR-4 is not always accompanied by CD-14 or MD-2 synthesis. However, sensitization with monomeric IgE importantly improves the capacity of MC to respond to ligands of TLR-4 receptors. Differential expression of CD-14 and MD-2 lead to important differences on the capacity of MC to secrete TNF in response to rough, semi-rough and smooth types of bacterial lipopolysaccharides (LPS). Manipulation of the secretory machinery with siRNAs against specific SNAREs shows that TLR-4-dependent cytokine secretion requires specific VAMP proteins. Our results show that MC responsiveness to innate immunity stimuli is affected by low-intensity stimulation of adaptive immunity receptors, such as Fc RI.

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Role of Wnt signaling into cellular subpopulation with increased ALDH activity from cervical cell lines

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The tumors display an intra-tumoral heterogeneity due to a cell subpopulation with stem cell properties. The stem cell properties are mainly the capacity of self- renewal and differentiate to generate multipotent progenitors (1). This cell subpopulation is called: Cancer Stem Cells (CSC´s). The CSC´s have been identified and isolated from a variety of malignancies, however have not been described for the cervical cancer.

The self-renewal capacity of CSC´s is regulated by a limited set of signaling cascades as Wnt pathway. The ligands Wnt´sare able to act dependent or independent of β-catenin. An Increased Wnt pathway activation and epigenetic silencing of Wnt antagonists have been reported in several tumor types. In cervical cell lines have been demonstrated the regulation of the nuclear level of β-catenin by the oncogenes E6 and E7 from HPV, suggesting a relationship between HPV and β-catenin dependent Wntsignaling.

The CSC´s represent a low percentage of cellular subpopulations; so the sphere formation allows enriching them. The aim of the present work is to determine the role of Wnt signaling in the CSC´s enriched through the sphere formation from cervical cell lines. The identification of the CSC´s is through the ALDH high activity, a common marker for both normal and malignant stem and progenitor cells in others types of tumor.

The results showed a major percentage of cellular subpopulation with ALDH high activity at 4 days upon sphere formation, correlating with an enrichment of CSC´s. At this time was observed an increase of total protein of β-catenin upon sphere formation inSiHa cells, while for HeLa cells it decreased. Additionally β-catenin enriched into the nuclear fraction from SiHa cells, likely by a Wnt signaling activation. It suggests a difference into Wnt signaling between both cell lines, however remains to elucidate if this difference is about the CSC´s of each one.

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GPR40 phosphorylation is induced by free fatty acids and activation of Protein Kinase C.

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The G protein coupled receptor 40 (GPR40), also known as free fatty acid receptor 1 (FFA1) is activated by medium and long chain fatty acids. GPR40 mediates the effect of free fatty acids on pancreaticβ cell insulin secretion. Altered function of GPR40 is considered to play a significant role in the development of metabolic diseases, such as diabetes type 2, obesity, and metabolic syndrome.

FLAG-GPR40 fusion protein expressed in HEK293 Flp-In T-REx cells was used in the present work.To study the receptor response to agonists we measured intracellular calcium increase in response to different concentrations of the fatty acids: docosahexanoic acid (DHA) and α-linolenic acid (α-LA). Both fatty acids were able to increase intracellular calcium. The responses were immediate reaching their maximum in a few seconds and decreasing to near-basal levels in approximately 80-100 seconds. Interestingly, DHA generated a higher calcium response as compared to α-LA, suggesting that DHA acts as total agonist while α-LA acts as partial agonist. Additional work showed that α-LA and PMA induced GPR40 phosphorylation. Whilethe PKC activator PMA (1 µM) induced a maximal GPR40 phosphorylation after 5 minutes,α-LA (100 µM) induced maximal phosphorylation after 15 minutes. Initial experiments showed that PMA-induced receptor phosphorylation, did not blocked the ability of DHA and α-LA to increase intracellular calcium. On the basis of studies using other GPCRs, such as GPR120, the possibility that PKC-mediated phosphorylation could be associated to receptor internalization is currently been studies. Similarly, it remains to be define the PKC isoform(s) that catalyzed GPR40 phosphorylation. To achieve this, broad and isoform-selective PKC inhibitors such as bis-indolyl-maleimide I, hispidin, and Gö 6976 will be employed.

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The expression of progesterone induced blocking factor is hormonally regulated and it increases the growth of human astrocytoma cells through IL-4R/STAT6 pathway

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Abstract

Astrocytomas are the most frequent and aggressive brain tumors in humans and constitute the leading cause of brain cancer related deaths. It has been reported that progesterone (P) induces the growth of astrocytomas through the interaction with its intracellular receptor (PR). Recently, it has been found that P induces the development of several tumors through the up-regulation of Progesterone-Induced Blocking Factor (PIBF), a protein related with the immunological and proliferative effects of P. A grade III human astrocytoma derived cell line (U373) was used to study the role of P in PIBF expression, effects of the latter in cell number, and its mechanisms of action. By using RT-PCR and Western blot, we found that P (10 nM and 100 nM) increased PIBF mRNA expression after 1 and 3 h of treatment, respectively, and this increase lasted 24 h. This effect was blocked by the PR antagonist, RU486. Two PIBF isoforms were detected: one of 57 kDa and the predominant one of 90 kDa. The content of the 90 kDa isoform increased after 12 h of P treatment, and RU486 also blocked this effect. We observed that PIBF was released into the extracellular medium, being the 57 kDa isoform the most abundant in this compartment. Inmmunofluorescence analysis showed that PIBF was localized in both the cytoplasm and nucleus. The effects of PIBF on U373 cell number were analyzed for five consecutive days. PIBF (200 ng/mL) significantly increased the number of U373 cells on days 2-5. Co-inmmunoprecipitation and Western blot assays revealed that PIBF was associated to IL-4 receptor and that it increases JAK1 and STAT6 phosphorylation at
20 min. Our results suggest that P regulates PIBF expression in U373 cells through PR, and that PIBF increases cell number through IL-4 receptor/JAK1/STAT6 signaling pathway.

Evaluation of heat shock proteins in human gastric adenocarcinoma cells that overexpress claudins

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The gastric adenocarcinoma cancer causes about one million deaths worldwide annually. In metastatic forms, tumor cells can infiltrate to the lymph vessels of tissues, spread to lymph nodes and into the bloodstream, after which the road is open virtually any organ in the body.

During the development of cancer, are performed multiple changes in cells that alters the expression of certain proteins, enzymes and essential routs resulting in a tumor phenotype such as the ability to invade and metastasize. To perform these processes is required the degradation of extracellular matrix (ECM), process that is carried out by metalloproteinases (MMPs), which alter cell-cell or cell-ECM contacts whit the help of proteins that assist in his activation.

In cancer, there is a close relationship between metalloproteinase and heat shock proteins (Hsp´s). The latter, play an important role in migration of cancer cells and are involved in the loss of cell-cell adhesion, activation of MMPs and migration and invasiveness of malignant cells in the body.

It has been reported that the overexpression of the tight junction proteins as claudins-6, -7 and -9 in gastric adenocarcinoma cells correlated whit the increase of proliferation, migration, cell invasiveness and the expression of MMP-2 and MMP-9. Because the Hsp´s are involved in the activation of MMP-2 and -9, in this work we evaluate whether overexpression of claudins-6, -7 and -9 correlated with changes in expression of heat shock proteins (mainly Hsp27, -40, -70 and -90) and if these claudins co-localize with the Hsp´s.

Western blot assay showed an increase in the expression of Hsp40 and Hsp90 with respect to AGS cells overexpressing claudin-9. The results obtained by inmunoprecipitation assay showed a differential regulation of Hsp´s expression. A colocalization of Hsp27 with claudin -7 and -9; Hsp40 withclaudin-6 and Hsp90 with claudin-6 and -9 was observed. In like manner, MMP-9 colocalize with Hsp27 in AGS that overexpressed claudin-9. The results collectively suggest that overexpression of claudins-6, -7 or -9 promote the activation of MMP-9 and

Identification of proteins involved in signaling pathways induced protein from *Bacillus thuringiensis* Cry1Ac activation in RAW 264.7 macrophages.

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Most infectious agents to enter the body through mucosal surfaces, so it is necessary to develop effective vaccination strategies that induce protective immune responses in these sites. Cry1Ac is a protein produced as protoxin crystal form by Bacillus thuringiensis soil bacteria that after being solubilized and proteolytically processed by trypsin is converted in toxin (t). Our laboratory group has focused on the immunological properties of the protein Cry1Ac and has been shown that the protoxin (p) is a potent immunogen and mucosal adjuvant. Up to now, the mechanism of immunopotentiation pCry1Ac is unknown, however it may be related to its ability to induce the upregulation of costimulatory molecules CD80 and CD86 and induce the production of proinflammatory cytokines TNF-α, IL-6 and MCP-1 in macrophages, in some cases at comparable levels to those obtained with lipopolysaccharide (LPS) stimulus. To help define the utility of pCry1Ac as a vaccine adjuvant we have proposed to understand the cellular basis of immunogenic and adjuvant mechanism seeking to elucidate the signal transduction pathways activated by this protein in RAW 264.7 murine macrophage, determining the role of NF-kB (nuclear factor-kappa B) and mitogen activated protein kinases ERK, JNK and p38 through the use of inhibitors of these pathways and by the identification of their phosphorylated forms, by flow cytometry and western blot assays.

Our data suggest that pCry1Ac-induced overexpression of CD80 and CD86 and MCP-1 cytokine production appear to be mediated in part by ERK while tCry1Ac-induced upregulation of CD80 appears to be mediated by p38, as inhibitors of these pathways partially inhibited the upregulation of these costimulatory molecules. Furthermore it was observed that the macrophages stimulated with both Cry1Ac toxin and protoxin induce ERK phosphorylation, which means that this pathway is involved in the Cry1Ac induced effects on macrophages. Treatment with the JNK inhibitor (SP600125) did not affect the expression of costimulatory molecules, but inhibited the production of TNF and MCP-1 stimulated by pCry1Ac. Cry1Ac and LPS appear to act by different mechanisms. Project supported by CONACyT 177612 and IN219013 PAPIIT.

Retinol Binding Protein type 4 (RBP4) induced insulin resistance in skeletal muscle cells of rats (L6), through the reduction in gene expression of elements of the signaling pathway activated by insulin.

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The inability of insulin to exert its biological effect on organs and target tissues (skeletal muscle, liver and adipose tissue), is known as insulin resistance (IR), a condition that precedes the development of *diabetes mellitus* type 2. A major risk factor for the development of IR is obesity, not knowing in detail the molecular basis involved. Elevated adipokines levels,produced in obese (leptin, TNF-α, IL-6 and RBP-4) induce insulin resistance in skeletal muscle, liver, pancreas and adipose tissue.

Under the effect of insulin, skeletal muscle is responsible for 80% of glucose uptake, as such, the purpose of the study is to define the molecular mechanism involved through which, RBP-4 induces insulin resistance in cells L6, from rat skeletal muscle.

Methodology: L6 cells were treated in presence and absence of different concentrations of RBP-4 as well as at different times. Stimulated glucose uptake by insulin was evaluated, and was determined the mRNA expression ofStra6and TLR4, and protein levels of TLR-4, Strat-6, Insulin receptor, GLUT-4 and SOCS-3.

Results: L6 cells express the mRNA and the corresponding protein STRA-6 and TLR-4. RBP4 induces decreased ability of glucose incorporation into L6 cells stimulated by insulin; the observed effect depends on the timing and concentration of peptide. RBP-4 induced changes in the expression levels of elements involved in the signaling pathway activated by insulin: Insulin Receptor, SOCS-3 and GLUT-4.

Keywords: Insulin resistance, RBP-4, Insulin signaling pathway.

Study of pH changes during human sperm capacitation

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Fertilization is the fusion of gametes to produce a new organism and this process is the most important event for sexual reproduction. The male gamete undergoes three important events prior to fertilization: 1) activation of motility, 2) capacitation and 3) acrosomal reaction. Sperm capacitation takes place within the female genital tract and it is a prerequisite to fertilize the oocyte. This process involves remodeling of the plasma membrane, cholesterol removal, plasma membrane hyperpolarization, extensive protein tyrosine-phosphorylation, as well as increase in intracellular pH (pH_i) and Ca^{2+} ($[Ca^{2+}]_i$). The precise function of these changes and how they are orchestrated and connected is still under study. However, most of the knowledge about capacitation derives from studies using mice as a model system. Despite the similarities among reproductive strategies in different mammals, it has recently become clear that there are important differences among species and studies must be performed in the species of interest. pH_i changes during capacitation are of utmost importance since regulation of sperm activity is strongly related to pH_i and HCO_3^- levels. For example, the two sperm specific channels CatSper and Slo3, essential for fertilization, are pH_i dependent. The molecular entities responsible for the capacitation associated pH_i changes are not clearly established and less is known in human sperm. Here we attempted to determine the pH_i changes that occur during human sperm capacitation, their kinetics and the identity of the transporters involved in this alcalinization and if this change modulates other capacitation parameters such as protein tyrosine phosphorylation and plasma membrane hyperpolarization.

Claudins -6 and -9 regulate the activation of MMP-2 and MMP-9 in human gastric adenocarcinoma cells.

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Claudins are integral membrane proteins responsible for the proper assembly of tight junctions. Changes in the expression of these proteins during tumor development is associated with disassembly of the EU and the loss of cell-cell adhesion processes play an important role in the invasiveness and metastasis. Claudins expression in some types of cancer has been associated with an increasein invasive ability andmetalloproteinase activity. It has been reported that claudins -1, -2, -3, -4 and -5 recruit and promote the activation of MMP-2. Overexpression of claudins-6 and -9 in gastric adenocarcinoma cells (AGS) promotes proliferation, cell migration and invasiveness and the endogenous claudin-1 overexpression. In this study we assessed whether the mechanism by which these claudins promote invasiveness is through activation of metalloproteinases -2 and / or -9. To this end we investigated: a) whether overexpression of claudin-6 and -9 induces interaction with MMP-2 and / or MMP-9, b) if the activity of these metalloproteinases is induced by the overexpression of claudins-6 or -9, and c)if endogenous claudin-1 expression is a potential intermediary in the activation of these MMPs and the signaling pathways involved in this process. The results show the interaction between claudin-9, MMP-2 and MMP-9 in AGS cells that overexpress claudin-9, and interaction between claudin-1 and MMP-2 in AGS cells that overexpress claudin-6. The activation assays showed a decrease in MMP-2 and -9 activation and an increase in the MMP-2 activation in AGS cells that overexpress claudin-9 and claudin-6 respectively. Immunofluorescence assays showed the co-localization of endogenous claudin-1 with MMP-2 and MMP-9 in cells that overexpress claudin-6 and -9. The results using specific inhibitors for p38 and PKCon AGS cells that overexpress claudin-6 indicate that claudin-1 expression decreaseswhenboth kinases are inhibited and MMP-2 expression decreases when only p38 is inhibited. The results suggest that claudin-1,as a consequence of claudin-6overexpression, has an important role in the activation of MMP-2 and/or -9 and this is regulated by p38 pathway.

Real time monitoring of the acrosomal reaction in human sperm

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Fertilization is an essential event for organisms that depend on sexual reproduction. This process is highly synchronized and involves a complex series of interactions between sperm and egg, culminating in the fusion of these gametes.
It has been shown that Ca^{2+} is essential in regulating processes required after sperm ejaculation that lead to fertilization, including the acrosome reaction (AR), which consists on the acrosomal granule exocytosis characterized by the formation of multiple fusion points between the plasma membrane and the outer acrosomal membrane in the anterior region of the sperm head. This process is necessary for sperm to traverse the egg envelope and to expose a fusogenic membrane that allows egg-sperm fusion.
Although one of the glycoproteins from the external egg matrix, ZP3, has been regarded as the natural inducer of the AR, other studies have shown that several compounds are able to induce this reaction with the consequent Ca^{2+} mobilization in the sperm. The physiological relevance of these inducers has been strengthened by recent results showing that at least in mouse, sperm that succeed to fertilize an egg are those that underwent the AR before reaching the egg. Therefore, it is important to further characterize other ligands encountered by sperm during its transit through the female reproductive tract known to induce AR.

The classical test to determine the acrosomal status is performed in fixed sperm using lectins coupled to fluorophores, which recognize glycosylated proteins present in the acrosome. However to characterize the real time dynamics of this process and to correlate it with Ca^{2+} mobilization, assays in living cells that allow simultaneous monitoring of AR and intracellular Ca^{2+} changes are required. Fluorescent dyes from the FM family display properties suitable to track plasma membrane dynamics during exocytosis and endocytosis in somatic cells. A previous work from our laboratory showed that FM4-64 successfully reports the occurrence of the AR in real time and that the associated intracellular Ca^{2+} changes can be followed with a Ca^{2+} sensitive dye at the same time.

In this study we monitor in single cell experiments the AR and the associated intracellular Ca^{2+} changes mediated by GABA, progesterone and PGE1 in human sperm.

We observed that progesterone and PGE1 induce AR with an associated Ca^{2+} increase while GABA elicit Ca^{2+} oscillations levels that do not lead to AR.

"Participation of ERK1/2, JNK and P38 in the formation of benzo[a]pireno metabolites in BEAS-2B cells".

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Benzo[a]pyrene (b[a]p), as the first identified carcinogenic component of polycyclic aromatic hydrocarbons (PAH), is the most extensively studied carcinogen in cigarette smoke and has been regarded as a critical mediator of lung cancer for a long time. b[a]p is metabolized by cytochrome P450 1A1(CYP1A1) and cytochrome P450 1B1 (CYP1B1) enzymes to B[a]PDE (benzo[a]pyrene diol epoxide), which is highly mutagenic and carcinogenic. This metabolic transformation is mediated by the Aryl hydrocarbon Receptor (AhR) through a cascade of events known as the AhR signaling pathway. However, studies have shown that PAH activates the ERKs and the JNKs kinases suggesting that other signaling pathways, particularly those mediated by kinases, are involved in the metabolism of HPA and those kinases are capable of modulating the transcription of genes such as CYP1A1. Cellular alterations induced by b[a]p are complex and they may be mediated by more than one signaling pathway activating multiple genes, so it is essential to know which pathways are involved in PAH´s metabolism to a better understanding of the basis of some molecular diseases like cancer. The present study aim is to determine the involvement of an alternative pathway by which b[a]p exhibits its toxic effects.

The cell line BEAS-2B was purchased from the American Type Culture Collection (ATCC) and maintained according to the supplier's instructions. Cells were treated with different concentrations and times of exposure (according to the test) of b[a]p using b[a]p stock dissolved in dimethylsulfoxide (DMSO). The presence and identification of interest variants of CYP1A1, CYP1B1, GSTμ, GSTτ and GSTπ was performed by PCR-RFLP and Multiplex PCR analysis. Cytotoxicity of b[a]p concentrations was determined using the MTT assay. Transcriptional activation of CYP1A1 and CYP1B1 was measured by RT-PCR analysis. Total RNAs were isolated using the RNeasy plus Mini Kit™ (Qiagen, USA) according to the instructions of the manufacturer. cDNA was synthesized by OneStep™ RT-PCR Kit (Quiagen, USA) and the phosphorylation and expression of kinases ERK 1 y 2 were determined by inmunoblotting.
The results so far suggest the presence of a GSTμ deletion in BEAS-2B cells. b[a]p is not cytotoxic to the cells, it activates the AhR, and transcription of CYP1A1 as well. Finally, b[a]p activates the ERK kinases 1 and 2 which could mean the participation of an alternative signaling pathway in the metabolism of b[a]p in BEAS 2B cells.

The presence of proinflammatory cytokines exacerbates the proliferative and migratory capacity of breast cancer cells

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According to World Health Organization (WHO) breast cancer is the leading cause of cancer death among women ages 20 to 59 around the world. In Mexico the National Institute on Cancerology reports that breast cancer currently occupies the first place in the malignant neoplasms incidence and represents the 11.34% of all cancer cases. The primary reason for the high mortality rate of breast cancer is metastasis.

Several lines of evidence obtained from experimental, clinical and epidemiological studies suggest that inflammation is strongly linked to the development and exacerbation of cancer. It has been demonstrated that mediators and effector cells from the inflammatory response are main components of the tumor microenvironment. Particularly, TNF-α and IL-6 have been detected within the tumor microenvironment of breast cancer.It is now accepted that IL-6 and TNF-α promote the proliferative and migratory capacities of the tumor cells, however the mechanism used to achieve such behavior is still unknown.

The main interest of this project is to evaluate if the presence of IL-6 and TNF-α induce the ability of tumor cells to undergo proliferation and migration within the triple-negative cell line MDA-MB-231 and to elucidate the signal pathway involved. The migratory capacity of the MDA-MB-231 cells was evaluated in presence and absence of TNF-α and IL-6by means of the wound healing assay. The presence of proinflammatory cytokines induced an increase of the closing speed of the wound regarding to the control condition. The changes observed in the migratory capacity of the cells were accompanied by a positive change in the expression levels of mRNA that codifies the α subunit of the voltage dependent sodium channel, Nav 1.5, and the Na^+/H^+exchanger NHE-1. These proteins have been related to cell motility in tumor and normal cells. As a complement of this project, the proliferative capacity of the MDA-MB-231 cell line was evaluated in the presence of TNF-α and IL-6, in this case a significant decreased of the cells treated with TNF-α was observed.

Leptin increase migratory activity of MDA-MB-231 breast cancer cells through voltage gated sodium channels overexpression and Na^+/H^+ exchanger

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Breast cancer is the main cause of death by cancer in women around the world. Breast cancers that develop before menopause are more often estrogen receptor negative and resistant to hormone therapy, it represents approximately 15% all invasive cancers. Many epidemiological studies have shown that, although there is no estrogen receptor and progesterone receptor expression, triple negative breast cancer presents high prevalence in obese women. Obesity is considered as a risk factor for the development primary tumor metastasis since this condition presents high adipokines levels, mainly leptin. On the other hand, invasive and migratory capacity has been related to several proteins expression among this voltage gated sodium channels and ionic exchanger Na^+/H^+.

The main objective of this work is determine if the molecular mechanism by which leptin increases migratory activity in tumor cells is due to a change over the functional expression of alpha subunit $Na_v1.5$ voltage gated sodium channel and the exchanger NHE1, we use as an experimental cell model of triple negative breast cancer the tumor cell line MDA-MB-231.

To study the leptin migratory capacity, we did a wound healing assay using different leptin concentrations (10-200 nM); we observed a dose-dependent effect. The leptin treatment produces an increased expression of mRNA encoding voltage gated sodium channel alpha subunit $Na_v1.5$ and Na^+/H^+ exchanger by RT-PCR assay.

In order to gain some insight into the signaling pathways mediating the migratory activity through leptin receptor activation on tumor cells MDA-MB-231, we used specific inhibitors for different signal pathways: a) PD98059 (MAPKs pathway), b) LY294002 (PI3K/AKT pathway), and c) STATTIC (JAK-STA pathway).

Regulation of ribosome biogenesis in maize embryonic axes during germination

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Ribosome biogenesis is a pre-requisite for cell growth and prolifetarion; it is however, a highly regulated process that consumes a great quantity of energy. It requires the coordinated production of rRNA, ribosomal proteins and non-ribosomal factors which participate in the processing and mobilization of the new ribosomes. Ribosome biogenesis has been studied in yeast and animals; however, there is little information about this process in plant. The objetive of the present work was to study ribosome biogenesis in maize seeds during germination, a stage characterized by its fast growth, and effect of insulin, homolog the endogenous maize factor ZmIGF in this process. Insulin has been reported to accelerate germination and to induce seedling growth. It was observed that among the first events reactivated just after 3 hours of imbibition are the rDNA transcription and the pre-rRNA processing and that insulin stimulates both of them. The transcript of nuclelin, a protein which regulated rDNA trancription and pre-rRNA processing, is among the messager stored in quiescent dry seeds and it is mobilized into the polysomal fraction during the first hours of imbibition (6H). In contrast, *de novo* ribosomal protein synthesis was low during the first hours of imbibition (3 and 6 h) increasing by 60 times in later stages (24 h). Insulin increased this synthesis at 24 h of imbibition; however, not all ribosomal proteins were similarly regulated. In this regard, an increase in RPS6 and RPL7 protein levels was observed, whereas RPL3 protein levels did not change eventhough its transcription was induced. Result show that ribosome biogenesis in the first hours of imbibition is carried out with newly synthesized rRN and ribosomal proteins translated from stored mRNA.

Leptin induces epithelial-mesenchymal transition in a FAK-ERK dependent pathway in mammary non-tumorigenic cells MCF10A

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Epithelial-mesenchymal transition (EMT) is characterized bythe loss of epithelial characteristics and the acquisition ofa mesenchymal phenotype. EMT is an essentialdevelopmentalprocess, wound healing and fibrosisby which cells of epithelial origin losetheir epithelial characteristics and polarity and acquire amesenchymal phenotype with an increase of cell migration. However, this process has been implicated with promestatatic properties, including increased motility andtumor invasion in breast cancer. EMT is characterized by the loss of epithelial markers such as E-cadherin, and upregulation of mesenchymal markers such as vimentin, MMPs secretion and cell migration.

Obesity is an important public health problem and major risk factor for postmenopausal breast cancer. Leptin is a hormone secreted by adipocytes and acts on the hypothalamus to influence food intake and energy expenditure. However, it has been involved in breast cancer progression inducing cell migration and invasion in breast cancer cell lines, nevertheless, no data exist about the role of leptin in EMT of mammary epithelial cells.

In this study, by using Western blot, we demonstrated that leptin 400 ng/ml induces an increase in vimentin expression, FAK and ERK activation and by using scratch wound assay we demonstrated that leptin induces an increase incell migration of MCF10A cells. Furthermore, we evaluate the role of FAK and ERK kinases on EMT in this cell line induced by leptin by employing a FAK inhibitor (PF-573228) and ERK inhibitor (ERKI). Our results demonstrate for the first time that leptin induces vimentin expression and cell migration in a FAK and

Molecular characterization of the Receptor for Activated C Kinase 1 (RACK1) in the jellyfish *Cassiopeaxamachana*

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The symbiosisbetween different organisms of the phylum Cnidaria, such as anemones, hydra, coralsand jellyfish,withdinoflagellate algae of the genus*Symbiodinium*,has profound ecological implications since it represents the basis of the energy supply for the coral reef.*Cassiopeaxamachana*is a jellyfish that lives in shallowwaters of the Caribbean, India andFlorida. Like other jellyfish,*C. xamachana*is dioecious and presents alternation of generations;that is, sexually maturejellyfish produce and releasesperm and eggs, and once external fertilizationproceeds,a ciliate free-livingaposymbiotic larvae is produced. Then, settlement on the appropriate substrate occurs and a polyp stage starts to develop. The polyp or scyphistoma can bud into new larvae and the cycle repeats many times. The polypcan only metamorphose to jellyfish when the symbiotic algae (*Symbiodinium*)are acquired. All these cellular processes require exquisitely regulated and diversesignaltransductionmechanisms for the correct response. Our aim is to study the role of RACK1, a protein involved in several signal transduction pathways,and characterize its binding partners before, during, and after the establishment of the symbiosis. We have begun to obtain partial sequence that encodes this protein usingoligonucleotides based on a multiple alignment with sequences fromphylogenetically relatedorganisms and PCR amplification.The full codingsequence will be cloned for expression to obtainpure protein for pull-down/immunoprecipitation assays to identifyits ligands.This will allow us to propose a role for RACK1 in the *C. xamachana* life cycle and its importance in the symbiotic process.

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Leptin induces cell migration in anEGFR transactivation-dependent pathway in MDA-MB-231 breast cancer cells

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Experimental and epidemiological studies have linked obesity and breast cancer progression. Leptin is a hormone secreted by adipocytes, which experimentally has been shown to promote cell migration of breast cancer cells. It is well established an important role of EGFR in tumor progression and drug resistant phenotype.Activated EGFR leading to the activation of several major pathways for tumor growth, progression and cell survival in breast cancer. Cell migration is associated with different steps of metastasis; one of the most important proteins associated with cell migration is focal adhesion kinase (FAK).**Objective**: To determine the role of EGFRin cell migration of MDA-MB-231 breast cancer cell line stimulated with leptin. **Materials and methods**: Breast cancer cell line MDA-MB-231 was used. Cell migration was assessed by Scratch-wound assays. EGFR activation was evaluated by measuring the phosphorylation of P-Tyr^{1173} by Western blot. EGFR inhibitor (AG1478) and MMPs inhibitor (GM6001) were used to evaluate the role of EGFR transactivation on cell migration. **Results**: We determinate that leptin induces EGFR activation in a time-specific manner and cell migration in a dose-specific fashion in MDA-MB-231 breast cancer cell line. Furthermore, our results demonstrate that leptin induces cell migration in anEGFR transactivation-dependent pathway. **Conclusion**: Leptin induces epidermal growth factor receptor (EGFR) activation and cell migration through anEGFR transactivation-dependent pathway.

Shh, Wnt and Notch pathways activation in acute lymphoblastic leukemia and their correlation to proliferative rate and chemoresistance.

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Keywords: Acute Lymphoblastic Leukemia, Wnt and Notch pathways, cell cycle, chemoresistance.

Overview: Around 30 to 50 % of children with acute lymphoblastic leukemia (ALL) die from causes related to complications of the disease and the treatment. The presence and development of resistance to the chemotherapyare the principal factors that trigger the therapeutic failure. Proliferation rate, differentiated cell ratio, survival and presence of ABC transporters are the principal conditions to influence forchemoresistance. Some of these characteristics are present in the Leukemic Hematopoietic Stem cells,these cellular population show alterations in the expression of oncogenes and antioncogenes involved in controlling proliferation, differentiation or survival. Deregulated events in signal transduction pathways are involved in the transformation process, alsoin the presence ofchemoresistance, like the Shh, Wnt and Notch pathways.***Objective:*** Identify the activation of self-renewal pathways (Shh, Wnt and Notch) and determine the proliferation ratio in lymphoblastic leukemia cells for correlate with the presence ofchemoresistance. ***Methodology:*** Primers were designed to amplify by RT-PCR the target genes of the Shh, Notch and Wnt pathways (Gli1, Hes1 and Wnt3Arespectively).Recognition of LLA immunophenotypeand the proliferating cells were made through the detection of differents CDs and Ki67,respectively, by flow cytometry from bone marrow (BM) aspirates of patients with ALL. ***Results&Prospects:*** We have identified by RT-PCR the expression of the target gene Hes1 in cells from patients with ALL, both in newly diagnosed and relapsed patient in the pre-B ALL and B-ALL. We didn't detect expression of the target gene Wnt3A in the same samples. We detected Ki67 in 5-85%. We are evaluating the expression of other target genes (Gli), receptors for Wnt and Notch pathways and testing the in vitro sensitivity to drugs used in chemotherapy by resistance assays (CSRAs) over BM aspirates samples of patients with ALL for correlate the presence of self-renewal pathways with the chemoresistance mechanisms.

Validation of the questions of an "extraordinay examination" in the subject of Biochemistry and Molecular Biology of the medical curriculum at the School of Medicine, UNAM.

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At the Department of Biochemistry of the School of Medicine, UNAM, an "extraordinary" evaluation in the subject of Biochemistry and Molecular Biology of the medical curriculum is perfomed annually for those students that did not credit the subject in the immediately preceding school period and those students that did not credit it in previous years. In the examination corresponding to the period 2010-2011, 169 students presented the "extraordinary" evaluation, of which 45 reached the required grade, that is 27%. The objective of the present study was to validate the 80 questions used in this examination. Validation was accomplished by applying four statistical criteria to the responses given by the students to the question. If the response to a question was correct it was assigned a value of 1; if incorrect, it was marked as 0. The applied criteria were: 1) index of difficulty (Pi), 2) index of discrimination (Di), 3) discrimination coefficient (rpbis), and 4) Cronbach's alpha; those questions that had at least three of the four criteria approved were considered as validated to be part of the evaluation database.

Of the 80 questions, 63 reached acceptance with the Pi criterion; 45 with the Di; 42 with the rpbis; and 42 with Cronbach's alpha; 41 questions had at least three of the four criteria, which means 51% of acceptance. With the obtained data, it can be considered that half of the "extraordinary" examination was of good quality. Those questions that did not reach the acceptance levels can be improved to reach their acceptance.

Validation of the conversion rate of weight live weight processed sea cucumber, *Isostichopusbadionotus,* through the moisture content.

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Abstract
The monitoring and control of fishing quotas sea cucumber in the Yucatan coast is via the conversion of processed weight to live weight, through a study INAPESCA each fishing season. However, this conversion factor used only processed weight loss, which can lead to differences with the producers because there is no standardized method. In this proyect, the moisture content of the different stages of the process was determined and related to weight loss. The weight loss results reported in this paper compares favorably with the conversion factor used by INAPESCA in 2012. Samples from different producers, which were determined moisture and could be that the product is at the stage were obtained process stated. Using the moisture content of the product related to the conversion factor given by INAPESCA serve to validate the conversion of processed weight to live weight, which would result in better monitoring and control of the quotas allocated to fishermen, prompting a fishing responsible and sustainable, providing certainty to buyers and processors.

Materials and methods
Sea cucumbers were collected in the areas of fisheries authorized to FisheriesCooperative. The harvested organisms were placed in containers with clean warm water of sea. The bodies were transported alive to the laboratory. A total of 35 bodies were weighed and marked individually to follow up during the process, a sample was taken to determine moisture. A semi-analytical balance was used to determine the weight and calculate the moisture content using techniques approved by the AOAC 2000.

The process used was as follows: Eviscerated First firing for a period of 2 hours, Drying beds with coarse sea salt for 48 hours , Second firing for a period of 30 min, Drying in the sun for a period of 13 days with a sun exposure 10.00 17.00. Additionally, samples of different processors according to the statements of the packing were obtained, these were in the passage of pre-cooked, all samples were frozen and transported to the laboratory in coolers for the determination of its moisture.

Conclusions
The conversion factor obtained in the laboratory is very similar to that reported by INAPESCA, determination of moisture in each step of the process provides important step to validate the process information. Products with higher humidity to 56.3% found before the first cooking. Also, products with humidity around 44% are savory. Less moisture 35.8% is required to determine that sea cucumbers has reached the second cooking. It is very difficult to determine the state of the process visually, as there is no reference as to the changes.

Significance of the findings and their economic and social impacts
The impact of having a method to validate the conversion rate has a great economic impact because if misclassified process step by applying the conversion decimal division is used, which increases the source of error and cause the authority to penalize fishermen for exceeding their quotas or otherwise, management plans would be affected to have erroneous data capture volume.

THE USE OF CLINICAL SCENARIOS IN BIOCHEMISTRY AND THE DEVELOPMENT OF COMPETENCIES (COMMUNICATION AND COLLABORATIVE WORK).

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World tendencies in education are oriented to the development of professional competencies and medical teaching is not out of these tendencies. National Autonomous University of Mexico Medicine Faculty's curriculum 2010 proposes a mixed educative model centered in student and articulated by competencies. Our working group has looked for new strategies for helping students to acquire knowledge and to develop some of the competencies proposed in the curriculum 2010. Among these strategies we utilize oriented discussion of clinical scenarios to apply Biochemistry to medical situations and to develop collaborative working and effective oral and written communication skills. We worked with a population of 471 students of Medicine first year of two generations distributed in 6 intervention groups and 6 control groups. The study themes were:"Glycaemia Regulation"; "Lipid Digestion, absorption and transport"; and "Cholesterol Metabolism". In intervention groups we used oriented discussion of clinical scenarios in collaborative groups to study these themes and the control groups had the traditional teaching based in conferences given by the professor. In intervention groups we also evaluate the progress in collaborative working and communication, by evaluation instruments designed for this purpose (rubrics, check lists). Results show that students develop gradually these competencies along the course and we found a positive correlation between a better communication skill and the appropriated biochemical information utilization measured by results obtained in the correspondent departmental exams. From these results we can conclude that the oriented discussion of clinical scenarios is a useful strategy to develop some competencies by Medicine first year students.

Analysis of theaccreditationcriteriaforthestudents in thefirstyear of the medicine school in thematter of biochemistry and molecular biology.

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Students in the first year of medicine in the Universidad NacionalAutónoma de México school the students bring a total of 8 subjects. To exempt any of these must obtain at the end of the year an average of 8.5 in the examinations. Biochemistry and Molecular Biology has a high rate of failing grades, and more than 80% of the students present the first final exam, along with 3 additional subjects (Anatomy, Embryology and Cell and Tissue Biology). This scheme leads to an approval rate of only a 10% of students on the first final examination of Biochemistry and a40% forthesecond final exam.

The bad grades in the final examinations generated frustration and stressin the studentsand they cannot continue with the second year of the medical career. In the field of Biochemistry and Molecular Biology about the 12% of the population gets a grade between 7 and 8, so these students must take the final exam, because they do not reach the required average 8.5. A situation that occurs frequently is that the students obtained a lower grade in their final examinations or even worsethey fail the course. Therefore, it is proposed that when students have passing grades with the teacher and departmental examinations, the average became the their final grade. However, the concerns arising from this proposal is that students with a grade of 6 will be bad. The reality is that only 4% of the population gets a 6, in this new system of evaluation and in Biochemistry does not necessarily reflects the knowledge and competences obtained by the students in the career.

Correlation between the grades attained at the high school of origin and the final grades reached in the subject of Biochemistry and Molecular Biology of the medical curriculum in a group of students at the School of Medicine, UNAM.

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Given the heterogeneity of the students that comprise the groups in the subject of Biochemistry and Molecular Biology (B and MB) of the medical curriculum at the School of Medicine, UNAM, a diagnostic test on general knowledge has been applied to group 1118 at the beginning of the course, for quite some time now. The grades obtained have no influence on the student's records in the subject; rather this internal evaluation aids the professor in knowing, at the beginning of the course, at what level to start teaching the subject. On the other side, on the registry form, students write down their average grade attained at the high school of origin. With the objective of establishing an association between the knowledge of students coming from the National Preparatory School (ENP, for its initials in Spanish), the College of Sciences and Humanities (CCH, for its initials in Spanish) of the UNAM, as well as of those coming from other schooling systems, and the final grade attained in B and MB, we performed a transversal, observational, descriptive, and retrospective study with students from different generations constituting the chosen student group (1118). The database comprises the grades of the students from 2006 to 2014. Data were collected on Excel spreadsheets and exported to the JMP statistical software. Data were analyzed first descriptively and, then, inferentially. In general, the average grades with which these students are admitted to the School of Medicine are very high (90% for ENP, 92% for CCH, and 86% for the other schools). Results of the analysis show that the average grade attained at the end of the high school is of 90%; that of the diagnostic test was of 54%, and that of the final grade in B and MB was of 65%. There is a 0.066 correlation ($p = 0.38$) between the final grade in B and MB and the grade obtained at the high school of origin. In addition, a correlation was performed between the diagnostic test and the final grade in B and MB, reaching 0.49 ($p < 0.001$). It is concluded that there is no relation between the grades attained at the high school of origin and the final grade in B and MB, but there is an association between the diagnostic test performed by the professor and the final grade of the B and MB subject.

Effect of senescence- associated secretory phenotype (SASP) from primary mice fibroblasts on cellular migration, proliferation and senescence on the transformed cell line L929.

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Senescence is an irreversible growth arrest state that is characterized by an altered morphology, gene expression pattern and chromatin structure, as well as an activated DNA damage response. Senescence functions as a powerful tumor suppressor mechanism that protects cells from becoming neoplastic and malignant. The amount of senescent cells found in early, pre-malignant tumor stages is related to the progression into malignancy.

An important aspect of senescent cells is the senescence associated secretory phenotype (SASP) characterized by abundant secretion of a large number of cytokines, chemokines, growth factors, and proteases. SASP may participate in multiple cellular processes such as activation of cell proliferation and cell migration, the formation of new blood vessels, and the induction of cellular senescence in neighboring cells, among others. At old age, SASP biological activity evades immune recognition to eliminate senescent cells, allowing their increment in the tissue.

The aim of this study was to evaluate the effect of SASP obtained from primary mice fibroblasts on the migration, proliferation and senescence induction of a transformed murine cell line (L-929).

Primary lung fibroblasts were obtained from CD-1 mouse and were allowed to senesce *in vitro* (replicative senescence), and SASP was recovered at different cukture stages (days 9, 15 and 21). L929 cells seeded on coverslips were exposed to fibroblasts SASP during 4 days. Cell proliferation was determined by trypan blue assay along with cellular migration by wound healing technique and cellular senescence by SA-beta-galactosidase assay. Our results showed that L-929 exposure to fibroblasts SASP significantly impacted cellular migration and senescence, as well as cellular proliferation.

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Neuroprotective effect of *Spirulina (Arthrospira) platensis*in intracellular redox state in rats undergoingendotoxic shock

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Abstract

*Spirulina (Arthrospira) platensis*is a cyanobacterium rich in antioxidants. Excessive generation of reactive oxygen species (ROS) and nitrogen (RNS) contribute to brain damage associated with endotoxic shock; the main cause of death in septic patients. In this condition proinflammatory cytokines are released to promote the presence of ROS and RNS.

The aim of this study was to investigate theneuroprotective effect of *Spirulina (Arthrospira) platensis* in redox state of rats with lipopolysaccharide (LPS) induced endotoxic shock. We used male Wistar rats, which were divided into 2 groups of 4 sub-groups each one: control, *Spirulina (Arthrospira) platensis*, LPS and LPS+*Spirulina (Arthrospira) platensis*; a dose of *Spirulina (Arthrospira) platensis*used 180mg/kg/p.o. once a day for 8 days to a group, and 22 days for the other group. The animals were sacrificed and brains were collected for analysis of lipid peroxidation, mitochondrial membrane fluidity, the activity ATPase hydrolysis, glutathione peroxidase, and nitric oxide.

The results show a similar behavior in the tests used to measure the intracellular redox state between the LPS+*Spirulina (Arthrospira) platensis*groupand the control group, showing differences in LPS-treated group.

We can conclude that the *Spirulina (Arthrospira) platensis*has antioxidant capacity in rats with endotoxic shock induced by LPS.

Keywords: *Spirulina (Arthrospira) platensis*, antioxidant,lipopolysacharide.

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Phospholipid Hydroperoxide Glutathione Peroxidase 2(GPX2) preserves yeast mitochondrial function and viability during chronological aging in the presence of PUFA

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The rate of aging in several species has been inversely correlated with the unsaturation degree of fatty acid from biological membranes. In a similar way, we have recently observed that incorporation of polyunsaturated fatty acids (PUFA) into the membranes of chronologically aged yeast lead to accelerated loss of viability, early mitochondrial dysfunction, enhanced ROS generation and increased sensitivity to lipid peroxidation, although it was observed a decline in the susceptibility to lipid peroxidation with aging progress, suggesting the activation of an adaptive response to this process. In this regard, threephospholipid hydroperoxideglutathione peroxidases (Gpx) have been identified in yeast. These enzymes play a role in the protection of membrane phospholipid against oxidative stress by catalyzing the reduction of phospholipid and fatty acid hydroperoxidesthat are produced in peroxidized membranes. Of these three isoforms, Gpx2p has been locatedat mitochondria, while the other two are present in the cytosol. Thus, the goal of this study was to demonstrate that Gpx2pmoreimportant for the survival of aged yeast in the presence of PUFA due to it may contribute to a better preservation of the mitochondrial function than the otherGpx isoforms. *S.cerevisiae* strain BY4741 and their respective mutants Gpx1Δ, Gpx2Δ and Gpx3Δwere chronological aged by cultivation in stationary phase up to 30 days in the presence of arachidonic acid.Cell viability,*in situ*mitochondrial membrane potential and*in situ* mitochondrial respiration were assessed every 5 days.The results shown thatthe viability was not affected in the first 15 days of culture in the mutant strains with respect to theparental cells; however, atthe $20^{th}$and 30^{th}days, it was observed a decrease of 70% and 90% in the survival of the Gpx2 Δmutant. This was accompanied by a parallel loss of mitochondrial function as respiration and membrane potential was severely impaired only in that mutant. Therefore, these data support the hypothesis that the maintenance of mitochondrial functions and membrane integrity by Gpx2p is essential for the survival of yeast during chronological aging.

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HSP70 protein levels in young and old monosodium glutamate (MSG) female and male treated mice: a link between obestiy and aging.

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Obesity has generally been considered an epidemic related to lifestyle, which not only occurs in the young and adult population, but is also observed in elderly people. Obesity is a health problem defined as an abnormal or excessive fat accumulation, due to an imbalance in energetic metabolism homeostasis, generated by multiple genetic and environmental factors.

Aging has been defined as the molecular, biochemical and cellular progressive decline during lifespan. Both, obesity and aging have been associated to low-grade systemic inflammation processes and represent risk factors for a wide range of diseases, including insulin resistance (IR), type 2 diabetes, dyslipidemia and cardiovascular disease.

To deal with a environmental and physiological stress there have been described several mechanisms to counteract the damage. One of these protector mechanisms is represented by the heat shock proteins (HSP), which increase their expression as part of the cellular protective effects against a wide variety of stressful stimuli, such as Inflammation, oxidative stress, hypoxia, infection, etc.

Previous data from our lab and others have aknowdlege the use of MSG-treated mice has a usefull model to study obesity. Therefore, the aim of this study was to evaluate HSP70 protein expression in the liver of young and old (4 and 20 months) MSG-treated female and male mice, in order to determine cellular stress effect during the obesity-aging process.

Our results showed differences in HSP70 protein levels between obese mice and controls in both genders. At 4 months old, an increment in HSP70 expression was observed probably as a response to obesity. However at 20 months of age HSP70 expression decreases in all mice gropus, but a greater decrease was observed in in obese mice.

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*Karwinskiahumboldtiana*administrationas a model of the induction of oxidative stress in *Sprague Dawley*rat: evaluation of melatonin and fish oil as preventive treatment

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Ingestion of the green or ripe fruit of Karwinskiahumboldtiana (Kh),also known astullidora, causes a neurological disordersimilar to the Guillain-Barre syndrome. In Mexico, the accidental ingestion of Kh causes several health problems. Kh contains significant amounts of phenolic antracenonas and / or anthraquinone.Oral administration of a slurry prepared with the seed of Kh in rats causes a uncoordinated motor activity in the hind followed by rapidly ascending flaccid paralysis and signs of respiratory distress[1].Furthermore, damage in organs such as liver (necrosis and fatty degeneration), lung (pulmonary hemorrhage), heart (necrosis) and kidney has been described. Alterations on energetic metabolism and oxidative stress are related with the mechanism by which Kh induces its cytotoxic effects[2,3]. These findings prompted us to investigate the effect of melatonin and fish oil on the alterations in membrane fluidity, lipoperoxideproduction and mitocondrial ATPase activities in rat liver mitochondriafollowing treatment with a slurry of Kh seeds. Adult male Sprague Dawley ratsthe following dose of dry powder of Kh fruit:1, 1.5 or 2 g/Kg body weight. Some rats were pretreated for 5 days prior Kh treatment with fish oil (0.0368 g EPA and 0.0184 g DHA, per day) or melatonin (10 mg/kg/day). Rats euthanized at day 7 received a second dose of Kh, 24 h after the first dose. After treatment, rats were sacrificed by decapitation and liverswere immediately dissected for mitochondria isolation. Lower dose of Kh on day 1 after treatment induced higher mitochondrial membrane fluidity than control group. This change was strongly correlated with increased ATPase activity and pH gradient driven by ATP hydrolysis. Mitochondrial membrane fluidity was hardly affected on day 7 after treatment with Kh. Surprisingly, the pH gradient driven by ATPase activity were significantly higher than controls despite an diminution of the hydrolytic activity of ATPase. Effect of melatonin and fish oil on the analyzed parameters will be discussed.

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Dynamic of reactive oxygen species in roots from *Arabidopsis* under Aluminum treatment.

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Aluminum toxicity is a major constraint for crop production in acidic soil worldwide. It has been described that the target for Al toxicity is the root tip and the elongation zone, mainly inhibiting cell elongation and cell division, which result in stunted plants accompanied by reduced water and nutrient assimilation. In plant cells reactive oxygen species (ROS) accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. (Mittler and Berkowitz, 2001). Furthermore, it has been described that ROS increases in plant root under toxic Al conditions.

Herein we report a new molecular probe to depict the ROS dynamic during root responses to Al treatment. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. This molecular probe was expressed as a transgen in Arabidopsis lines (1) under fluorescence microscopy. Herein we report the dynamic distribution of H_2O_2 in the root after Al treatment and the different responses in the root hair cells, apical region and the elongating region. A clear decrease at the early stages and increases after several days were found, these results will be presented and discussed.

1. Hernandez-Barrera, A., Quinto, C., Johnson, E. A., Wu, H. M., Cheung, A. Y., & Cardenas, L. (2013) *Methods Enzymol* **527,** 275-290.

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Tissular and biochemical changes in maize scutellum during transition of germination to the postgermination.

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The last event in germination is the protrusion of radicle or embryonic root; this is critical due to the nutritional demand that implies. In maize, the radicle growth is associated to anatomical changes in the scutellum detected between 18 to 24 h after imbibition. The epidermis of scutellum adjoining the starchy endosperm is transformed in small zones, these changes includes modifications of cuboid isodiametric cells to cells with broader basal poles until cells acquire the finger-like structure. Besides, enzymatic changes were detected, among them class III peroxidase activity increased, catalase activity remains without changes and decreased superoxide dismutase activity. Part of class III peroxidase is extruded to the apoplast between epidermis and fibrous layer, structure that limit and separates the starchy endosperm from the scutellum. Also, in this apoplastic space we detected superoxide and phenols including flavan 3-ol compounds. Detection of peroxidases (through *in situ* activity and immunolocalization) shows that in controlled microenvironments this enzyme can be a pro-oxidant factor in the generation and accumulation of superoxide in the apoplast, and it are in accord with the diminution in activity of superoxide dismutase and with a less immunoreactivity in scutellum epidermis. The presence of those conditions correlates with anatomical changes in small zones in the fibrous layer, where changes in proportion and/or properties of endogenous compounds were detected, thus allowing free flow of substances between scutellum and starchy endosperm. After 36 h of imbibition, the three enzyme activity increased in the scutellum, the epidermis is became papillate cells and with this, transforming the epidermal cells into a functional epithelium with a fibrous layer that allows interchange of signals, hormones, enzymes and substances between both compartments.

Effect of the specific NADPH oxidase inhibitor (VAS2870) on intracellular calcium changes: a connection between ROS and calcium signaling

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Cytoplasmic Free Calcium ($[Ca^{+2}]_{cyt}$) is a key second messenger in animal and plant cells. On the other hand, reactive oxygen species have emerged as important signaling molecules which together with calcium constitute key players in cell signaling. In plants the Reactive Oxygen Species (ROS) are generated by NADPH oxidases enzymes which are known like Rboh (*Respiratory burst oxidase homolog*). These ROS are involved in many processes, such as, stomatal aperture, pollen tube and root hair polar growth, including pathogen responses. The NADPH oxidasas in plants can be regulated
by phosphorylation, small GTPases, and calcium changes. In root hairs a model has emerged where calcium increases activates the NADPH oxidase that is involved in ROS formation, and this ROS is an important player in opening calcium channels that allow the entering of $Ca^{+2,}$ which again activates the NADPH oxidase activity. In plants the inhibition of the NADPH oxidases is limited to DPI, which is a general inhibitor for the flavor-enzymes, and thus unspecific.

In this work, we measured the cytoplasmic $[Ca^{+2}]$ in root hairs from *Arabidopsis thaliana* that express the Camaleon YC3.60, which is a widely used molecular probe for measuring intracellular Ca^{+2} in transgenic plants. In this work we used these transgenic lines and visualized the intracellular calcium gradients in root hairs under normal condition and evaluated the effect of a new generation of NADPH oxidase inhibitor, the VAS28070 and compared with the traditionally inhibitor DPI. The connection between Ca^{+2} and ROS will be presented and discussed.

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Comparative DNA repair capacity of human adipose derived mesenchymal stem cells to remove oxidative damage induce by different oxidative insults

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DNA is a metabolically active molecule that exists in acellular environment rich in water and reactive oxygen, surrounded by a plethora ofmetabolic products that can cause hydrolytic and oxidative modifications in DNAbases, phosphodiester bonds, and pentose moieties.In addition to this endogenous sources of DNA lesions, DNA can be damagedby exogenous (environmental) factors, such as ionizing (IR) and ultraviolet (UV)radiation, as well as a myriad of chemicals. Exogenous factors can induceDNA damage both directly and indirectly. For example, IR may directly ionize basesand/or deoxyribose groups that absorb radiation energy. However, the most damagingeffect of IR is indirect, through radiolysis of water and the production of reactiveoxygen species (ROS), such as oxygen superoxide, hydroxyl ions, electrons, andhydrogen peroxide, all of which cause oxidative damage.DNA lesions resulting from oxidative stress, as well as single strandbreaks are repaired via the base excision repair (BER) pathway. Many of these cellularDNAdamage defense mechanismshave been studied in somatic cells,however, far less is currently knownand understood about the maintenanceof genomic integrity in stem cells.Multipotent stem cells are long-lived and function to replace damaged or dead cellsover an organism's life; also they are exposed to a lifetimeof accumulation of DNA lesions. Defective DNA repaircan promote accumulation of mutations that interfere with self-renewal and differentiation,or induce malignant transformation.The aim of the study is compare the repair capacity of adult mesenchymal cells to handle oxidative damage induces by IR and $FeCl_3$. Due to most of the adultstem cells have low metabolic activity and low production of ROS. Our results show the basal DNA oxidative lesions and compare their changes after both oxidative insults IR and $FeCl_3$. DNA repair capacity was determined by DNA-single strand breaks extinction across recovery time.

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Malfunctioning assembly of the iron–sulfur cluster machinery in *Saccharomyces cerevisiae* produces mitochondrial oxidative stress by an iron-dependent mechanism, affecting respiratory function and increases sensitivity to ethanol and ROS.

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The Fe-S proteins are involved in many cellular processes such as oxidative phosphorylation, Krebs cycle, DNA replication and repair and amino acid biosynthesis, among others, with an important role in electron transport, catalysis and regulatory processes in the cell. Biogenesis and recycling of the Fe–S clusters plays an important role in iron homeostasis mechanisms involved in mitochondrial function. In *Saccharomyces cerevisiae*, the Fe–S clusters are assembled into Fe–S-containing proteins by the iron–sulfur cluster machinery (ISC), which has been extensively studied and involved in the incorporation of the Fe-S centers into apoproteins in both bacteria and eukaryotes. In eukaryotes, two main systems of Fe-S protein biogenesis have been described, the cytosol/nucleus (CIA) and the mitochondrial (ISC) machineries.

The present study aimed to determine the effects of mutations in the ISC genes and iron release under oxidative stress on mitochondrial functionality in *S. cerevisiae*. Reactive oxygen species (ROS) generation caused by ethanol, H_2O_2, or menadione was associated with a loss of iron homeostasis and exacerbated by ISC system dysfunction. *ISC* mutants showed increased free Fe^{2+} content, exacerbated by ROS-generator compounds, causing an increase in ROS, which was decreased by the addition of iron chelator. In addition, the activity of the complexes II and IV from the electron transport chain (ETC) was impaired or totally abolished in the *ISC* mutants; however, increased oxygen generation was observed.

Our study suggests that the free Fe^{2+} content it was originated mainly from mitochondrial Fe–S cluster apoproteins under conditions of oxidative stress. In mitochondria from *SSQ1* and *ISA1* mutants, the content of [Fe–S] centers was decreased along with III_2IV_2 respiratory supercomplex formation, but not in the iron-deficient *ATX1* and *MRS4* mutants. These results indicate that the ISC system is important in both iron-homeostasis and in the assembly of the supercomplexes III_2IV_2 and III_2IV_1, thus affecting the functionality of the respiratory chain.

Effects of DPI (NADPH oxidase inhibitor) on post-fatigue tension of slow skeletal muscle

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Fatigue is conceive as decrease of the power-force during an exhaustive activity. Reactive oxygen species have been implicated in this process. NADPH oxidase (NOX) present in skeletal muscle has been considerated has an important source of ROS production. Therefore the purpose of this work was to determine the effect of diphenylene iodonium (DPI) and NOX inhibitor on post-tension fatigue of skeletal muscle.

Anterior Latissimus Dorsi (ALD) muscle of chicken (2-3 weeks old) was dissected and mounted on an experimental recording chamber by placing the proximal end to the bottom of the chamber and the distal end hook mechanic-electric transducer (Grass FT03), which through an amplifier and a 320 CyberAmp analog-digital interface (Digidata 1322A) allowed to acquire the muscle tension generated by a computer (Pentium 4) and a "software" of data acquisition (AXOTAPE, pCLAMP 9.2). We performed a fatigue protocol by twitches, which consisted of repetitive electrical stimulation (pulses of 100 V, 300 ms duration, frequency of 0.2 Hz). The bundle was stimulated until the force decreased by 60 %, then was applied the study drug for 6 min to observe its effect. Concentrations 15, 25 y 50 µM were used to analyze a concentration-response curve.

An increase of post-fatigue tension was observed with all the doses tested, having 25 µM dose the maximum increment, 25 ± 3.55 % for peak tension and 39.51 ± 16.58 % for total tension with respect to fatigue force ($p<0.05$; $n=5$), 15 y 50 µM did not shown a statistical significance. Results indicated that ROS are necessary for a good functioning of the muscle. Also it shown that with a medium concentration of DPI force is recovered once the muscle was fatigued. The outcome of this experimental series indicates that NOX plays an important role in the production of ROS in skeletal muscle.

MAIZE POLYAMINE OXIDASES ARE IMPORTANT FOR TUMOR FORMATION IN THE MAIZE-*Ustilago maydis* INTERACTION

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Ustilago maydis is a dimorphic and biotrophic pathogenic Basidiomycete fungus responsible for common smut or “huitlacoche” in maize. The maize-*U. maydis* interaction is characterized by the hyperproduction of anthocyanins, the development of chlorosis and, the formation of large plant tumors (product of cell enlargement and proliferation), which are filled with dark pigmented sexual teliospores produced by the fungus.

Polyamines (PAs) are small aliphatic amines implicated in the regulation of many physiological processes in plants and appear to play important roles in their defense mechanisms against biotic and abiotic stress. Cellular PAs are tightly regulated, and recently their catabolisms has been related with biotic stress responses. PA degradation is mediated by diamine (DAO) and polyamine oxidase (PAO) enzymes, which yield hydrogen peroxide (H_2O_2), an important signalling molecule that may act as a mediator of programmed cell death, lignification, wall stiffening and cellular defense.

Herein, we characterized five maize PAO genes orthologous to the Arabidopsis PAO gene family. The maize PAO genes show specific expression paterns in different maize tissues. In order to determine their participation in the maize-*Ustilago maydis* interaction, their expression pattern was analyzed in early stages of the infection (chlorosis and anthocyanins production) and in maize tumors. It was found that mainly the *ZmPAO1* gene is expressed in tumors, while most PAO gene expression is down regulated at early stages of infection. Previous data generated by our research group indicated that PAO activity was increased ca. 2 fold in maize tumors. In these regard, we used a specific PAO inhibitor to block PAO activity in maize plants prior to *U. maydis* inoculation. If was found that inhibition of maize PAO activity negatively affects tumor formation, as well, diminishes H_2O_2 production in infected tissues. It is important to mention that the inhibitor does not affect *U. maydis* development and mating. We propose that H_2O_2 production trough PAO activity in maize is important for lignification and wall stiffening processes leading to tumor formation in the maize-*U. maydis* pathosystem.

Antioxidant Compounds of Timbe (*Acaciellaangustissima*) and Hypoblycemic Effect

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Currently the chronic complications of diabetes such as diabetic nephropathy and heart failure are the main causes of death in Mexico, which are closely related to inflammatory processes from the increase in oxidative stress generated in diabetes, however, reactive oxygen species (ROS) can be inactivated by natural defense mechanisms or through antioxidant compounds present in various foods or drugs, such as Timbe.Several studies have demonstrated the antioxidant and hypoglycemic of the specie of Acacciella, which belongs Timbe having a high content of bioactive compounds called phenols, which act as chain terminators of free radical reaction, transforming ROS to more stable products, conferring antioxidant activity that helps protect cells from oxidative stress and improve capillary resistance and inhibit inflammatory processes in diseases like diabetes The objective of this study was to determine the hypoglycemic effect of methanol extract of Timbe (*Acaciellaangustissima*) in rats with induced hyperglycemia. To do this, we determined the concentration of condensed tannins, total phenolic, flavonoids and antioxidant capacity of the extracts of Timbe, where the methanol extract (EMA) showed values 5, 3, 8 and 1.2 times, respectively, compared with the aqueous extract (EAA). For *in vivo* studies worked with streptozotocin-induced diabetic animals 45 mg / kg (with ≈ 250 mg / dl glucose), which were fed with the EMA added to the diet at the concentrations of 25, 50 and 100 mg / kg b.w., quantified weekly fasting blood glucose. At the end of treatments the concentration of insulin, urine protein and lipid profile (total cholesterol and triglycerides, HDL and LDL) and lipid peroxidation. EMA consumption in the highest treatment (100 mg / kg b.w.) produced a hypoglycemic and hypolipidemic effects in diabetic rats and a decrease in markers of kidney damage such as protein in urine, in addition to total cholesterol, LDL and triglycerides. Also, it was observed that was able to significantly attenuate the increase in the level of TBARS, obtaining similar values to control healthy rats. These results suggest that EMA exhibits a strong antioxidant activity due to their high content of phenolic compounds, indicate greater protection against inflammatory processes such as renal damage. It is suggested that this species can be applied in pharmaceutical industries for their bioactivity.

Key words:Diabetes, Oxidative Stress, Antioxidants, *Acacciellaangustissima*, Hypoglycemic, Kidney damage, Methanol extract.

Influence of Different Elicitors on phenolic compounds in sweet peppers (*Capsicum annuum*L.) cultivated in greenhouse conditions.

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Modifications in growing techniques can affect the yield and nutritional quality of various cultivated plant species. Due to its functional characteristics, sweet pepper (*Capsicum annuum* L.) was used in this study as a model plant to investigate the effect of electric conductivity (EC) and application of elicitors (SA and H_2O_2) on antioxidant capacity under greenhouse conditions. However, sweet pepper is an important vegetable crop, not only because of its economic importance, but also due to the natural value of its fruit(I). Plants are frequently exposed to different environmental stresses, which can be both biotic and/or abiotic. These stresses cause biochemical alterations as generation of hydrogen peroxide (H_2O_2) resulting in an early response of the plant defense mechanism. Oxidative burst, the generation of reactive oxygen species (ROS)(2). Sweet peppers contain a wide array of phytochemicals with well-known antioxidant properties. In this research functional trait such as flavonoids, tannins and phenolic content as well as the antioxidant properties were determined in sweet pepper (red). The correlation of the contents of flavonoids, tannins and total phenolics and the ability to remove free radicals of the sweet pepper was significant ($r = 0.99$, $P < 0.000$) in the T7 showed a correlation between the DPPH and ABTS. The T7 showed to be more effective than the treatment 1 (control), as in the treatment 1 antioxidant capacity by ABTS only affected by tannins and antioxidant capacity by DPPH is affected by flavonoids and tannins, therefore, T7 has synergizes of the three secondary metabolites in the two methods of determining the antioxidant capacity researched and could be considered a very good source of antioxidant bioactive compounds. Sweet pepper showed the
capability of preventing oxidation and reduces dietary risk factors for heart disease and cancer formation(3). Also, the sweet pepper could decrease the degradation of fatty acids that may cause deterioration of food flavor and taste. The high EC application did not affect the production the phenolics compounds in the T7. However, it may be concluded from the above
discussion that salicylic acid enhances the growth and productivity secondary metabolites of plants. Exogenous application of salicylic acid induces the resistance in plants, thereby provides a considerable protection against various biotic and abiotic stresses. The lower concentrations of salicylic acid have proved to be beneficial in enhancing the photosynthesis growth and various other physiological and biochemical characteristics of plants.

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ASCORBATE-GLUTATHIONE CYCLE IN MITOCHONDRIA OF TOMATO FRUIT IN TWO DIFFERENT RIPENING STAGES

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Plant mitochondria host some of the most important biological processes, i.e, oxidative phosphorylation, citric acid cycle and fatty acid oxidation. Although chloroplasts and peroxisomes are the major reactive oxygen species (ROS) producers in plant cells under light periods, mitochondrial metabolism significantly accounts for the total ROS generation. To maintain the cellular redox homeostasis and avoid an oxidative stress that could cause molecular damage, plant mitochondria possess a set of antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD), Trx/Prx/Srx system and enzymes of the ascorbate-glutathione cycle (ASC-GSH). The ascorbate-glutathione cycle is catalyzed by a set of four enzymes, ascorbate peroxidase (APX) monodehydroascorbate reductase (MDHAR), glutathione-dependent dehydroascorbate reductase (DHAR), and glutathione reductase (GR). These antioxidant enzymes scavengers together with metabolites such ASC and GSH, respond to the stress situations by regulating the level of ROS and modulating the redox signaling. Mitochondrial ROS generation can be perpetuated throughout a broad number of reactions yielding different reactive species that serve as substrates for the specific antioxidant enzymes. The mitochondrial antioxidant system, through superoxide and peroxides detoxification, has a pivotal role affecting redox signaling. In this work we showed the activity of the ascorbate-glutathione cycle enzymes and ASA/DHA, GSH/GSSG ratios in purified mitochondria from tomato fruit (Pony Espress F1) harvested in Murcia Spain in two different ripening stages (turning and orange).

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Determination of antioxidant activity in larvae and vermicompost of the insect *Tenebrio molitor*

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In foods of animal origin, we find the bugs and their derivatives; consumption practice is known as entomofagia. Studies have shown that insects contain high nutritional values, highlighting its protein value. Currently, many researches focus on the role of food antioxidant, which has attracted much attention since they have the ability to protect cells against oxidative damage that causes aging and chronic degenerative diseases. In this context, the antioxidant activity of the insect *Tenebrio molitor* in their larval stage and the product obtained by feeding (vermicompost) was evaluated by determining the inhibitory potential of 2,2-diphenyl-1-picrihidrazilo radical (DPPH$^{\bullet}$) and the 2'-azinobis-3-ethyl-benzthiazoline-6-sulfonate radical (ABTS$^{\bullet+}$). We found that antioxidant activity is greater in the vermicompost than in the larvae of *Tenebrio molitor*. This study provides information of bioactive or antioxidant potential of insects and their derivatives.

Chronic administration of S-allycysteine (SAC), do not causes histological damage in eight tissues, and induces Nrf2 factor and antioxidant enzymes in brain of male rats

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S-allylcysteine (SAC) is the most abundant compound in aged garlic extract. A large number of studies have demonstrated the protective effect of SAC, which has been associated with the prevention or amelioration of oxidative stress. One of defense mechanisms to prevent the oxidative damage involves the activation Nrf2 factor that enhances the transcription of the antioxidant and phase II enzymes genes. We evaluated the toxic effect and the ability of SAC to activate Nrf2 factor - a master regulator of the cellular redox state-and increase the antioxidant defense.

Male Wistar rats (90-100 g) were administered with SAC (25, 50, 100 and 200 mg/kg- body weight each 24 h, *i.g.*) by 90 days (this time was used as a model of a long administration useful in several chronic diseases, such as stroke or neurodegenerative diseases). Histological damage (in eight tissues), renal and hepatic function, and Nrf2 activation and the activities of glutathione peroxidase, glutathione reductase, glutathione S-transferase, catalase and superoxide dismutase (in hippocampus,striatum and cortex) were evaluated.

SAC treatment not induced morphological damage in kidney, liver, lung, heart, bazo, pancreas, hippocampus, striatum and cerebral cortex, neither changed renal and hepatic function. Hematic parameters evaluated were not changed with neither SAC doses used. On the other hand, SAC induced a transitory activation of Nrf2 factor in hippocampus, and increased the activity of antioxidant enzymes since the dose 25 mg/kg in the regions studied. The major increase was observed with SAC 50 mg/kg in striatum and SAC 100 mg/kg in hippocampus and cerebral cortex. Finally, the mayor changes in the enzyme activities occurred in hippocampus and striatum.

In conclusion, SAC didnot induce damage, was able to activate the Nrf2 factor in hippocampus, and could be activating other pathways in striatum and cerebral cortex. All data together emphasize its potential use as therapeutic agent in chronic diseases associated with oxidative stress.

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Effect of moderate physical exercise as Tai chi and Walking on Biological markers of Oxidative stress and chronic inflammation in the elderly

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ABSTRACT

Background: moderate physical exercise has been noted as an alternative beneficial to health, even has been pointed out as antioxidant and anti-inflammatory, however this effect in the elderly has not been fully demonstrated.

Objective: To determine the effect of moderate exercise: walking, Tai Chi and a combination of both modalities on biological markers of oxidative stress (OxS) and chronic inflammatory process (CIP) in older adults without chronic diseases out of control.

Methods: A longitudinal study was carried out in a population of 137 elderly people assigned to convenience to the following groups of interventions: i) 23 in the control group ii) 32 Tai Chi group iii) 51 walking group and iv) 32 combined activity group. We did anthropometric measurements, blood chemical and hematic biometry. Also it was measured plasma lipid peroxides (LPO), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS), and antioxidant gap, as biological markers of OxS, and interleukin 6 (IL6), interleukin 10, tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) pre and post intervention. The three groups carried out the assigned physical activity 5 days a week, in sessions of 50 for 6 months. The control group performed no physical activity. The data were subjected to a one-way ANOVA and covariance (ANCOVA), using SPSS V.16.0.

Results: We observed a significant decrease in total cholesterol, low density lipoproteins (LDL) and systolic blood pressure (SBP) in subjects who practiced Tai Chi compared to controls ($p < 0.05$). Levels of IL-10 were significantly higher in who practiced Tai Chi and the combined routine versus the control group ($p < 0.05$), also the concentration of TNF-α was significantly lower in the group who practiced Tai Chi compared to control ($p < 0.05$). With regard to markers of OxS adjusted baseline, SOD activity was significantly greater in subjects who practiced Tai Chi and those who practiced walking compared with controls ($p < 0.05$), while the GPx was significantly greater in the walking group relative to control ($p < 0.05$).

Conclusion: The three kinds of activities have antioxidant and anti-inflammatory effect in this population. Tai Chi produced a better anti-inflammatory and antioxidant response.

ROS generation in cell transformation induced by metal mixture and OLA1 expression

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Transformation assays have been proposed as a method of in vitro for the identification of potentially carcinogenic compounds. The two-stage Balb/c 3T3 cell assay is a validate approach to identified initiators and promoters of cellular transformation. Cell transformation is initiated by mutagenic stimulus of MNNG and promotion is induced by TPA, the assay induces a transformed phenotype after 25 days of cuture. Recent studies from our laboratories have validated procedure and have indicate that oxidative stress play a major role in the development for the metal mixture (2 µM NaAsO2, 2 µM $CdCl_2$, and 5 µM $Pb(C_2H_3O_2)_2 \cdot 3H_2O$) and transformation potential. The aim of the present study is determine differences between ROS changes through the transformation assay induced by MNNG and TPA as initiator and promoter stimulus and the transformation induced by the metal mixture as both (initiator and promoter). In addition is our interest determine the expression leves of OLA1, a negative regulator of the cellular antioxidant response independent of transcriptional processes through the transformation of Balb/c 3T3 induced by metal mixture. We found that cell transformation induced by MNNG and TPA did not generates ROS, while in the transformation induced by metal mixture show ROS generation that increase in the promotion phase more importantly in the cultures where metal mixture acts as promoter stimulus. During initiation, both ROS and OLA1 levels had the lower values, however across promotion is possible observe an increase of both parameters in transformation induced by metal mixture. At respect to expression levels of the OLA1 we determine the same behavior that ROS generation that is directly related.

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Selection of a thermotolerant yeast and evaluation of the oxidative stress and antioxidant response

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Yeast is an important microorganism, as a model for higher eukaryotes and a useful microbe in biotechnology.Yeasts are used in many industrial processes, such as the production of alcoholic beverages, biomass and metabolic products. In terms of industrial applications, stress tolerance is vitalfor yeast cellswhen they areexposed to different environments. High temperature is one of the most important stresses that raise the yeast susceptibility to inhibitory compounds. It affects growth rate and biomass yield, two of the physiological features of key importance in industrial processes. High temperature produced oxidative stress, and overexpression of antioxidant systems that increases thermotolerance. Such the environment of high temperature is expected to exert a natural selection pressure, to evolve as thermotolerant strains.In this work, wepresent one yeast isolatedaccording to their resistance to high temperature, that has less oxidative stress and more antioxidant response, e.g.*Saccharomyces* (MC4) and *Kluyveromyces* yeasts (OFF1 and SLP1). The resistance to high temperature was determined by specific growth rate (μ), biomass yield (DW), volumetric productivity (Qx) and doubling time (T2). To evaluate the oxidative stress was determined the production of reactive oxygen species (ROS), carbonylation and lipoperoxidation. The antioxidant response was determined by the superoxide dismutase and catalase activities, and glutathione production. The strain SLP1 (*Kluyveromyces*) showed higher specific growth rate, biomass yield, biomass volumetric productivities and less doubling time at 40 °C, whit less ROS, lipoperoxidation, and more glutathione production and catalase activity, in heat shock. The carbonylation and superoxide dismutase activity were not different between the strains. In conclusion, the SLP1 strain showed better thermotolerant adaptation, compared to the other strains.

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The hormone prolactin is a novel survival factor for the retinal pigment epithelium.

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Retinal pigment epithelial (RPE) cells form a polarized monolayer that is adjacent to the photoreceptors and indispensable for vision. Degeneration of RPE cells results in retinal disorders such as age-related macular degeneration. Because a major feature of this disease is the increased formation of reactive oxygen species (ROS), the identification of endogenous factors able to protect RPE cells from oxidative stress is important. Cell transplant strategies have potential therapeutic value for such disorders but an inadequate supply of donor cells limits their therapeutic success; therefore, identifying factors that help RPE cells to proliferate could provide a renewable source of cells for transplantation. Since we detected the hormone prolactin (PRL) and its receptor in RPE cells and previous studies reported PRL to be a survival factor, we postulated that RPE cells are a target for the PRL trophic effect. Using a cell line derived from human RPE (ARPE-19), we found that recombinant human PRL increases ARPE-19 cell viability and proliferation in a bell-shaped, dose-dependent manner. In addition, a competitive antagonist for the PRL receptor blocked the effect of PRL on ARPE-19 cell viability and proliferation. Next, we showed that conditioned medium from ARPE-19 monolayers contains PRL and that a specific anti-PRL antibody or the competitive PRL receptor antagonist reduces ARPE-19 cell viability. Also, we found that PRL prevented both the reduction in viability and proliferation and the increase in apoptosis of ARPE-19 cells induced by the oxidant hydrogen peroxide. ARPE-19 cells were also protected from hydrogen peroxide when PRL was applied after the oxidant. Notably, RPE cells from mice lacking the PRL receptor showed higher levels of apoptosis than RPE cells from wild-type animals. This effect was exacerbated in aging animals. Taken together, these results indicate that PRL is a novel endogenous survival factor for the RPE that is able to protect it from oxidative damage.

Mitochondrial DNA oxidation and OGG expression change (8-oxoguanine DNA glycosilase) during heart failure development.

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Introduction. Recent reports indicate that presence of mitochondrial DNA (mtDNA) oxidized in the cytosol, induce inflammation (IL-1) generating heart failure (HF) [1]. Furthermore, it is known that the most abundant form of DNA oxidized is 8-oxo-deoxiguanosina (8-oxoG) and an increase in oxidation leads mtDNA fragmentation and release from mitochondrion [2]. On the other hand, cells have DNA repair systems such as 8-oxo-guanine glycosilasa (OGG), whose regulation could be decisive for HF development. Recently, described that OGG overexpression inhibits cardiac fibrosis [3]. Therefore, the objective of the present work is to follow mtDNA oxidation as well as OGG contents during progression to HF.

Materials and methods. Coartation aortic (CAA) plus high salt diet (NaCl 1%) was used as hypertension model. After 3 or 6 weeks of CAA, 8-oxoG total and mitochondrial were analyzed by ELISA and OGG content was measured by Western blot.

Results: After three weeks of CAA, cardiac mass increase 19% ($p<0.05$), DNA oxidation and OGG content did not changed and mitochondrial function only was affected in state 3 (10 %, $p<0.05$). After 6 weeks cardiac mass increased significantly 23 % ($p<0.05$), DNA total oxidation does not changed, however mtDNA oxidation decreased significantly (97 ± 3; 82 ± 4 ng8-oxodG/10µgDNA, $p<0.02$), OGG total do not showed changes but mitochondrial OGG content increase 3 times ($p<0.05$) and mitochondrial function was not affected. Indicating that in initial stages, mitochondrial dysfunction occur without altering mtDNA structure and form this evolves there is a compensatory period marked by an increase in the enzyme OGG and decrease mtDNA oxidation, thus avoiding damage mitochondrial. **Conclusion**: The mitochondrial DNA damage by hypertension-induced is compensate for by an increase in the antioxidant system and a higher content of mitochondrial OGG.

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Does Bcl-3 activate p50 during oxidative conditioning hormesis response?

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Oxidative stress (OS) is a process in which reactive oxygen species (ROS) are in a major proportion than the antioxidant cell defenses. OS has been related to the etiology of several degenerative diseases like Alzheimer's disease, cancer and the aging process. In the past few years our laboratory developed an hormetic model, where L-929 fibroblasts were preconditioned with a hydrogen peroxide (H_2O_2) sub-lethal dose which activated the antioxidant and survival cell responses against lethal oxidative challenges. Part of the cellular response was carried out by the anti-apoptotic protein Bcl-2, whose expression was induced by the transcription factor NFkB, particularly by the p50 subunit, which was observed to form homodimers during the oxidative conditioning hormesis (OCH) response. Intriguingly, p50 subunit lacks of a DNA binding domain. Hence, the aim of this work was to find a protein that could work as an NFkB co-activator during the hormetic response. Bcl-3 is a protein that has been shown to interact with numerous members of the NFkB family, it has a DNA binding domain, and it also has several cysteines that might regulate its participation during the OCH response. All the previous suggest that Bcl-3 might be the ideal candidate to interact with p50 as the searched co-activator. Therefore, here we induced the OCH in L929 cells and determined Bcl-3 participation. Interestingly, our results showed that even tough we found a nuclear p50 and Bcl-3 immune-localization, and a NFkB activation by an Electrophoretic Movement Shift Assay (EMSA), this assay did not show a retardation with Bcl-3, suggesting that: 1) Bcl-3 interaction with p50 homodimer might be locking out the protein region recognized by the antibody and therefore the signal is not been retarded, or 2) Bcl-3 might not be the searched co-activator that we are looking for. Silencing Bcl-3 might be useful to determine if Bcl-3 is indeed p50 co-activator during the hormetic response.

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Dynamics of the intracellular H2O2 levels in the root of *Arabidopsis thaliana* in response to a specific NADPH inhibitor.

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Evaluación de la producción de especies reactivas del oxígeno en mitocondrias aisladas de hepatocitos de conejos con una dieta moderada en grasa.

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The reactive oxygen species (ROS) are created as a natural sub-product of metabolism; when the ROS concentrations exceed the antioxidant response tooxidative stressconditionis favored, this could be cause damage in the principal biomolecules. We know that when an organism has a lipid-rich diet produces damage in a short time, accompanied byoxidantive stress and chronic inflammation. However at this moment no one had realized studies for determinate which are the effects of a diet with a moderate concentration of lipids, in this work was evaluated the effect of moderate lipids added to the diet over the mitochondrial functionality and the ROS production; also we determined if the use of an nonsteroidal anti-inflammatory (acetylsalicylic acid) is able to reduce the damage in the mitochondrial function caused by the intake to lipids for a year. We used 4 groups Control, Grasa, GrasaAspirinaand Aspirina. Where production of ROS was evaluated, we found that the experimental group Grasa presented a high production that Grasa/Asipirna however Aspirina presented the higherlevel production than of all them. The mitochondrial functional evaluation demonstrated that in the experimental groups Grasa and Grasa/Aspirina exists a dysfunction in the mitochondrial complex III.

HEPATIC INJURY RESPONSE AGAINST A PRO-INFLAMMATORY STRESS IN RATS FROM DIFFERENT AGES

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Liver plays a unique role as metabolic center that carries out must of xenobiotics and drugs biotransformation and detoxification. The lipopolysaccharide (LPS), which localizes in the gram-negative bacterial external membrane, is a known immunogen that induces inflammatory responses and enhances reactive oxygen species (ROS) generation. Host response to LPS includes the expression of a variety of pro-inflammatory cytokines. When this process is exacerbated it turns into local or systemic tissue damage, leading to physiological detriment. However, the liver is able to induce tissue regeneration by activating different transcription factors that regulate proliferation and repair genes expression; nevertheless this repair capacity decreases with age. Therefore, the aim of this work was to study some of the factors that are regulating cellular processes after liver damage depending on age. An hepatic damage model was developed administrating LPS (0.5 mg/kg) a at different time-points 12, 24, 48, 72 and 96 h. Young (4 m) and adult (12 m) Wistar rats were used. Plasmatic pro-inflammatory cytokines such as IL-6 y TNF-α were determined, and an increase was observed at 12 h for both ages, but adult animals presented a higher increment. ALT and AST were determined for general liver injury as well as 8-OHdG for oxidative DNA damage, which was higher in the adult rats suggesting that DNA repair mechanism was slower for these animals.
One of the proliferation mechanisms after liver injury is the Delta-4-Notch pathway, so those two proteins were also evaluated; our results showed a higher expression for both of them in young than in adult animals. Since most cell death after LPS treatment is due to apoptosis, Bax and caspase 3 were also evaluated along with p53 as a damage reporter of damage DNA. In these regard we found that damage and death response in young animals is quicker and sustained, which was not the case for the adults.
However it might be important to continue with this study and determine other repair mechanisms activated by Delta 4-Notch.

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ROS detoxification analysis in the non-conventional yeast *Debaryomyces hansenii*.

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Oxidative stress can be defined as the imbalance between the production and the neutralization of reactive oxygen species (ROS) derived from nitrogen and oxygen; in our group we work with the non conventional yeast *Debaryomyces hansenii*, that seems to exhibit a better ROS neutralization system than *Saccharomyces cerevisiae* through catalase activity. Using flow cytometry, we demonstrated that on saline stress, O_2^- is the major ROS compared to the H_2O_2 content. Superoxide dismutase (SOD) catalyzes the O_2^- decomposition producing H_2O_2, which is further transformed to water and oxygen by catalases. Preliminary work in our lab has shown that catalase T is predominant during growth on glucose as a carbon source while catalase A is the main enzyme in ethanol as carbon source. Both enzymes are inhibited when NaCl is present. However, by Northern analyses we found contradictory results at transcript level. The aim of this work is to analyze if the carbon source and NaCl affect the expression of SOD and both catalases.

To answer this question, *D. hansenii* cultures grown until exponential and stationary phases on YPD (glucose), YPD + 0.6 M NaCl, YP-ethanol and YPEtOH + 0.6 M NaCl were starved and RNA was extracted.Samples were used for RT-qPCR performance, using primers for the identification of SOD and Catalases A and T transcripts.

Our first results confirm that *Dh*CTT is overexpressed in glucose media when NaCl is present while *Dh*CTA is overexpressed on ethanol–NaCl, suggesting that NaCl up-regulates at transcriptional level and down-regulates at posttranscriptional level, supporting the differential expression of catalases previously observed. We are currently running experiments to confirm these findings and to test the SOD role in these phenomena.

TETRASPANIN PLS-1 IN THE REGULATION OF NADPH OXIDASES IN *Neurospora crassa*

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The NADPH oxidases (NOX) are enzymes that produce reactive oxygen species (ROS) from O_2 and NADPH. ROS are key players in the regulation of cell differentiation in microbial eukaryotes.

N. crassa contains two *NOX* genes: *nox-1* and *nox-2*. The first is required for the fruiting body (perithecia) development, normal polar growth, asexual sporulation and cell fusion and the second is essential for sexual spores (ascospores) germination.

The tetraspanins are small ubiquitous proteins with four conserved transmembrane domains Acting as organizers of membrane signalling complexes, these proteins have been involved in regulation of cell morphology, motility and cell fusión. Three tetraspanin families (PLS-1, TSP-2 and TSP-3) have been identified in fungi and some of them have been linked to NOX function. In *Podospora anserina*, mutants lacking NOX-2 or tetraspanin PLS-1 share the same ascospore-specific germination defect, while in *Botrytis cinerea* Δ*nox*-B and Δ*pls*-1 mutants show similar phenotypes, being defective in plant cell penetration.

The tetraspanins are small ubiquitous proteins with four conserved transmembrane domains Acting as organizers of membrane signalling complexes, these proteins have been involved in regulation of cell morphology, motility and cell fusión. Three tetraspanin families (PLS-1, TSP-2 and TSP-3) have been identified in fungi and some of them have been linked to NOX function. In *Podospora anserina*, mutants lacking NOX-2 or tetraspanin PLS-1 share the same ascospore-specific germination defect, while in *Botrytis cinerea* Δ*nox*-B and Δ*pls*-1 mutants show similar phenotypes, being defective in plant cell penetration.

We show that the phenotypes of mutants lacking NOX-1 or the tetraspanin PLS-1 are virtually identical, indicating that PLS-1 is required for NOX-1 function. We generated *N. crassa* strains containg versions of the tetraspanin PLS-1 tagget at its C- and N-terminus, expressed from *pls-1* gene promoter, and show that these proteins are non-functional. In both cases the proteins were detected in hyphal septa, where they might co-localize with the NOX-1 protein. Our results indicate that intact PLS-1 N- and C-termini are critical for proper function. The localization of PLS-1 at hyphal septa remains to be confirmed using new functional tagged versions of this protein.

Oxidative stress by nicotine in rat gingiva

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Periodontal disease such as gingivitis and periodontitis, are associated to numerous factors; external as well as their own body.One of the external factors that are strongly linked as a risk factor is cigarette because it induces oxidative stress in the body (increased reactive oxygen species and / or decreased antioxidant system such as superoxide dismutase (SOD) and catalase (CAT)).

In the present research seeks to understand the involvement of oxidative stress in the damage caused by nicotine in rat gingiva.For that, the gum above the upper incisors of the vestibular area and palate was used. Both tissues were incubated with different concentrations (0.05 uM-5 mM) of nicotine for 2 to 3 h.Reactive oxygen species were measured to determine the concentrations and time to study.Concentrations of 0.05, 0.5 and 5 uM for 3 h were selected to evaluate different oxidative stress markers. It was observed that increases the production of hydrogen peroxide, superoxide and hydroxyl to 10 times in gingiva and palate.

The SOD and GPx antioxidant enzymes are inhibited by nicotine in the gum and are unchanged on the palate. The CAT enzyme is induced in the gingiva and palate.

Dynamic of reactive oxygen species in root hair cells and pollen tubes are essential for polar growth.

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Many responses in animal and plant cells depend from reactive oxygen species (ROS). These ROS can activate calcium channels and receptors involved in signaling processes and metabolism. In plant cells ROS accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response (Mittler and Berkowitz, 2001). In guard cells from *Viciafaba*regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS levels generate and maintain an apical calcium gradient. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes.
Herein we report a new molecular probe to depict the ROS dynamic during root hair cell and pollen tube apical growth. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. With this probe in root hair cells an apical gradient of H_2O_2 is observed and contributes to the polar growth, furthermore we were able to visualize the ROS oscillation, which are couple to growth oscillations. In pollen tubes we found a different ROS distribution, however their oscillations were clearer and couple to growth oscillations. In both tip growing cells, the apical domain result the site with the more dynamic ROS changes. Finally we analyzed the whole root and found a general oscillation in the complete root, probably regulating the plant root growth and development.

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The release of cytochrome c is altered in the liver of rats with metabolic syndrome: role of cardiolipin

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Mitochondria are essential for liver cell survival by producing energy and maintaining redox status. Moreover, accumulating data suggest that altered mitochondrial function results in reduced ATP biosynthesis and in increased reactive oxygen species (ROS) generation. Oxidative stress and ROS generated by mitochondrial respiratory chain have been described to be an important factor to determine the release of cytochrome c from the inter-membrane space into the cytosol[1].

This study was aimed to investigate the mechanism by which cytochrome c is released from liver mitochondria and especially in sucrose-fed rats (SFR) which have been shown to generate ROS at a higher rate than control rats.

Cytochrome c release from mitochondria of SFR liver is significantly lower when compared to control mitochondria oxidizing pyruvate/malate or succinate. The addition of Ca^{2+} to induce mitochondrial membrane transition increases cytochrome c release from both SFR and control rat mitochondria but cytochrome c release remains lower in SFR mitochondria compared with control mitochondria. The difference in cytochrome c release from SFR and control mitochondria can also be due to changes found in the composition of cardiolipin molecular species, components known to be involved in cytochrome c interaction with the mitochondrial inner membrane. The proportion of palmitic acid-rich cardiolipin species is found increased in lipid membrane from SFR mitochondria while linoleic acid-rich cadiolipin species is found decreased in SFR mitochondria. Furthermore the amount of BAX, found lower in mitochondria and higher in supernatant homogenate from SFR, may also be involved in the reduced cytochrome c release from SFR mitochondria.

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Participation of Fe-S ProteinsAco1p, Lip5p, Sdh2p and Rip1p in Ethanol toxicity in *Saccharomyces cerevisiae*.

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The yeast *Saccharomyces cerevisiae* has served as a model organism todiscover the steps occurring in several metabolic pathways shared betweeninferior and superior eukaryotes.Among these metabolic processes, one of the most conserved is the biogenesis of Fe-S proteins. This process is primarily carried out in the mitochondria and requires sophisticated systems comprising at least 14 proteins with diverse functions, encoded by a set of genes called ISC (Iron Sulfur Cluster).Previous reports in our working group have shown that Ssq1p, Jac1p and Isu1p proteins,which belongs to the biogenesis of Fe-S centers, are involved in ethanol tolerance.

The goal of the present work was to determine the involvement of the Fe-S proteinsAco1p, Lip5p, Rip1p Sdh2p in the mechanism of ethanol toxicity in *S. cerevisiae*, as these proteins are involved inROS generation, the reduction of NAD^+ and FAD^{2+} and in functioning ofthe electron transport chain (ETC).Growth kinetics, ethanol and H_2O_2 tolerance, Fe release and ROS productionwere assayed in the wild-type strain BY4741 and the mutant strains lip5Δ, aco1Δ, sdh2Δ and rip1Δ. The results shown higher sensitivity of the mutant strains to these substances relative to the wild-type strain. The magnitude of the ROS generated was directly related to the amount of free iron in the cell and these values increasing with the ethanol treatment. Finally the activity of each protein (aconitase, succinate dehydrogenase-complex II, cytochrome-c oxidoreductase-complex III and cytochrome c oxidase-complex IV) was determined. The results show low activity for aco1Δ mutant and no activity for sdh2Δ and rip1Δ mutants, respect for the wild type and mutant lip5Δ, indicating a synergistic effect between the activities analyzed for these proteins.We conclude that the proteins with Fe-S: Aco1p, Lip5p, and Rip1p Sdh2p are involved in ethanol tolerance by decreasingROS generation and Fe^{2+} release.

Oxidative DNA damage and antioxidant enzyme activity in two vampire bats species *(Desmodusrotundus y Diphyllaecaudata)*

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Oxidative DNA damage accumulation has been related to alterations in physiological processes within cells and organs, resulting in the deterioration associated to aging. This kind of damage can be caused by an imbalance between reactive oxygen species (ROS) and antioxidant enzymes, which is called oxidative stress. Vampire bats are a very interesting model; they are long-lived species (15-20 years) compared to other mammals its size. Even though they feed exclusively on blood, which contains high levels of iron. Iron is a ROS inductor through the Fenton reaction, causing DNA damage. There are only three known species of vampire bats in the world and two of them live in Mexico, *Desmodus rotundus* that feeds on mammals blood and *Diphyllae caudata* that feeds on wild birds blood, so it is interesting to study the mechanisms by which these species are able to counteract ROS damage in order to and live long.
Hence, oxidative DNA damage was quantified measuring 8OHdG by HPLC in liver, brain and intestine of those two vampire bat species. The activity of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase was also determined. Our results indicated that DNA damage increased with age which was not the case for the antioxidant enzymes.

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OXIDATIVE STRESS IN LIVER AND BRAIN IN RATS FED WITH A DIET SUPPLEMENTED WITH 30% SUCROSE

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It is a social priority to attend the health problemaof obesity.Multiple resources have been dedicated to elucidate the cellular and molecular aspects of this malady. Evidence has been published demonstrating that oxidative stress (OS) could be an important factor in the physio-pathology of obesity. It is known that OS plays a role in the pro-oxidant damage that characterize several immflamatory conditions such as the overweight and obesity.
Experimental models of obesity usually involve modified animals lacking the expression of key genes, or protocols with high proportion of fat or sugars in the diet. It has been reported that 30% sucrose can cause alterations that are similar to the symptoms of metabolic syndrome in humans.
In this project, we have studied the effect of obesity with the pro-oxidant reactions that take place in different subcellular fractions of the liver and in some brain areas. In addition, we correlated these findings with alterations in chronobiological parameters such as the rhythms of locomotor activity and food intake.
We used 30 male rats with an initial weight of 200 g. Each animal was ubicated in a cage to record the locomotor activity. Every week the glucemic levels were measured as well as the body weight. Each 2 weeks, the rats were subjected to a glucose tolerance test.
Chronobiological parameters were evaluated by the ActiView program. Pro- oxidant reactions were determined by TBARs, conjugated dienes and carbonyls in hepatic subcellular fractions. Mitochondrial structure was evaluated by electronic microscopy. In addition, histological stains were implemented (hematoxilin-Eosin and Red oil).
Preliminar results showed increased OS in brain homogenates and the hepatic cytosolic fraction of the obese rats fed with sucrose. The data are suggestive that obese animals are able to control the pro-oxidant reactions almost as in normal conditions. Evaluation of antioxidant factors are needed to have a more complete perspective of these experiments.

Role of ZnT1 in the regulation of hepatic Zinc after an experimental inflammatory process

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The phase acute response that is produced after the inflammation during a experimental surgery in animals, has been related with a decreases of zinc in plasma due to the redistribution of this metal to different organs and tissues, mainly liver. However at this time is unknown the mechanism involved in the return of zinc to basal conditions when homeostasis is recovered. Therefore the main objective of this work was to evaluate the role of the zinc transporter ZnT1 during this process. Animals received a 3-cm ventral laparotomy and at different times after surgery were sacrificed, and the liver from each animal excised. Hepatic zinc levels were determined by atomic absorption spectrophotometry, the intracellular distribution of zinc was evaluated using zinquin and dithizone stains and the metallothionein levels by the Cd saturation method. RNAm of the ZnT1 exporter was determined by RT-qPCR and the protein expression by immunohistochemistry and western-blot. Twenty hours after experimental surgical trauma was produced an increase of zinc in liver and remain untill 36 h post-surgery, returning to basal levels at 48 h. At intracellular level, zinc that is weakly binding to proteins showed increases between 20 to 28 h, meanwhile, zinc compartmentalized (inside cellular organelles) increased from the 28 h to 32 h, decreasing 36 h post-surgery. Twelve hours after surgery, hepatic metallothionein had an increase and this increase remained until 36 h. Experimental surgery had a positive effect on RNAm and protein levels of ZnT1, which presented an increase after 16 h post-surgery and remained until the 36 h, although ZnT1 protein remained increased at 48 h. The changes found in the expression of the zinc transporter after an experimental surgical trauma in animals, shows that this metal exporter can have an important role in the regulation of hepatic zinc concentrations in the inflammatory process.

Effect of oestradiol and testosterone on the activity of GPx and levels of MDA in CBA/Ca mice infected with *P. berghei* ANKA.

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Malaria is an infectious disease originated by the parasite*Plasmodium*, is responsible of almost one million of deaths every year. Although the incidence is similar between both sexes, men develop higher pathology and mortality than women; this phenomenon is called sexual dimorphism. Given that, the main mechanism for parasite elimination by the immune response is oxidative stress, and because of the major differences between sexes are dictated by gonadal hormones,in the present work, we studied the effect of oestradiol or testosterone on the activity of the antioxidant enzyme glutathione peroxidase (GPx) and on the levels of malondialdehide (MDA) in the blood, liver and spleen of CBA/Ca mice infected with *P. berghei* ANKA. To this end, groups of female and male mice were treated with oestradiol or testosterone and sesame oil as control. The activity of GPx and the levels of MDA were analysed by spectrophotometric techniques.

The activity of GPx was higher in the blood and liver of male compared with female mice. Administration of testosterone in female mice increased the activity of GPx, on the contrary, in males testosterone decreased the activity of GPx in all the tissue analysed, suggesting that testosterone levels modulates the activity of this anti-oxidant enzyme. The administration of oestradiol in female mice decreased GPx activity in the blood and spleen. Conversely, the administration of oestradiol increased the activity of GPx in spleen but decreased it in the liver of male mice. These results suggest that oestradioldifferentially modulate the oxidative stress in distinct tissues from the same organism.

Furthermore, the administration of testosterone did not modify the MDA levels in female or male mice. In contrast, the administration of oestradiol increased the MDA levels in females and in the blood and liver of male mice. In conclusion, female and mice exhibit important differences in oxidative stress after malaria infection, and both testosterone and oestradiol affect differentially the oxidative stress in male and in female mice infected with *P. berghei* ANKA. These results provide knowledge about the role of hormones on the oxidative stress during malaria infection.

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Glycosylation pathways in T lymphocyte-associated signal transducer and activator of transcription-6 in a mouse model knockout

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Surface glycoproteins regulate various cellular functions and the formation of such molecules is carried out by the process of glycosylation. T cells are key regulators of the adaptive immune response helping in the defense against pathogens intra or extracellular. STAT-6 is a transcription factor responsible for the activation of GATA-3 favoring Th2 response. Other hand STAT-6 is involved in the development of inflammation or bronchoconstriction airway tissue.

Last years it has been determined that the transcription factor T-bet and GATA-3 involved in the expression of membrane as sugars selectin ligand Sialyl Lewis-X. Furthermore, subpopulations of Th1 and Th2 cells have a profile of differential glycosylation lactosaminicos and sialic acid residues, respectively. The aim of this work is to determine the glycosylation profile that regulates STAT 6 during the activation of CD4 and CD8 T lymphocytes as well as in a Th2-type response. To analysis of the glycosylation of membrane following T lymphocytes play lectins that recognize sialic acids, lactosamines, GalNAc, GlcNAc and mannose alpha for subsequent analysis by flow cytometry was used.

The results showed that STAT-6 regulates the expression of the saccharide ligands on T $CD4^+$ and $CD8^+$ activated, since the absence of this transcription factor produces a higher density of ligands, lectins, suggesting that the expression of ligands containing sialic acids, fucose, galactose, mannose, *N*-and *O*-glycans is finely regulated by the activity of STAT-6. Finally, simulating a natural microenvironment for inducing a Th2 response, we used an in vivo model of immune Th2 response and try to find out whether the absence of STAT-6 could induce a change in glycosylation of membrane of T lymphocytes $CD4^+$ and $CD8^+$ however to date only been together two independent experiments and all I can add is that a seemingly opposite trend to that found in our model of in vitro activation with anti-CD3 antibodies observed in this model anti-CD28 , since both T $CD4^+$ and $CD8^+$ lymphocytes from WT mice showed a higher glycosylation pattern of cells STAT-$6^{-/-}$.

Effect of oestradiol and testosterone on the activity of catalase and the levels of haemoglobin in CBA/Ca mice infected with *P. berghei* ANKA.

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Malaria is a parasitic disease that induces the death of approximately 1 million of people around the world every year. Although the prevalence of *Plasmodium* infection does not differ between the sexes, parasitaemia and pathology are usually higher in men compared with female, suggesting that circulating sex steroids may influence outcomes. Given that anaemia is a feature of pathology in malaria and that oxidative stress is related to the elimination of this parasite. In the present work, we studied the effect of increase the levels of oestradiol or testosterone on both, the levels of haemoglobin (Hb) and the specific activity of catalase in mice infected with *P. berghei* ANKA. To this end, CBA/Ca female or male mice were administered subcutaneously with oestradiol or testosterone and then infected with *P. berghei* ANKA. Groups of female and male mice administered with the vehicle and infected with the parasite were used as controls. On day 9 post-infection, mice were sacrificed and samples of blood, spleen and liver were used to quantify the catalase specific activity, blood was also used to evaluate anaemia detected by the haemoglobin levels.

Male displayed higher levels of Hb than female mice. The administration of oestradiol increased the concentration of Hb in female mice, whereas in male the effect was the opposite. The treatment with testosterone did not modify the haemoglobin concentration in male, but in female increased the levels of haemoglobin. Relative to catalase activity, no significant differences were detected between male and female mice. The administration of oestradiol did not mofify the activity of the enzyme. However, the treatment with testosterone decreased the activity of catalase in blood of male, whereas in spleen the effect was the opposite.

In summary, the increment in testosterone or oestrogen in female mice increase the levels of haemoglobin and consequently decrease anaemia in female contrary to male mice. The administration of oestradiol affects differentially the catalase activity of different tissues. This work provides evidence that sexual hormones affect anaemia and oxidative stress in malaria and this knowledge could be useful for the future design of new drugs or vaccines against malaria.

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Analysis of Branched-chain amino acid metabolism and mTOR activation during macrophage polarization

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Macrophages can be polarized to an antiinflamatory (M1) or proinflammatory (M2) phenotype. During obesity, macrophage polarization in adipose tissue is prone to the M1 phenotype producing an inflammatory state. However, little is known about the mechanism that favors this M1 phenotype. Furthermore, several biochemical parameters are increased during obesity, and one that has attracted much attention are the branched chain amino acids (BCAAs). Among BCAAs, leucine plays an important role in protein synthesis because is a potent activator of mTOR. Recently was highlighted that mTOR plays an important role in regulating macrophage polarization. Thus, the aim of this study was to analyze BCAA metabolism and mTOR activation during macrophage polarization and to evaluate if an increase in the availability of BCAAs could impact the polarization of macrophages. To test our hypothesis, bone marrow-derived macrophages were polarized to M1 subpopulation by stimulating whit INFγ and LPS or polarized to M2 with IL-4. BCAT and BCKDH expression and the oxidation, incorporation to lipids or proteins of [U-14C]-leucine were determined to evaluate BCAA metabolism at 4, 24 and 48hrs to after the induction of polarization. Phosphorylation of S6K and AKT was determined to evaluate the activation of mTORC1 and mTORC2 at the times mention above. Finally, genes markers such as IL-12, iNOS, Fizz1, Arg1 and YM1 and cytokine production of TNFα and IL-10 were measured after the polarization of macrophages in the presence of high concentrations of BCAAs to evaluate changes in the phenotype of the subpopulations. Our results show that M1 macrophages have higher expression of BCKDH, increased leucine oxidation and higher activation of mTORC1, reflected in an increasedleucine incorporation to proteins. Interestingly, there wasn´t any changes in these parameters in M2 macrophages. Finally, we found that increased concentrations of BCAAs had not affected macrophage polarization. However, there was a decrease in the expression of M2 macrophage gen markers (Arg and FIZZ1) and decreased secretion of TNFγ and IL -10 in M1 subpopulation in the presence of BCAAs. In conclusion, M1 macrophages are able to metabolize BCAA in a higher rate than the M2 subpopulation and high concentrations of BCAAs have little effect on macrophage polarization.

Effect of sexual hormones on thevariationofweight and temperature in CBA/Ca mice infected with *P. berghei* ANKA.

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Malaria is a global health problem; every year causes almost one million deaths in the world. Fever andweight loss are some of the main characteristics of this disease. Although, the incidence of malaria is similar between sexes, men display higher pathology than women, this phenomenon is called sexual dimorphismand it is important because could affect the treatment by using drugs or even vaccines. Given that testosterone and oestradiol are the main sexual hormones responsible of major differences between sexes, in this work, we studied whetherthe administration of testosterone oroestradiolinduce changes in fever and weight lossin female and male CBA/Ca mice infected with *P. berghei* ANKA. Temperature was measured using an infrared thermometer and weight loss was detected using an electronic balance.

Female mice showed higher temperatures than male mice from day 0 to day 7 post-infection.Afterwards, male displayed higher temperatures than female mice. The administration of testosterone increased the temperature with exception of days 3 and 9 in male mice, whereas in female increased the temperature only on days 2 and 8 post-infection. The treatment with oestradiol decreased the temperature from day 3 to 6 post-infection in female mice, whereas in male mice, oestradioldecreasedthe temperature only on day 4 post infection.

Female developed higher body weight than male from day 0 to 7.Afterwards, female lost more weight compared with male mice. The administration of testosterone increased the body weight only on days 2 and 3 post-infection in male mice, whereas in female the treatment with testosterone increased the weight compared with vehicle treated mice. Finally, the administration of oestradiol decreased the body weight in both female and male mice.

In conclusion, female developed higher temperatures and lower weight than male mice, oestradiol is the hormone that affects more the temperature and weight in mice infected with *P. berghei* ANKA. This work shows evidences that levels of sexual hormones are critical forimportant features of pathology such as fever or weight loss.

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Cortactin regulates intestinal epithelial permeability by stabilizing tight junctions

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Chronic inflammatory diseases of the intestines such as inflammatory bowel disease (IBD) are characterized by disturbed epithelial contacts and increased intestinal permeability. The actin cytoskeleton and its regulators are critical for these features. Analyzing cortactin-KO mice, we found that the actin-binding molecule cortactin (CTTN) controls vascular permeability. However, it is unknown if CTTN also regulates intestinal epithelial permeability. Thus, we generated a stable CTTN-depleted Caco-2 cell line that showed reduced transepithelial resistence (TER) and increased paracellular flux of 4 kDa FITC-dextran. In these cells, we also observed a less developed cortical actin ring and internalization of occludin and ZO-1. However, CTTN depletion did not cause increased apoptosis. CTTN-KO mice also showed increased basal permeability in the colon. In healthy human colon tissue biopsies, we found that CTTN colocalized with actin and the tight junction protein ZO-1. By contrast, in IBD patients, we observed strong internalization of CTTN resulting in a loss of colocalization with actin and ZO-1. Our data imply that CTTN regulates intestinal permeability by controlling actin dynamics at cell contacts to stabilize epithelial junctions.

Virulence, immunopathology and transmissibility of selectedstrains of *M. tuberculosis* fromepidemiologicalstudies in Colombia evaluated in a murinemodel

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Untilrecentlythe*M. tuberculosis* (Mtb) complexwasconsideredgenetically a highlyconservedgroup of bacteria, however, overthelastdecade, a widevariety of genotypeshavebeendiscoveredamongMtbstrains. Now, differentepidemiological and molecular data haveshownthatvariations in transmissibility and virulence of differentstrainscould be relatedtothegeneticbackground of theorganism. **Objetctive:** Describe and assesifthere are variations in theprogression of thediseasecausedbythedifferentstrains.
Methodology: SixMtbstrainsthatcauseddifferentclinical/epidemiologicalpatterns of pulmonary tuberculosis weretested in a wellcharacterized BALB/c mouse model of progressivepulmonary tuberculosis to examine thecourse of infection in terms of strainvirulence (mouse survival, lungbacillaryloads, histopathology) and immunological response (cytokineexpression). Also, a mouse model of transmissibilityconsisting of prolongedcohousing of infected and naïveanimalswastested. **Results:**Allthetestedstrainscauseddistinctdiseasepatterns, regardlessof havingthesamegenotype (LAM): 1) two of thestrainsshowedhighbacterialloads, more tissuedamage(necrosis) and a strong and fastproinflammatory response;
2) in contrast, therest of theisolatesshowedlowerbacterialloads and formation of granulomas and pneumoniaaroundthe 3rd week; 3) allsixstrainscould be transmittedtonïveanimals.
In conclusion, geneticallydifferentMtbstrainsmayaffecttheprogression of thedisease, and establishingthe link betweenvirulence and transmissionisimportanttoreachthegoalfor Tb control.

Effect of the E6 and E7 oncoproteins of HPV16 on dendritic cells in skin of transgenic mice models

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The high risk human papillomavirus (HR-HPVs) are strongly associated with the development of epithelial cancers. Various studies have demonstrated that the E6 and E7 oncoproteins of HR-HPVs cooperate to exert cell immortalization and transformation. These oncoproteins alter cellular processes like apoptosis, chromosomal stability, transcription, cell differentiation and immune response. The activation of dendritic cells, such as Langerhans cells (LC), is required to eliminate HPV-infected cells by the immune system; however, HR-HPVs have developed different mechanisms to prevent the action of antigen presenting cells. Therefore, the aim of this study was to determine the differential effect of the E6 and E7 oncoproteins on dendritic cells in skin using the K14E7 and K14E6 transgenic mice models. Hyperproliferation on skin of K14E7 and K14E6 models was determined by immunohistochemistry and hematoxylin-eosin. The expression of MHC-II was analyzed by flow cytometry, and a reduction of this marker was observed in samples of K14E7 and K14E6 mice as compared with control mice (FvB). The MHC-II and DEC-205 markers used for immunohistochemistry in epidermal sheets showed morphological alterations in dendritic cells of K14E7 mice samples, however, this was not observed in K14E6 and control mice. Together, these results suggest that the E6 and E7 oncoproteins could affect antigen presentation; moreover, the E7 oncoprotein is able to modify the morphology of the Langerhans cells. Additional experiments are needed to determine whether the function of these cells is affected by oncoproteins in our models.

Effects of plant antimicrobial peptides on the innate immune response of bovine mammary epithelial cells infected with *Staphylococusaureus*

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Antimicrobial peptides (APs) are small molecules that typically have positive chargeand contain hydrophobic residues. These peptides exhibit antimicrobial and cytotoxic activities against bacteria, fungi, viruses and cancer cells. To date, more than 2,400natural AP have been described, which are abundant in eukaryotes, but bacteria can also produce them.Recently, these peptides have been named as host defense peptides (HDPs)in mammals,due to their immunomodulatory properties besides their antimicrobial activity. In general, the immunomodulatory concentrationsof these HDPs are lower than those with antimicrobial effects. In plants, the immunomodulatory effects of AP (plant APs, PAPs) have been poorly explored in mammal cells. Considering that secondary and tertiary structures of these peptides are conserved among family members in eukaryotes such as defensins, the aim of this work was to evaluate theimmunomodulatory effects of the PAPs γ-thionin produced by*Capsicum chinense* andthionin Thi2.1 from*Arabidopsis thaliana* on innate immune response of mammal cells. To do this, we employed a host-pathogen model: bovine mammary epithelial cells (bMEC) infected with *Staphylococcus aureus*. In this model, we used a clinical *S. aureus* strain (ATCC 27543) from bovine mastitis, and analyzed the effect of chemically synthesized γ-thionin and Thi2.1 on the internalization of bacteriain bMEC treated 24 h with these PAPs.Previously, we performed viability assaysin order to determine possible cytotoxic and antibacterial effects of these PAPs against bMEC and *S. aureus,* respectively. Using MTT and trypan-blue exclusion assays, we did not detect cytotoxic effects of these PAPson bMECat a range of concentrations from 100 ng/ml to 10 μg/ml, neither antimicrobial effects for bacteria using turbidimetric analysis or CFU counting. The internalization of *S. aureus*was inhibited 80% in bMEC treated 24 h with Thi2.1 at concentrations of 5 and 10 μg/ml, and a similar result was obtained using γ-thioninat concentrations of 0.5 and 100 ng/ml and5 μg/ml.In order to determine if the reduction in *S. aureus* internalization is due to the immunomodulation of these PAPs, we analyzed by ELISA the concentration ofthe pro-inflammatory cytokine IL-1β.The PAP Thi2.1 at 500 ng/ml increased (~1.5 fold) the secretion of IL-1 βto culture medium.Now we are performing analysis of real-time PCRto determine the expression of several genes representative of the innate immune response, in order to analyze if the reduction in bacterial internalization correlates with the immunomodulatory properties of these PAPs. The genes analyzed include: chemokines, pro and anti-inflammatory cytokines and host antimicrobial peptides.In conclusion, these results indicate that PAPs could be modulators of the innate immune response in mammals in addition to their antibacterial activity.

Activity of GPx and MDA levels in mice knockout for the prolactin receptor infected with *P. berghei* ANKA.

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Malaria is the infectious disease responsible of almost one million of deaths every year. In pregnant women the mortality increases.Furthermore, in this particular condition prolactin levels also augment, suggesting that prolactin may be involved in this outcome. Both Th1 and Th2 immune responses are important for the elimination of *Plasmodium* (the parasite that causes malaria). However, when the pro-inflammatory responses mediated by Th1 citokinesare not regulated by Th2 anti-inflammatory cytokines the mortality increases. Prolactin in the immune system is considered as a pro-inflammatory Th1 cytokine and because is increased in pregnant women, it is possible that plays an important role in the outcome of malaria. In addition, oxidative stress is related to the elimination of *Plasmodium* for the immune system. To study whether prolactin affects the oxidative stress in malaria, we infected knockout prolactin receptor mice (KO) with *P. berghei* ANKA and measured the activity antioxidant of glutathione peroxidase (GPx) and the levels of malondialdehide. We found that GPx activity and MDA levels werehigher in of KO mice compared with their wild type counterparts. These results suggest that prolactin is involved in the modulation of oxidative stress in CBA/Ca mice infected with *P. berghei* ANKA.

This work provides knowledge about the role of prolactin in malaria which is important to understand the higher mortality in pregnant women infected with *P. berghei* ANKA.

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PlantDefensins: Structure, Function and Heterologous Expression

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Plant defensins are clustered as a rich cysteine protein superfamily with more than 500 members. CADEF1 from *Capsicum annuum* is a 47 amino acid peptide with four disulfides that shows antifungal biological activity *in vitro*. Besides pathogen infection, CADEF1 transcript was also detected in response to salicylic acid, methyl jasmonate, hydrogen peroxide and other compounds. It has not been possible to purify this peptide from plants. Recently, the homologous defensin (83% of amino acid sequence identity) from*Medicago,* MtDef4, was characterized as a phospholipid binding protein and a mutant with high selectivity for phosphatidic acid was generated [Sagaram*et al*., 2013]. A related peptide from *Nicotiana,* NaD1, is known to bindphosphatidylinositol 4,5- bisphosphate(PIP_2). Its dimerization, and sequentialoligomerizationto obtain an active peptide is PIP_2 concentration dependent [Poon*et al*., 2014]. This membrane penetrating peptides emerge as possible factors in signaling pathways and gene expression.In this work, the efforts for CADEF1 heterologous expression in *Escherichia coli*are discussed.Recombinant expression system factors such as fusion protein, solubility, disulfide pattern and yield of the product areemphasized. The structural motifs for oligomer formation and phospholipid binding site are proposed.

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Immunomodulatory effect of an adenosine derivative (IFC-305) in isoproterenol induced myocardial infarction

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Cardiovascular diseases are the principal cause of decease in the world, 16.4% of the worldwide population dies every year as consequence of myocardial infarction (MI) (WHO, 2013).In the myocardium infarction a vascular occlusion originates isquemic necrosis and decrease of coronary flow. Several models had been developed to research this pathology, nonetheless not all models offer the possibility to evaluate each stage of MI, which limits the understanding of this phenomena. In 1956, Rona found that isoproterenol (ISO), a beta adrenergic agonist, is able to induce MI in rats. This model, characterized biochemical, histological and physiologically (Chagoya, 1997) allows the study of every stage of MI: pre-infarction, infarction (12 hours after ISO administration) and post-infarction.

Correct inflammatory responses during the post infarction phase leads to the formation of a scar with enough force to prevent the extension of the infarct, which is necessary for an accurate healing process (Anzai, 2013).It has been reported that adenosine has a strong role in the inflammation process.(Haskó, 2004).

In previous studies achieved in our laboratory it was observed that an adenosine derivative, IFC-305 favor the hepatic tissue remodeling , modulates the energetic state (Pérez-Carreón JI, 2010; Master thesis Pérez-Cabeza de Vaca, 2010) and immune response in hepatoxicity, this suggest a possible role of the IFC-305 against the injuries that take place during MI.

EXPERIMENTAL MODEL: Male rats Wistar of 200g receive a subcutaneous administration of ISO 67mg/kg, and one hour after they receive an IFC-305 intraperitoneal injection. We worked with the following groups: 1. Control (Saline solution), 2. ISO, 3.IFC and 4. ISO+IFC. Rats sacrifice was performed 6, 12, 24 or 96 hours after ISO administration. Hearts and plasma samples were recollected.

We evaluated the levels of IL 1β, IL-6, INF γ, TNF α, and IL 10, in male Wistar rat plasma and heart, through every stage of the infarction process induced by isoproterenol. The results show alterations in the former cytokines levels as consequence of isoproterenol administration. Also, the restoration of the physiological levels of these cytokines was reached with the administration of an adenosine derivative: IFC-305.

In conclusion, our results show evident alterations in cytokines levels as reflect of immune response that take place during the MI and that the IFC-305 is able to modulate this response in a favorable way. Still, there are necessary more studies about the immnunomodulatory role of the IFC-305 during MI.

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Regulation of steroid hormones on the secretion of proMMP-9 produced from human fetal membranes during infection with *Escherichia coli*.

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Introduction

The human fetal membranes actively respond to various infectious stimuli by secreting inflammatory cytokines (IL-1β, IL-8, TNFα), uterotonic (PGE) and collagenolytic metalloproteinase (MMP-2 y -9). Has been shown that steroid such as progesterone (P_4) and estradiol (E_2) regulate the secretion of IL-1β, IL-8 and TNFα. However, this regulation has not been determined in human fetal membranes.

Objective

To evaluate the effect of the P_4 and E_2 on the secretion of collagenolytic metalloproteinase after infection with *Escherichia coli*

Materials and methods

Term human fetal membranes obtained by cesarean section were mounted in Transwell system generating two amino magazines and coriodecidua which were infected with Escherichia coli by the following groups 1) Control; 2) infection coriodecidua; 3) infection in the amnion. In each group were incubated with P_4 (4.80 x 10-7M), E_2 (2.5 x 10-7M) and in combination. After 24 hours incubation, the culture medium was recovered and the activity of metalloproteinase was determined by gel activity.

Results

In the region of the coriodecidua E. coli increases the secretion of MMP-9. The P4 ($p = 0.03$) and E2 ($P = 0.03$) significantly reduced the secretion of MMP-9; however, the simultaneous incubation of P_4 + E_2 not reduce secretion. In the region of the E. coli amnion increases the secretion of MMP-9. E_2 ($p = 0.03$) reduced the secretion of MMP-9; however, the simultaneous incubation of P4 + E2 not reduce secretion.

Conclusions

The main findings in this study are: 1) P_4 and E_2 regulate the secretion of proMMP-9; 2) there is a differential effect being the amnion and coriodecidua; and 3) the simultaneous incubation of the two hormones inhibits the effects observed.

Proinflammatory cytokines responses induced by *Pseudomonas aeruginosa* in human type II pneumocyte cells.

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Introduction: The epithelial surface of the alveoli is composed of alveolar pneumocytes type I and type II cells (PTII). The PTII play a key role at synthesis, storage and secretion of pulmonary surfactant (PS). Besides its function at stabilizing the alveolar epithelium, the PS takes part in the establishment of innate defense mechanisms on the lung. [1] *Pseudomonas aeruginosa* express a wide range of virulence factors, which are able to significantly compromise host defense and induce potent activation of inflammatory responses [2], such as pro-inflammatory cytokines.

Objectives: To evaluate the secretion profile of the pro-inflammatory cytokinesIL-1β and TNF-α in human type II pneumocyte cell line A549 infected with three different concentrations of *Pseudomonas aeruginosa* through time.

Material and methods: A culture of PTII (A549) was infected using three different concentrations of *Pseudomonas aeruginosa* (10^2, 10^4 y 10^6 CFU/mL), collecting the culture media at 3, 6 and 24 hours post infection and working simultaneously with the control group (Not infected cells). The secretion profile of pro-inflammatory cytokines IL-1β and TNF-α (IL-1β y TNF-α) was held using ELISA test with the collected culture media.

Results

The infective environment produced by Pseudomonas aeruginosa over the pneumocytes cell culture increases significantly the production of IL-1β with a bacteria concentration of 10^4 CFU/mL (15.42; p=0.002) and with 10^6 CFU/mL (16.39; p=0.002) compared with the control group 24 hours post infection. TNF- α secretion increases significantly when infecting with of 10^4 CFU/mL (4.67; p=0.002) and with 10^6 CFU/mL (5.17; p=0.002) compared with the control group 24 hours post infection.

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Identification and characterization of allergenic proteins in latex gloves Christian Galicia Díaz Santana, Guillermo Mendoza Hernández, José Federico Del Río Portilla, Adela Rodríguez Romero.
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Natural Rubber Latex (NRL) is obtained from the *Hevea brasiliensis* tree and it is used for the manufacture of about 40 thousand products. Many of the proteins present in NRL may lead to an allergic reaction, mainly through the use of medical equipment such as gloves. Recent reports indicate a NRL allergy prevalence of 3-17% for health care workers and 40-50% for patients with multiple surgical interventions as in the case of children with spina bifida. In our laboratory some of these proteins (allergens) have been characterized and their structures were reported. Thermal and chemical processes are part of the manufacture of latex gloves, which potentially modifies the structure of an allergen, as reported for other proteins such as food allergens. Therefore, the purpose of this work is to characterize the allergens present in NRL gloves, determine their structural integrity and their ability to be recognized by allergic patients in comparison with the natural non-processed allergens.

Some of the known allergens of *H. brasiliensis* were identified in the gloves, and two of them were purified. The allergen Hev b 2, a β-1,3-glucanase, was identified and purified using a ConA affinity column. Three bands could be observed in a SDS-PAGE gel after the purification method, the three of them corresponding to Hev b 2 or fragments of Hev b 2 as confirmed by protein sequencing. It is important to mention that the allergen and its fragments maintain its glycosylation sites, this is essential for IgE recognition as previously reported by our group. The second allergen purified from gloves, Hev b 6.02 was found to be the most abundant and also the most structurally conserved, this is a 4.7 kDa protein with four disulfide bridges that makes it very stable. The protein (purified from gloves) was crystallized and the structure solved by X-ray crystallography at 1.45 Å resolution using the S-SAD method to determine the phases. When the model is aligned to the one previously reported for Hev b

6.02 (non-processed) we can observe that the protein maintains its tertiary structure having a

C_α RMS= 0.2. ELISA experiments were performed for both Hev b 2 and Hev b 6.02, clearly

showing that they can be recognized by allergic patients after going through the manufacture process. Interestingly, some patients recognize better the non-processed allergens and others

have more affinity to the gloves allergens. This is an indicative that while some epitopes are

conserved during the manufacturing process, others might be destroyed.

Analysis of gene expression and activity of MMP9 in human corio-amniotic membranes.

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Introduction. Preterm birth is the leading factor causing neonatal mortality and morbidity. Inflammation plays a central role in stimulating uterine contractility and premature rupture of corio- amniotic membrane, this mechanism involves the proinflammatory release of IL-1 B, IL-6 and TNFα that activates MMP9 gene expression and production. We examine the extrinsic factors that promote the MMP9 production in membrane obtained by cesarean or parturition to elucidate the physiological events that participate on premature rupture. The aims of this study were: 1) Analyze the MMP9 expression and 2) The effect of trypsine and pro-MMP9 (and its activator) in the production of MMP9 and membrane rupture by *in vitro* model and zymogram.

Methods.

Reverse Transcription- Polymerase Chain Reaction (RT-PCR). We recovered 3 cesarean-procedure and 3 parturition corio-amniotic amembrane from pregnant women at 37 weeks of gestation and preserved it at -80°C.

We analyzed the MMP9 gene expression and obtained RNA, by isolation using the phenol-chloroform extraction method with TRIzol (Life Technologies), evaluated RNA concentration and purity by UV Spectrophotometry and integrity by electrophoresis and reverse transcription using oligo (dT) primers and Superscript II – Reverse Transcriptase (Life Technologies). We assayed the expression of specific complementary DNA (cDNA) by PCR using the selected gene-specific primers. Our internal expression control was ribosomal RNA.

In vitro model for MMP9 production

The transwell assay technique provides a simple in vitro approach to performing in vitro model for extracellular matrix. Barrier employed in this assay was a bilayer of corio-amniotic membrane on the permeable support with the corion cellular layer facing upward and the amniotic cellular layer facing downward, embedded on different concentrations of trypsin as an activator of MMP9. Supernatant was recollected from both compartments and gelatine zymogram was performed to identify the active form of MMP9.

Results

We observed the MMP9 expression only in 2 cesarean membranes and analyze MMP9 production on different conditions by transwell assay. We tried different trypsin concentration and pre-MMP9 with orwithout U937 (macrophages MMP9 producers) and observed the membrane integrity at 24h, 48 h and 72 h without any apparent changes. To analyze enzyme activity we measured protein concentration by Bradford using 5 μg of total protein for the zymogram assay at different times. We observed that MMP9 activity was higher at 48 and 72 hr rather than at 24 hrs further studies should confirm this observation.

Inhibitory effect of Galectin-1 over IL-6 expression in LPS-treated decidual cells by reducing IκBζ.

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Role of the α7 nicotinic receptor on the LPS-induced TNF production in bone marrow-derived mast cells.

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Introduction. Mast cells (MCs) respond to bacterial lipopolysaccharides (LPS) from Gram-negative bacteria with the secretion of a number of pro-inflammatory mediators. Some of those compounds are pre-synthesized and kept on secretory granules (histamine, serotonin), whereas other are newly formed after the activation of specific enzymes (prostaglandins and leukotrienes) or transcription factors (cytokines). Some cytokines, such as the Tumor Necrosis Factor (TNF) are both kept in granules and synthesized after cell stimulation. TNF secretion requires the participation of MAPK (ERK and p38), IKK and TTx-sensitive VAMPs. Production of TNF by MCs is associated to important acute and chronic inflammatory reactions leading to the defense against bacteria but also to the development of chronic-degenerative diseases. Our group has shown that, in vivo, MCs-dependent secretion of TNF after TLR4 activation is negatively controlled by the cholinergic anti-inflammatory reflex. In this work we aim to characterize the molecular mechanism of the inhibitory role of the nicotinic α7 receptor (nAchRα7) on the LPS-triggered TNF production utilizing murine bone marrow-derived mast cells (BMMCs). **Material and methods**. BMMCs obtained by culture of bone marrow with IL-3 were pre-incubated with buffer or different concentrations of nicotine, GTS-21 (an agonist for the nAchRα7) and MLA (nAchRα7 antagonist) before to be stimulated with LPS (500 ng/mL). To assess TNF secretion, supernatants of treated cells were collected after 4 hours after LPS addition. To analyze protein phosphorylation, total cell extracts were obtained after 15 minutes of LPS treatment. Calcium mobilization after nAchRα7 addition to BMMCs was measured by fluorometry using Fura 2-AM as calcium tracer. **Results.** The stimulation of nAchRα7 with GTS-21 (100 µM) and MLA (1 mM) in BMMCs inhibits the secretion of TNF after LPS stimulus. nAchRα7 stimulation with nicotine, at the concentrations used (10 nM-100 µM) had no effect on this response in BMMCs. GTS-21 treatment also inhibited phosphorylation of ERK and P38 evoked by LPS. nAchRα7 stimulation with GTS-21 (100 µM) but not with MLA (1mM) provoked Ca^{2+} mobilization in BMMCs. **Conclusions**. The inhibition of GTS-21 on LPS-induced TNF secretion involves the blockage of ERK phosphorylation induced by TLR4 receptor. The molecular mechanism by which MLA and GTS-21 prevents TNF secretion seems to be independent on [iCa^{2+}] increase. Supported by Grants ANR-Conacyt 188565 (CGE) and CB2010/155255 to (CLR).

Intestinal innate immune response against rotavirus infection: Role of dendritic cells from mesenteric lymph nodes

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Rotavirus infects and destroys enterocytes of the small intestine, which makes it an excellent model to study the intestinal immune response. It is well known that B and T cells play an important role in protection against the infection, however, the initial response by the innate immune cells has been poorly studied. Dendritic cells (DC) are key players in the induction of innate immune responses and therefore it is very likely that the DCs of the Gut-Associated Lymphoid tissue (GALT) play an important role in the initial steps of the protective immune response against rotavirus infection. Previous work of our Laboratory showed in the mouse model that the rotavirus infection induced a strong activation of DCs from Payer´s patches (PP) at 48 hrs post-infection, since the maturation markers CD40, CD80, CD86 and MHC Class II were up-regulated and pro and anti-inflammatory cytokines were expressed.

The aim of this work was to analyze the DC response from mesenteric lymph nodes (MLN) within the first 48 hrs after rotavirus infection in an adult mouse model and compare it to the previous results in PP. Thus, adult BALB/c mice were orally infected with the murine rotavirus $EDIM_{wt}$, and cells from MLN were obtained at 0, 12, 24 and 48 hrs post-infection. The levels of CD40, CD80 and CD86 in $CD11c^{+}/CD103^{+}$ were evaluated with specific mAb and analyzed by flow cytometry. It was found that the DC from MNL increased the levels of the maturation markers since the 12 hrs reaching a peak at 24 post- infection. These results suggest that after the rotavirus infection of the enterocytes of the small intestine the $CD103^{+}$ DC beneath the epithelial layer capture rotavirus particles and migrate to the MNL at least 24 hrs before than the activation observed in the DC from PP. The meaning of these results in term of the innate immune response against rotavirus in the intestine will be discussed.

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Recognition sites for murine IgG and IgE in the allergen Hev b 8 profilin.

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Abstract

The incidence of allergy worldwide has increased dramatically over the past 30 years. Allergy or Type I hypersensitivity to latex proteins is a major health problem that has arisen with food allergies. In the latex of the rubber tree, *Hevea brasiliensis,* there has been described several allergenic proteins like Hev b 8 that belong to the family of profilins, and have been classified as panallergens. It has been demonstrated its involvement in the cross-reactivity pollen-latex-fruit syndrome. Using immunoassays or *in silico* analysis was defined IgE recognition sites in profilins. These areas are mainly found in conserved regions in the surface of the allergen and share certain structural identity with other allergens favoring cross-reactivity. In this study we determined the three dimensional structure of the allergen Hev b 8.0102 into two space groups, one of which shows a dimer in the asymmetric unit. The dimerization of profilin has been described before, having an effect in the cross linking of the specific IgE antibodies that are bound to the receptor FcεRI at the surface of mast and basophil cells. This inmuno-complex activate the cross-linking of IgE Abs in the cells and the release of inflammatory mediators (histamine), proteases, and proinflammatory cytokines that causes the symptoms of allergy. Furthermore, profilin-specific IgG and IgE murine monoclonal antibodies have been obtained, allowing us to assign the recognition regions by interaction studies in solution or in crystalline state.

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Dissociation of immunosuppressive and nociceptive effects of fentanyl, but not morphine, after repeated administration in mice: Fentanyl-induced sensitization to LPS

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Morphine inhibits several parameters of the innate immune response against Gram negative bacteria. Acute morphine administration decreases important proinflammatory cytokines in sepsis such as tumor necrosis factor (TNF). Our previous work showed that acute administration of morphine blocks early LPS-induced TNF release in murine peritoneal resident mast cells (MCs) and this could be related to morphine analgesic efficacy. However little is known about other opiate analgesics and the effect of chronic opiate treatment using different administration schedules. The aim of this work was to compare the acute and chronic effects of morphine and fentanyl to inhibit LPS-induced TNF release in murine peritoneal resident mast cells.

Our results show that fentanyl was equally effective and 1000x more potent than morphine to inhibit i.p. LPS-induced TNF release and this was dependent on intraperitoneal MCs. Repeated morphine administration induced tolerance to both antinociception and inhibition of response to endotoxin. Repeated fentanyl injection did not induce significant antinociceptive tolerance, but, interestingly, produced sensitization to LPS.

In conclusion: 1) opiates with different analgesic potency also differ in their potency to induce immunosuppression; 2) MCs are the cellular target of the immunosuppresive actions of fentanyl; 3) in contrast with morphine, tolerance to fentanyl's immunosuppressive actions can be dissociated from tolerance to its antinociceptive effects. Current experiments are in progress to elucidate a crosstalk between the signaling pathways involved.
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Immune sensing of *Candida non-albicans* and members of the *Sporothrix schenckii complex*

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The cell wall of the opportunistic pathogen *Candida albicans* is composed of chitin, β-glucans and glycoproteins, which are enriched with *N*- and *O*-linked mannans, and these are considered the main pathogen-associated molecular patterns that the innate immune system recognizes to establish a protective anti-*Candida* immune response. It has been demonstrated that *C. albicans O*-linked mannans are recognized by TLR4, *N*-linked mannans by mannose receptor, DC-SIGN, dectin-2, and dectin-2, and β1,3-glucan by dectin-1. Interestingly, chitin is able to block the proper recognition of *C. albicans* by immune cells. Despite this knowledge, little is known about the relevance of cell wall components during the immune recognition of *Candida non-albicans*, and other fungal pathogens such as the causative agent of sporotrichosis, *Sporothrix schenckii*. Here, we studied the relevance of cell wall components of *S. schenckii*, *C. parapsilosis sensu latto*, *C. tropicalis, C. guilliermondii* and *C. krusei* during cytokine stimulation and phagocytosis by human monocytes and monocyte-derived macrophages, respectively. Our results showed that these organisms have similar cell wall composition, but different degrees of cell wall porosity, and this physical parameter correlates with the ability to stimulate cytokine production. Removal of *O*-linked or *N*-linked glycans affected the stimulation of cytokines in a species specific-manner. As previously reported in *C. albicans*, *S. schenckii* stimulated cytokine production in a morphology-dependent way.

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Effect of cobalt chloride-induced hypoxia on the secretory machinery and the cytokine profile secreted by mast cells activated through TLR-4 and FcεRI receptors

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Introductión: Mast cells (mc) are important effectors of innate immunity because they contribute to the initiation of inflammation and help to fight against distinct bacterial infections. Mc recognize the bacterial lipopolisaccharide (LPS) through the Toll-like receptor 4 (TLR-4), which promotes the synthesis and release of proinflammatory cytokines, such as the tumor necrosis factor (TNF) and the CCL-2 chemokine. This cell type also participates in Type I hypersensitivity reactions due to the fact they secrete inflammatory mediators after the crosslinking of the high affinity IgE (FcεRI) receptor. Mc express v-SNARE proteins (VAMP-8, VAMP-3) and t-SNAREs (SNAP-23 and STX-4), which contribute to the fusion of the secretory granules with the plasma membrane. Although the presence of mc has been extensively documented in inflammation sites characterized by low levels of oxygen (hipoxia), it is not known if this condition modifies the synthesis and localization of SNARE proteins, besides to change the cytokine secretory profile. **Objective.**To evaluate if hipoxia modifies the expression an subcellular localization of VAMP-8, STX-4 and SNAP-23 and if this condition alters the secretion of TNF and CCL-2 in response to the activation of TLR-4 and FcεRI receptors. **Material and Methods.** Bone marrow-derived mast cells (BMMCs) from C57BL/6J mice were utilized. Cells were sensitized with IgE (100 ng/mL) before to be stimulated with LPS (500 ng/mL) or DNP-HSA (27 ng/mL) in conditions of normal culture or hypoxia (induced by $CoCl_2$ 100 μM). Expression and localization of SNARE proteins was determined by western blot and cytokine secretion was analyzed by ELISA. **Results.**hypoxia did not modify the synthesis of VAMP-8, VAMP-3, SNAP-23 and STX-4 in basal conditions, but caused the release of βhexosaminidase, a change in the localization of VAM-8 and the secretion of CCL-2 in mc stimulated with LPS. On the other hand, hypoxia diminished the secretion of TNF in mc activated with DNP-HSA. **Conclusion.**Hypoxia induces the translocation of VAMP-8 to lipid rafts, the release of β-hexosaminidase and facilitates CCL-2 secretion after TLR-4 receptor triggering, whereas diminishes TNF secretion induced trhough the activation of the FcεRI receptor in mc.

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Production mechanisms of anti-Non-bilayer phospholipid arrangements IgG antibodies

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Anti-lipid IgG antibodies are produced in some mycobacterial infections and autoimmune diseases (lupus, anti-phospholipid syndrome). However, few studies have addressed the mechanisms that lead to the production of these immunoglobulins. Anti-lipid IgG antibodies are consistently found in a mouse model of lupus induced by chlorpromazine-stabilized non-bilayer phospholipid arrangements (NPA). NPA are transitory lipid associations found in the membrane of most cells; when NPA are stabilized they can become immunogenic and induce specific IgG antibodies apparently involved in the development of the disease [(1)]. We used this model of lupus to investigate the *in vivo* mechanisms that lead to the production of these antibodies. Oftenly, in response to most protein antigens B cells are activated via germinal centers or extrafollicular reactions. In germinal centers, a T cell-dependent response leads to isotype switch, somatic hypermutation, affinity maturation and memory generation [(2)], whereas these events do not usually occur in extrafollicular reactions [(3)].

In this mouse model of lupus induced by chlorpromazine-stabilized NPA, we found ($Gr1^-$, $CD19^-$, $CD138^+$) plasma cells producing NPA-specific IgGs in the inguinal lymph nodes, the spleen, and bone marrow. We also found a significant number of (IgD^-, $CD19^+$, PNA^+) germinal center B cells specific for NPA in the inguinal lymph nodes and the spleen, as well as the presence of NPA in these same germinal centers. In contrast, we found very few ($B220^+$, $Blimp1^+$) extrafollicular reaction B cells specific for NPA. Altogether, our data suggest that, in this murine model of lupus, B cells produce anti-NPA IgG antibodies mainly via germinal centers.

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Effects of *Bacillusthuringiensis*-derived Cry1Acproteins and identification of putative receptors inmurine macrophages.

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One of the biggest challenges for immunologists in the vaccine design is the feasibility to induce protective immune responses at mucosal sites. One of the most studied approachesis the use of adjuvants. To date few mucosal adjuvants have been developed, Cholera toxin is the most powerful known,but it is not safe for clinical use, thus, innocuous and effective mucosal adjuvants are still required. In previous reportsour group showed that Cry1Acprotoxin (133kDa) and Cry1Ac toxin (65kDa) of *Bacillus thuringiensis*are potent mucosal and systemicimmunogens. Cry1Ac protoxin can act as an effective and protective adjuvant in three murine parasitic infection models. The family of Cry proteins have been widely used as biopesticides,although they were considered apparently innocuous for vertebrates their effects on the immune system haven not been fully studied and the exposure to these toxins is being increasing due to the intake of transgenic plants expressing Cry genes.In an effort to characterizethe adjuvant mechanism of Cry1Ac protoxin we have reportedits ability to induce the activation ofmacrophages frommucosaland systemiccompartments.Cry1Ac protoxin activates ERK1/2and p38 signaling pathways in Raw 264.7 macrophages suggesting theexistenceofapossiblereceptorinmammalsforCry1Ac protoxinand toxin. OBJECTIVE: Identify the putativereceptorforCry1Ac protoxinandtoxininmurine macrophages. METHODOLOGY: Cry1Acprotoxinis derived fromthe recombinant strainof*E.coli*JM103, solubilized andproteolyticallyprocessed into toxin and are free of LPS.We determined the effectoftoxinandprotoxin Cry1Ac on activation of RAW 264.7 cells analyzing theupregulation of costimulatory molecules and theproduction of proinflammatory cytokines. To identifya putative receptor we performedimmunoprecipitationandaffinity chromatographyassays (with Cry1Ac conjugated to Sepharose) along withligandblot assays intotal and membrane isolated proteins,and estimatedthe relative molecular weights of the bands on SDS-PAGE 10%.RESULTS:Cry1Acprotoxinandtoxinbinds tothe membrane surfaceonRAW 264.7 macrophagesand induceoverexpressionof costimulatory moleculesCD80andCD86plus CD11b andincreasesthe production of IL-6, IL-12, TNF-α, MCP-1 andnitric oxide. The estimated Kd of Cry1Actoxin to RAW264.7 cells isof634nM. Different union proteinsfor Cry1Ac were identified fromligandblot analysis, inparticularby separationof the union proteins to toxin-sepharose column.We principally identified threebandsof47, 43and68 kDa. CONCLUSIONS:Cry1Acproteinsbind tothe membraneand induce theclassical activationinRAW264.7 macrophagesin a dosedependent waythat can be mediated byareceptor in asaturable mannerwitha moderateKdfor Cry1Ac toxin.

Effect of high glucose conditions on classical and alternative activation of human macrophages *in vitro*.

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Diabetes mellitus (DM) is a non-communicable chronic disease characterized by a persistent hyperglycemia condition. In diabetic patients, nervous and vascular tissues are affected by high blood glucose levels, which promote complications such as chronic renal failure, cerebrovascular accident and myocardial infarction. In addition to this, the role of macrophage in nerve and blood vessels damage during hyperglycemia has been studied, due to its ability to infiltrate and recruit inflammatory cells to lesion focus. However, whether high glucose levels have *per se* a direct effect on the macrophages activation, has not been explored. Therefore, the aim of this research was to establish the direct effect of high glucose levels on the activation of human macrophages *in vitro*. In order to know this, Human monocytes were isolated from buffy coat of healthy blood donors and differentiated into macrophages with macrophage colony-stimulating factor (M-CSF) an interleukin (IL) 3. After 6-days differentiation, macrophages were cultured with normal (5.5 mM) and high (15 mM) D-glucose conditions, while a control of pressure was performed cultivating macrophages in 5.5 and 9.5 mM of D-glucose and D-mannitol, respectively. Macrophages exposed to high glucose conditions showed increased on CD11c, decreased on IL-10 and increased on TLR4, nuclear factor kappa B (NFκB), aldose reductase (ADR) and sorbitol dehydrogenase (SDH). These results have shown that high glucose is able to restrict anti-inflammatory activity of human macrophages *in vitro*, possibly due to cell polarization toward a pro-inflammatory profile. These findings suggest an additional pathophysiological mechanism through which macrophages could participate in tissue damage during hyperglycemia.

Toll-like Receptor-2 and Toll-like Receptor-4 Expression on Human CD16+ and CD16- Monocyte Subsetsfrom Peritoneum in Homeostatic and Inflammatory Conditions

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Introduction

Recently, two subsets of human monocytes (Mo) has been identified in peritoneum cavity based on CD14 and CD16 expression: the classical CD16– Mo and the minor CD14+CD16+ Mo. Phenotypically, CD16+ are considered more mature cells than CD16– Mo by their low expression of CD11b and CD33 and their high expression of CD16 and MHC class II molecules. Human Mo subpopulations also differ in that CD16+ Mo are the main source of TNF and IL-1 when stimulated with TLR2 and TLR4 ligands. Together, with a reduced ability to produce IL-10, these data led to the conclusion that CD16+ Mo are potent inducers of inflammation. Mo are plastic cells that modify their functional activity in response to environmental signals, because it our results show that CD16– and CD16+ Mo respond differently in terms of phagocytosis and cytokine production.

Toll-like receptors (TLR) are pattern recognition receptors in mediating the innate immune response and their activation can lead to production of cytokines. TLR4, in conjunction with CD14, is well characterized as the receptor for Lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria. TLR2 has been demonstrated to act as receptor for components of Gram-positive bacteria such as peptidoglycan (PGN) and lipoteichoic acid.

Materials and Methods

Cells from the peritoneal cavity of twelve patients on peritoneal dialysis were obtained after their informed consent. The patients' condition was classified into inflammatory (six patients) or homeostatic (six patients), according to the presence or absence of neutrophils in the peritoneal lavage, respectively. The cause of inflammation in the patients was not determined. A fraction of the peritoneal cells was incubated with mAb to CD14 labeled with PerCP (BD Biosciences), to TLR2 coupled to APC,to TLR4 coupled to PE and to CD16 labeled with Pacific Blue (BD Biosciences).The expression of CD16, CD14, TLR2 and TLR4 was evaluated in these cells by FACS analysis.

Results

Both subsets of Mo were found in the two conditions, but in homeostatic peritoneal fluid the CD16+ subset was nearly absent.TLR4 was found to be expressed 1.6 times higher in homeostatic Mo compared to inflammatory CD16+ and 3 times higher compared to inflammatory CD16-, while TLR2 expression was found to be 1.8 times higher in homeostatic Mo compared to inflammatory Mo.

Discussion

There are a minimum number of monocytes in the peritoneum cavity of patients with peritoneal dialysis without inflammation with a high pattern recognition receptors expression. We hypothesize that Mo are the first cells in recognize pathogens like bacteria and yeasts, but are weak to opsonized microbes, because his lack of CD16 on his surface. Therefore, in case of infection, many monocytes CD16- and CD16+ migrate from the bloodstream to peritoneum.

Participation of germinal centers in mice with Chagas disease treated with NIPOx-B

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Chagas disease is a parasitic disease caused by *Trypanosome cruzi*. It is endemic in Latin America, where it is a major health problem since it affects 8-10 million people, causes 50,000 deaths per year and about 25% of the population is at risk of acquiring the disease [(1)]. It remains practically incurable, mainly because of the limited interest in developing new drugs (it is considered a neglected disease) and because the drugs available for its treatment, Benznidazole and Nifurtimox are inefficient to cure patients [(2)]. The acute phase of the disease appears shortly after infection, and the chronic phase appears after a silent asymptomatic period that may last several years. During the chronic phase, the heart, oesophagus, colon and peripheral nervous system are irreversibly affected, and patients usually die from heart failure. The chronic phase may be established by the evasion of the adaptive immune response by the parasite, and secondly because Benznidazole and Nifurtimox generate free radicals that can affect the cells of the adaptive response which could favour the chronic phase. Our research group synthesized the benzyl ester of N-isopropyl oxamic (NIPOx-B) that is an effective inhibitor of alpha-hydroxy acid dehydrogenase II enzyme, which is a key enzyme in the glycolytic pathway of the parasite [(3)]. This inhibitor lacks the toxicity of Benznidazole and Nifurtimox so it do not affect the cells of the adaptive response and it also cause the death of the parasite, so the cure of the disease can be achieved more efficiently [(3)]. On the other hand, germinal centers are structures that are formed in the secondary lymph organs, and are mainly responsible of the formation and production of IgG antibodies that are more specific and important to resolve Chagas disease. It is important that actually there are just a few studies of germinal centers in parasitic diseases. So in this work we studied the germinal centers in mice with Chagas disease, which contributes to a better understanding and treatment of this disease.

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The Effect of BCG Vaccination on the Virulence of Different Genotypes of *Mycobacterium tuberculosis*

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Diverse world regions face major tuberculosis outbreaks; meanwhile BCG, *Bacille Calmette-Guerin*, the only vaccine approved for prevention of this disease, has shown inconsistencies in the protection range in different populations of the world. There are several theories which attempt to explain the variation in the efficacy of BCG, among others BCG may protect against most, but not all *Mycobacterium tuberculosis* (MTB) genotypes, therefore selecting genotypes able to circumvent BCG-induced immunity. In this case, the immune response elicited by BCG may produce a harsh environment for MTB, exerting a selective pressure on the microbe to which it must adapt, yielding higher virulence microorganisms as a response to the stressful environment of a vaccinated host.

Objective: Measure the direct effect of BCG immunity on the virulence of MTB.

Methods: Four strains were selected from different genotypes and used to challenge BCG vaccinated mice. After 2 months, vaccinated animals were sacrificed and bacteria isolated from infected lungs. Naïve mice were infected intratracheally with these bacteria. The disease´s course was compared with animals infected with bacilli isolated from non-vaccinate animals and mice infected with the stock-original strain. Survival and pulmonary bacilli loads were used to define the level of virulence.

Results: We observed variability in the effect of BCG vaccination on the bacterial virulence; in the case of the 2 Beijing strains studied, both showed virulence increase when exposed to BCG immunity. The other strains (LAM genotype and H37Rv strain) did not show a virulence increase when exposed to BCG vaccinated mice.

Conclusions: BCG immunity appears to have a direct effect on the virulence of certain genotypes of MTB, in this case the Beijing genotype, that escape the harsh immunity effect, inducing higher degrees of virulence when exposed to an activated immune system.

Interaction of neutrophil extracellular traps with *Trichomonas vaginalis*

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Trichomonas vaginalis causes trichomoniasis, the most frequent no viral sexually transmitted disease. In 2008 the World Health Organization estimated a global incidence of 276.4 millions new cases of trichomoniasis; in Mexico it is the 16º cause of morbidity among the main 20 communicable diseases. In pregnant women, trichomoniasis is a risk factor for premature delivery and low weight newborns. Additionally, this infection increase the acquisition and transmission of HIV (human immunodeficiency virus) and is associated with HPV (human papilloma virus) infection and consequently with the risk to develop cervical cancer and bacterial vaginosis.

Trichomoniasis is characterized by a heavy infiltration of polymorphonuclear neutrophils at the site of infection. Neutrophils act against foreign agents by phagocytosis, degranulation and the formation of extracellular traps. This process involves the release into the extracellular space of networks of DNA containing antimicrobial peptides and proteins as a strategy to prevent microbial spread. However, the effect of traps in *T. vaginalis* is unknown. The aim of this study was to determine if neutrophil extracellular traps interact with *T. vaginalis* and the outcome of the interaction.

Extracellular traps formation was induced in human purified neutrophils. After a 3 h interaction of *T. vaginalis* with extracellular traps, we observed the association of the parasite with the traps by confocal microscopy. After the interaction, the parasite growth was compared to appropriate controls, observing a reduction of about 60% in *Trichomonas* growth. These results demonstrate the susceptibility of *T. vaginalis* to neutrophil extracellular traps.

Searching potential inhibitors of arginase II through virtual screening. The search for new drugs against endothelial dysfunction.

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Introduction. The endothelium plays an important role in hemostasis. Integrity of structure is essential for its function as a barrier between the bloodstream and surrounding tissues. One of the greatest contributions to endothelial dysfunction is the reduction of nitric oxide level, a potent vasodilator and essential in the regulation of vascular tone and blood pressure. Nitric oxide in the endothelium is produced by nitric oxide synthase (eNOS) from L-Arginine. Endothelial Arginase II also uses the substrate L-Arginine competing with eNOS.This situation provokesa decreasein nitric oxide levels, thereby arginase II is an attractive target for drug design to treat endothelial dysfunction.
Methods.A rigid docking virtual screening protocol was made in MOE software (www.chemcomp.com) using the crystal structure of Arginase II (PDB code 4HZE) and the small molecules library “Diverset” from Chembridge (www.chembridge.com). The search of potential inhibitors was performed having the enzyme catalytic site as target.
Results. From the molecules docked, compounds 2652,4241, and 4620had the best binding energy, -16.8, -16.7, and -16.7 Kcal/mol, respectively. The structural analysis of the enzyme-ligand complex showed that hydrogen bonds with Ser156 and Ser154, and π-π interactions with His145 were common among the three compounds and the enzyme.
Conclusions. The three compounds found trough virtual screening, have the potential to inhibit arginase II and could be used as hits to obtain new drugs against endothelial dysfunction.

EFFECT OF SUCROSE-INDUCED METABOLIC SYNDROME UPON AMP-ACTIVATED PROTEIN KINASE (AMPK) AND INSULIN SIGNALING IN THE RAT SKELETAL MUSCLE.

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INTRODUCTION.
Metabolic syndrome (MS) is a cluster of risk factors for developing cardiovascular diseases such as obesity, hypertension, dyslipidemias and type 2 diabetes, all them characterized by an energy unbalance. Insulin resistant has been proposed as the underlying mechanisms leading to development of MS, as well as other cellular energy disturbances including AMPK signaling. AMPK is considered as a metabolic and nutritional pivotal sensor in the cell that control energy production and expenditure. These energetic alterations differentially affect target organs such as skeletal muscle (SM), liver and adipose tissue. In this sense, SM plays an important role in controlling insulin response and energy expenditure. Thus, in this work we aim to elucidate the changes in SM energy pathways due to development of metabolic syndrome induced by sucrose feeding in the rat.

METHODS.
MS was induced by feeding male Wistar rats (250 g) with 30% sucrose in their drinking water during 16 weeks. Age-matched animals having tap water served as controls. Both groups were fed with standard lab chow at libitum. MS was confirmed by serum triglycerides, insulin, HOMA, and visceral fat content. From these animals we isolated the extensor digitorumlongus (EDL) and soleus (SOL) muscles and evaluated AMPK and AKT expression, as well as their basal activation by Western Blot using specific antibodies for total and phosphorylated forms. Data was compared using a t-student test and difference was considered significant when a $p \leq 0.05$ was obtained.

RESULTS.
EDL and SOL muscles from rats with MS exhibited a similar content of total AMPK to their control matches, as well, total AKT content in EDL from MS rats had no significant changes compared to the control ones. However, in SOL from MS rats, a small but significant increased of total AKT was observed compared to SOL belonging to control rats. Regarding basal AMPK activation, SOL muscles from MS rats showed a slightly increased in the phosphorylated basal state compared to the control.

CONCLUSIONS. MS seems affecting total AKT expression in SOL by increasing the protein levels likely to compensate the insulin resistance posed by MS in the animal, this may be related to larger basal activation of AMPK, suggesting that SOL muscle was early affected than EDL. Further investigation is deserved to examine the muscle response to energy challenges.

Antifibrotic activity of flavonoids and saponins extracted from the seed coat of black bean (*Phaseolus vulgaris*) in fibroblasts from rat heart.

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Introduction: The importance of functional food is well recognized as promoterof health. Food may help prevent chronic disease or reduce risk of disease progression. In this regard, black bean is a good source of bioactive compounds such as flavonoids and saponins. It has been proven that particularly saponins present cardioprotective properties, increasing the strength of contraction of isolated heart muscles from murine models of cardiotoxicity. Also, there is evidence in animal models about potential benefits of flavonoid intake on cardiovascular disease through antioxidant and immunomodulatory mechanisms.

Previously our group described antifibrotic properties of a black bean extract (BBE) on a murine model of heart failure. Nevertheless, compounds of BBE with antifibrotic activity have not been identified. Therefore, this work aims to define the specific compound or compounds in the BBE, with antifibrotic activity in cardiac fibroblast *in vitro.*

Methods: Cardiac fibroblasts were cultured until reaching a confluence of 80% or more. Fibrosis induction was accomplished by decreasing to 1% of fetal calf serum (FCS) for 24h. Under these experimental conditions, cells were treated with the BBE, flavonoid-enriched extract (FBBE)and saponins-enriched extract (SBBE). Subsequently, cell viability was evaluated with Alamar Blue. mRNA expression of collagen type I, collagen type 3, TGFβ and TNFα will be evaluated by qRT-PCR.

Results: Expression of collagen I mRNA was increased following stimulation with 1% FCS. The addition of increasing doses of BBE resulted in potent inhibition of collagen I expression; significant suppression of collagen I expression was observed at doses as 40μg.ml^{-1} of BBE. In addition, an increase in cell viability was observed following FCS induced-fibrosis at 40μg.ml^{-1}of the BBE.Changes in the expression of collagen type 3, TGFβ and TNFα as pro-fibrotic gene markers will be presented, also its effects in a time and -dose dependence of the FBBEand SBBE extracts.

Reduced activity and Ca^{2+} sensitivity of ryanodine receptors in cardiomyocytes from metabolic syndrome rats

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Metabolic syndrome (MetS) is related to the development of a subclinical cardiac dysfunction characterized by an early left ventricular (LV) diastolic dysfunction developing prior to impaired LV systolic function (1). Regarding LV systolic dysfunction, we have reported that it is associated with a decrease in both the rate of rise and the amplitude of electrically stimulated (ES)-cytosolic Ca^{2+} transients (1). Moreover, we have shown that impaired ES Ca^{2+} transient is due, in part, to a decrease in the sarcoplasmic reticulum (SR) Ca^{2+} release mediated by ryanodine receptors (RyRs) (2). Nevertheless, the mechanisms underlying the alterations in RyR-mediated SR Ca^{2+} release are not clear. Thus, in this work we evaluated whether the decrease in SR Ca^{2+} release in MetS heart involves alterations in RyRs function and whether these alterations are related to changes in the Ca^{2+} sensitivity of these channels. To this end, we used a sucrose-fed rat model of MetS and recorded the resting activity of RyRs, manifested as diastolic Ca^{2+} sparks or spontaneous Ca^{2+} waves, in isolated cardiomyocytes. In addition, we evaluated the effect of different approaches that increase the Ca^{2+} sensitivity of RyRs, such as caffeine and Ca^{2+} overload, on Ca^{2+} sparks and spontaneous Ca^{2+} waves. Our data show that cardiomyocytes from MetS rats exhibit a decreased Ca^{2+} spark frequency (CSF) compared with control cardiomyocytes. Nevertheless, spontaneous Ca^{2+} wave frequency (CWF) is similar in both groups. Caffeine (0.5 mM, 1 min) induces an increase in CSF in cardiomyocytes from control rats, but does not affect CSF in cardiomyocytes from MetS rats. Moreover, cellular Ca^{2+} overload induced by incubation of cardiomyocytes with a high external [Ca^{2+}] (10 mM, 5 min) increases CSF and CWF in control and MetS cardiomyocytes. However, these effects are more pronounced in control cardiomyocytes. These data suggest that systolic dysfunction in MetS is associated with a diminished RyR activity which is accompanied by a decrease in the sensitivity of these channels to Ca^{2+}.

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MT-ND3 sequence analysis in tumor tissue and peripheral blood from patients with prostate cancer.

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Introduction: Prostate Cancer (PCa) is a highly heterogeneous disease and the second cause of cancer mortality worldwide. The etiology of the disease is unknown; however, it is considered a multifactorial disease, in which genetic predisposition and environmental effects (like obesity) play an important role in the developmental of this cancer. For PCa, obesity is known to be associated with higher-grade and late stage disease at the time of diagnosis (Capitanio et al., 2011). Moreover, obesity regardless of genetic and age variations is associated with a significant decrease in the number of copies of mitochondrial DNA (mDNA) (Pietiläinen et al., 2008). Also, given the central role of the mitochondria in energy production (Shutt & Shadel, 2010) and apoptosis (Riemer et al., 2009), it has been associated with carcinogenesis process. The study of genetic variants that alter cellular bioenergetics and therefore the molecular biology of the tumour, are important for the understanding of PCa. There are reports describing mutations in MT-ND3, that are associated with PCa (Gómez-Zaera et al., 2006)

Objective: Analyze the complete sequence of MT-ND3 in both peripheral blood and tumor prostate tissue in patients with PCa.

Subjects and Methods: It is an observational, cross-sectional study. After informed consent, fifteen patients with PCa diagnosis, were studied. All of them were diagnosed by transrectal prostate biopsy and met the inclusion criteria. DNA was isolated from blood leukocytes and paraffin-embedded tumor tissue. PCR for MT-ND3 was performed using specific oligonucleotides derived from the sequence of the MT-ND3 gene. Direct sequencing from PCR products was performed. The sequence of the MT-ND3 was compared with that reported in the database http://www.mitomap.org/bin/view.pl/MITOMAP/LocusND3.

Results: Most of our patients had overweight or obesity, and a Gleason scale of 6-7. Four out of the 15 studied patients presented changes in their DNA sequence, in both tumor tissue and peripheral blood. Patient 1 and patient 8 showed the genotypes: G10398A and T10400C, and patients 4 and 15 G10398A. Sequence modification that causes aminoacide changes between threonine and alanine, has been associated with other types of cancer, especially breast cancer. This change in protein sequence of complex I avoids the normal complex function. There is no junction and oxidation of NADH, which is in charge of giving the first pair of free electrons to complex I.

Conclusions: We did not find somatic mutations in our population. Our results did not find association of any genetic variants of MT-ND3 gene with an increase of PCa aggressiveness in patients with overweight or obesity.

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Docking studies in shikimate dehydrogenase from *Enterobacter cloacae* to find potential inhibitors.

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Introduction.*Enterobacter cloacae* is the main microorganism isolated in nosocomial infections in our country. These infections are due in large part to the widespread use of antimicrobials, which leads to the emergence of resistant strains creating an increased necessity to the development of a new antimicrobial therapy. Inthis regard,shikimate dehydrogenase (SDH), which catalyzes the conversion of 3-dehidroshikimate to shikimate, belongs to the Shikimate Pathway, a pivotal route in thebiosynthesis of aromatic amino acids, folates, and ubiquinone. Besides, this pathway is absent in humans, making the SDH from *E. cloacae* (EcSDH)an excellent target for the design of microbicides agents.
Methods. Since there is no crystal structure of EcSDH, a model prediction strategy named homology modeling was used, using differents programs such as Swiss model, EsyPred3D, Prime and MOE. Validation of the obtained models was conducted with Qmean score and the Ramachandran plot. The model with themore favorable validation was used for the realization of the virtual screening. This was performed using MOE (www.chemcomp.com) and Glide programs (www.schrodinger.com), on the active site of the enzyme. The small molecules "Drug like" subset of the ZINC database was employed, with around of 15 million compounds. The top 3 compounds which coincided in both programswereselectedtoperformanInducedFitprocedureusingInducedFit program (www.schrodinger.com).
Results. Structuralanalysis of InducedFitdata showedthatthecompoundsZINC34616948, ZINC83442116, ZINC15206727 presented abindingenergyof -6.706, -7.755 y -6.090 Kcal/mol, respectively. Compound ZINC34616948 made hydrogen bonds with Lys65, Asn86 and Tyr215; while ZINC83442116 formed it with Ser15, Ser17,Thr62 and Lys66. Finally, compound ZINC15206727 made hydrogen bonds with His14.Predicted drug likeness score from these molecules was in the range to be considered as potential drugs.
Conclusions. These molecules could be potential inhibitors of EcSDH and serve as a guide in the search of a new chemotherapy against nosocomial infections.

Effects of steam cooking on the proximate and fatty acid composition of rainbow trout (Oncorhynchus mykiss) from Huasca de Ocampo, Hidalgo, México.

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Fish has been recognized as a valuable source of high quality of protein in the human diet. Also, fish consumption has been linked to health benefits such as reduced risk of coronary heart disease, hypertension, type 2 diabetes and insuline resistance (1). This is attributed to polyunsaturated fatty acids (PUFA´s) present in fish oil, specially. Studies of nutrients intake from fish are frequently carry out with raw food. However, the nutritive values of fish can be affected by cooking method.
Rainbow trout (Oncorhynchus mykiss) is an extensively cultured species in Huasca de Ocampo from Hidalgo state, Mexico. It can be easily provided alive or very fresh from pond to table. The fish is commonly cooking by steam. However, the people has minimal knowledge about nutritive values of raw and cooked fish. This study was conducted to determine the effect of this method of cooking on the proximate and fatty acid composition.
The change in moisture, ash, fat and protein contents of raw and cooked rainbow trout are shown in table 1. The composition of the raw trout is similar to other researches (2). The moisture content of the rainbow trout decreasing after cooking.

Table 1. Proximal composition of rainbow trout

	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
Raw	72.75 ± 0.41	1.31± 0.012	4.39 ± 0.09	15.86 ± 0.34
Steaming	67.84 ± 0.65	1.39 ±0.021	7.86 ± 0.10	17.63 ± 0.75

Values are means ± standard error (n=3)

The decrease in the moisture content has been described as the most prominent change that makes the protein, fat and ash contents increase significantly in steam cooking fish (3)
The profile of fatty acid of rainbow trout was carry out by gases chromatography. The most abundant fatty acid in raw rainbow trout were palmitic acid (C16:0), arachidic acid (C20:0), oleic acid (C18:1n9) and linoleic acid (C18:2n6). However it had low levels of ω3 fatty acid as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Steaming marginally affected the fatty acid content, some fatty acid that were not detected in raw trout were found in low levels this heating treatment. The change observed must be a consequence of the water lost produced by this process.

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The Post-conditioning in the Treatment of Acute Myocardial Infarction in an Experimental Model with Metabolic Syndrome.

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The post-conditioning maneuver (PC) is a cardioprotective strategy against reperfusion injury, resulting in a decrease in infarct size. Most of the studies where this mechanical maneuver applies cardioprotection has been done in animal models that have no co-morbidity factors (e.g. diabetes or metabolic syndrome). It is important to mention that there are some reports of the application of PC in humans, but has not been documented no clinical study in patients with co-morbidity.
The aim of this work is to investigate the effectiveness of the PC on cardiac function in animals with metabolic disorders (metabolic syndrome), related the effectiveness of PC with markers of tissue and cell damage.
In this study we generated a metabolic syndrome model in male Wistar rats, with sucrose in drinking water - as reported previous-until the appearance of at least four of the symptoms that define the metabolic syndrome (16 week).Having established the model, we evaluated the effect of PC. The hearts were mounted into the Langendorff system and subjected to 30 minutes of ischemia and 60 minutes of reperfusion. PC consisted of 5 cycles of 30 seconds ischemia/20 seconds reperfusion, then the hearts were reperfused.
The cardioprotection was evaluated by measuring the contractile performance of the heart, infarct size, mitochondrial activity and activation of cell death pathways (release of cytochrome C, insertion of pro-apoptotic proteins such as BAX, BAD).
The results generated in this study represent the scope of applying the PC in murine models with co-mobility. Under these conditions, the PC reduced the infarct size, remained mitochondrial function and prevented the onset of apoptosis in rat hearts with metabolic syndrome.

Antineoplastic copper coordinated complexes (Casiopeínas) uncouple oxidative phosphorylation and induce mitochondrial permeability transition in cardiac mitochondria and cardiomyocytes.

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Copper-based drugs, Casiopeinas (Cas), exhibit antiproliferative activity *in vitro* and antineoplastic activity *in vivo* conditions. Unfortunately, the clinical use of these novel chemotherapeutics could be limited by the development of dose-dependent cardiotoxicity. The molecular mechanisms responsible for Cas anticancer activity as well as those underlying Cas-induced toxicity, particularly cardiotoxicity, are unknown. Here, we explore the potential role of mitochondria energetics in the molecular mechanisms underlying Cas-induced cardiotoxicity.

To explore the properties on mitochondrial metabolism, we determined Cas-effects on respiration, membrane potential, membrane permeability and redox state on cardiac mitochondria and cardiomyocytes. On isolated mitochondria, Cas IIIEa, IIgly and IIIia inhibited uncoupled NADH-linked respiration with an IC_{50} of 22±2, 42±4 and 216±12 µM, respectively; however, FADH-linked respiration exhibited 4-fold less inhibition. Cytochrome oxidase activity was inhibited by Cas IIIia, IIgly and IIIEa with an IC_{50} of 223 ± 5, 295 ± 102 and 440 ± 23 µM, respectively; suggesting a Cas-inhibition of respiratory chain at several levels (NADH dehydrogenase>Succinate dehydrogenase>cytochrome bc1 complex> cytochrome oxidase). At lower concentrations, Cas showed a marked increase on state-4 respiration and a significant reduction of membrane potential, suggesting that Cas acts also as potent mitochondrial uncouplers. Cyclosporine A, a classical blocker of permeability transition pore, significantly prevented the drop in membrane potential and Ca^{2+} efflux triggered by Cas; indicating that Cas induces permeabilty transition. Cas produced a marked increase on reactive oxygen species and oxidation of thiol groups, including components of mitochondrial permeability transition pore i.e., adenine nucleotide translocase (ANT) at Cys^{56}. Exposure of adult rat ventricular myocytes to Cas (40 µM) resulted in profound dissipation of membrane potential, only prevented in IIIia treatment by tiron.

In conclusion, the cardiotoxic effects of Cas depend on the inhibition of several components of the electron transport chain and produced a significant loss of membrane potential due to mPTP opening, mediated by ANT thiol groups oxidation.

Identification of Enteropathogenic Bacteria with DNA Microarrays.

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It is a fact that the current and future growth in the world population begins and will experience serious problems for the production of food, drugs and other supplies for human consumption. A key factor in the distribution and consumption of these inputs is to ensure the safety of themselves, derived from this concern, various methods have been established for the detection of pathogens. Of these the most effective procedures are based on identification of genetic material from the site or for any pathogens present in the sample. The techniques commonly used are the endpoint PCR and quantitative qPCR. While these technologies have proven to be efficient, an additional problem may be the number of samples to be analyzed and the time required to do the same analysis with conventional methods.

In our group, we designed a methodology that combines PCR amplification in a multiplex format with DNA microarrays. For which a number of species-specific sequence of the most common foodborne pathogens were identified: as *Yersinia enterocolítica, Campylobacter jejuni, Staphylococcus aureus, Citrobacter freundii, Clostridium perfringens, Listeria monocytogenes, Salmonella, Shigella flexneri, Vibrio cholerae, Escherichia coli* variedad O157, O121, O127, O26, O111, O103. The system consists of a multiplex PCR reaction in which a fluorescent molecule is incorporated and amplicons are hybridized on a microarray on which probes have been printed in the form of digital numbers, which allows an easy interpretation as each microorganism is identified by a code.

Additionally we manufacture the chips are printed on a 12 arrays per chip, enabling simultaneous 12 test biological samples. Our methodology allows detection of microorganisms of interest in minimum conditions of material and equipment, so you can move anywhere without affecting the reliability of the results.

Effectof chronic immobilization stress andsucrose consumptionon the development ofhepatic steatosisinmale infant rat

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Modern life stress combined with high calorie diets are key factors associated with the development of metabolic and neuroendocrine abnormalities. Many of these abnormalities are mediated by hepatic metabolism. Stress increases enzymatic activity of 11B-hydroxysteroid dehydrogenase-1 (HSD1), which has been associated with increased glucocorticoids and development of hepatic steatosis. In animal models, consumption of sucrose during early age decreases serum corticosterone and induces an inflammatory liver damage. Moreover, exposure to stress and high fat diet induces an increase in corticosterone. However, there are no studies assessing the combined effects of stress and a diet rich in carbohydrates on liver damage in infant rat. Here we investigated whether chronic immobilizationstress combined with high sucrose diet leads to the development of hepatic steatosis during childhood in rats. We used male Wistar rats aged 21 days (n = 32). Rats were divided into the control (C, n=8), chronic stress (St, n=8), high-sucrose diet (S30, n=8), and chronic stress and high-sucrose diet (S30/St, n=8). The animals were subjected to chronic stress for four weeks by immobilization. Liver was extracted and fixed in Bouin, dehydrated in ascending alcohols, cleared in xylene and embedded in paraplast. Histological sections were stained with hematoxylin-eosin and inmunohistochemistry to determine the oxidative damage. Corticosterone concentration and HSD1 activity was measured by ELISA and RIA, respectively. We found that stress causes steatosis, inflammation and oxidative stress in the liver parenchyma accompanied by an increase in the concentration of serum corticosterone and activity HSD1 in the liver. Contrary to our predictions, no effect of sucrose consumption on hepatic damage was found.These results suggest that feeding high-sucrose diets do not contribute to development hepatic steatosis. Funding: CONACYT a CPA (417844), CONACYT-225126. PNPC C-122/2014.

MOLECULAR IDENTIFICATION OF RESPIRATORY VIRUSES IN PATIENTS WITH CLINICAL DIAGNOSIS OF INFLUENZA

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Keywords: respiratory virus, H1N1

The most common etiologic agents associated with respiratory infections include: Influenza type A, B, and C, adenovirus, bocavirus, coronavirus, enterovirus (ECHO virus), metapneumovirus, parainfluenza type 1, 2, 3, and 4, Rhinovirus and Virus respiratory syncytial,those that mainly affect immunocompromised patients, the elderly, children and infants**[1]**. Previous studies suggest that these viruses are underdiagnosed when conventional methods(direct immunofluorescence, culture, aetc.) are used, so recently developed commercial methods are employed for rapid and timelyidentification.**[2]**. Because of this and its clinical importance, the molecular identification of respiratory viruses was carried out in patients with clinical suspicion of influenza H1N1 in Juarez Hospital of Mexico by PCR.**Material and method.**118Nasopharyngeal swabs were evaluated, DNA and viral RNA were extracted using the commercial**RTP® Pathogen Kit from STRATEC Molecular**, which can determined was used for identification the Seeplex®RV15 OneStep ACE Detection kit (corea), which can determines 16 was used for identification types of respiratory viruses simultaneously with endpoint PCR.Samples that tested positive for influenza type A, genotyped for seasonal and pandemic influenza virus using real-time PCR.**Results.**It was found that 50% of samples were positive (59/118) for respiratory viruses, 50 samples tested positive for influenza type A of which; 37 positive H1N1 type, 8 for type H3, 5nontypable type and 4 for seasonal influenza type B**.**Five patients with other respiratory viruses were observed; Respiratory Syncytial Virus 1 for 1 Rhinovirus, 2 Coronavirus, 1 Parainfluenza. **Conclusions**. The molecular identification of patients with clinical symptoms of influenza and other respiratory viruses is important to give proper treatment fast and timely manner, coupled to the influenza virus A H1N1 remains a public health problem especialling in the months between December and March in our population.

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Sleeprestriction induces glucosetolerance in rats

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Sleepisclassicallydivided in non-rapideyemovement (nREM) and REM sleep. nREMsleepischaracterizedbyslowwavesnamed delta in the EEG. REM sleepisalsonamedparadoxicalsleepbecauseits EEG is similar towakefulness. Has beenreportedthatsleeprestriction (SR) orsleepdeprivation (SD) modifiescognition, immune response, hormonal secretion and metabolism. In humans, SR or SD increasesthe basal levels of glucose and foodingestion, induces glucosetolerance and insulinresistance. However, thestudies in animals are contradictory. In ordertoassesstherelationshipbetweensleeprestriction and glucosemetabolismweused Wistar ratsdeprived of REM sleepfor 20 hrs per day (rREM) for 21 or 42 consecutivedays. Two control groupswereused, onesimulatedthecondition of REM restrictionbutratsreached and maintained REM sleep (butlossabout 30-40% of total sleep, SR), and theother control groupwasconformedby males thatremained in their home cages (CON). Ourresultswith 21 days of SR showed no differencesbetweengroups, althoughwith 42 days, rREM males showed a slightincrease in the basal levels of glucose and rREM and SR groups show a slowreturnto basal levels in theglucosetolerance test. Theresults show a directrelationshipbetweendecrease of sleep time and theglucosetolerance, withoutincreasingfoodingestion, suggestingthatsleeprestrictionis a cause todeveloptype 2 diabetes.

Evaluation of the effect of avocado oil on renal vascular reactivity and mitochondrial dysfunction induced by angiotensin II

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Hypertension (HT) is a chronic degenerative disease with a major economic impact due to the high costs associated with the development of chronic renal failure.Dysfunction in the formation and/or metabolism of nitric oxide (NO) is believed to be involved in the pathogenesis of the HT.Chronic administration of L-arginine derivatives, especially ester Nω-nitro-L-arginine methyl (L-NAME) in rat inhibits nitric oxide synthase (NOS) and reduces the formation of NO, resulting in endothelial dysfunction.Angiotensin II (AngII) alsoplays an important role in the development of renal complications during HT and its overproduction increases the mitochondrial formation of reactive oxygen species (ROS) and contributes to renal failure.It has been proposed that electron transport chain (ETC) is one of the main sites of ROS generation in the kidney damage during HT.Recently, we have shown that dietary administration of avocado oil decreases the deleterious effects of oxidative stress generated during diabetes in kidney mitochondria, which has been attributedto the presence of a wide range of antioxidants carotenoids in this oil.Thus, the main goal of this work was to study whether avocado oil administration modifies ROS formation, oxidative stress, renal vascular reactivity to AngIIand kidney mitochondrial dysfunction in hypertensive (HT) rats.Avocado oil decreased renal vascular reactivity only in control rats, while in HT rats, renal reactivity was higher than in control rats and avocado oil did not modified this parameter.Moreover, mitochondria from HT rats exhibited impaired membrane potential and this effect was fully prevented by avocado oil.These effects were associated with animprovement of the activity of the complex IV in the same groups. Moreover, mitochondria from HT rats exhibited higher levels of oxidative stress and ROS production and these effects were attenuated by avocado oil supplementation.Together these results suggest that avocado oil intake decreases oxidative stress and improves mitochondrial function of HT rats without having an effects of vascular reactivity to AngII.

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ProteomicAnalysis of RatSerumTreatedwithDNAse I and Papain (preliminaryresults)

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Recent studies have demonstrated that treatment with proteases and DNAse I decreases the concentration of cirulating DNA (cDNA), which plays the role of promotor in the model of cancer progression. Our research group has proposed a treatment with papain (a protease) and DNAse I to reduce tumor volume, however it is not known if this treatment has side effects on other serum proteins. The aim of this study is to identify significant changes in the proteome after Papain and DNAse I treatment in a cancer rat model.

Male Wistar rats were injected with 2 x 10^6 C6 cells to generate subdermic tumors. After tumor induction, rats were treated with a single dose of papain and DNAse I then serum samples were taken at different times during 96 hours. Digestion patterns and identification of serum proteins were confirmed by one and two dimention gels.

Most of serum proteins are digested by Papain, having the main effect at 30 minutes. The presence of the proteins previously detected by MS and MS/MS in rats with tumors, is going to be evaluated in samples taken at this time point.

Expression and distribution of tight junction proteins in peritoneal cells of diabetic rats.

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Background: Peritoneal dialysis (PD) is the most commonly used alternative therapy in patients with chronic renal failure, and diabetes is one of the most common causes of this condition. Due to the progressive damage caused by chronic exposure to peritoneal dialysis solutions (PDS)with high glucose content on the peritoneum, the efficiency of PD is limited by alterations of the transport properties of water and solutes of the peritoneum. Tight junction (TJs) proteins are critical to maintain ions, molecules and water transport through the paracellular pathway in epithelial and endothelial cells. Exposure to PDS with high glucose damages the TJs and decreases the transepithelial electrical resistance (TER) in peritoneal mesothelial cells (PMCs)monolayers. For these reasons it is very important to study thePMCs which are the most important cells of the peritoneum through which the exchange of solutes during dialysis occurs. We explored the function, amount and distribution of TJs proteins: claudin-1, claudin-2 and occludin, in PMCs monolayers at early stages of diabetes.**Methods**: Female Wistar rats were used.Diabetes was induced by tail vein administration of streptozotocin (60 mg/Kg). Omentum was used to obtain primary culture of PMCs. Cells were cultured in DMEM medium.TER measurement, immunostaining(by confocal microscopy) and Western blot (WB) analyses of claudin1;claudin 2 and occludin expression were performed. **Results:**Diabetesdecreased TER ($6\Omega \cdot cm^2 \pm 1.65\Omega \cdot cm^2$) compared to control group ($10\Omega \cdot cm^2 \pm 1.45\Omega \cdot cm^2$). By confocal microscopy, claudin-1 immunostaining did not change in both groups. Claudin-2 was overexpressed (evaluated by WB and confocal microscopy) under diabetic conditions compared to control group. In contrast, the expression and distribution of occludin decreased under diabetic conditions. **Conclusion:**It was found that diabetes decreases TER which might be related to changes in the distribution and expression of the tight junction proteins in PMCs.

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Sucrose consumption plus and chronic stress induces histological changes in the adrenal gland in the male rat

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Recently, we have shown that consumption of 30% sucrose, weaning for three months, increases visceral adiposity and alters the histology of the adrenal gland, without changing the concentrations of serum corticosterone and leptin. In animal models it has been shown that stress combined with caloric diet, increases the weight of the adrenal gland, the alteration or the concentration of leptin and corticosterone. However, these results are dependent on the intensity, frequency and duration of stress and diet. We are now interested in evaluating the function of the adrenal gland in a situation of stress. The objective was to determine the effect of sucrose consumption more stress at weaning in the histological features of the adrenal gland, leptin and corticosterone. Thirty-two recently weaned (21 days age) Wistar male rats were divided in 4 experimental groups (8/group): control (C), stress more drinking water (St), 30% sucrose in drinking water (S30) and 30% sucrose in drinking water more stress (S30/St). Animals were subjected to a chronic stress during for 4 weeks. At sacrifice the adrenal gland was removed; both right and left adrenal glands were stained with Masson's trichrome to evaluate the histological organization and the presence of connective tissue of adrenal glands for each rat. The concentrations of leptin and corticosterone were measured using a commercially available enzyme-linked immunosorbent assay (EIA) Kit. Statistical significance was determined by a two-way ANOVA followed by post hoc Newman-Keuls tests ($p < 0.05$). Stress increased corticosterone levels, the 30% sucrose in drinking water increased leptin levels, and the interaction increased the weight of the right adrenal gland. Stress, the 30% sucrose in drinking water and interaction modify the histological variables of the gland. Apparently, the sucrose consumption reverses damage caused by stress, by regulating the concentration of corticosterone, through leptin locally and through the axis hypothalamus-pituitary-adrenal. Finance: CONACYT a YDA (366801); PNPC C-122/2014, CONACYT-225126.

Low ADAR 1, ADAR 2 and ADAR 3 levels are associated with a different expression of microRNAs in Idiophatic Pulmonary Fibrosis.

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Introduction: Idiophatic pulmonary fibrosis (IPF) is the most aggressive and lethal, age-relating lung disease of unknownetiology without treatment. Evidence points out that the fibrotic response is driven by abnormally activated alveolar epithelial cells. These cells produce mediators that induce the formation of fibroblast and myofibroblast foci which secrete excessive amounts of extracellular matrix proteins, mainly collagens, resulting in scarring and destruction of the lung architecture. One of the most important profibroticmolecule is transforming growth factor 1 (TGFβ1), this is regulated by microRNAs such as miR21, miR-155 and Let-7d. It has been demonstrated that they are differentially expressed, miR-21 2 fold upregulated in IPF, and Let-7d, downregulated in alveolar epithelial cells. Doble chain RNA are edited by a family of proteins denominated Adenosine DeaminasesActing on RNA (ADAR), And this edition impairs miRNAs ability to bind to targets. There are three different ADAR, 1, 2 and 3, and are expressed in lung. Using software “Inosine predict” (http://bioserver.hci.utah.edu: 8080/Bass/InosinePredict), it was determined that miR- 21, miR-155 and Let-7d have sites whit a probability higher to 50% to be edited by ADAR 1 and 2. Aim: Determine if the expression of ADAR 1, 2 and 3 have an association with the expression of miR-21, miR-155 and Let-7d. Localize the subcellular sites of ADAR 1,2 and 3 in the lung fibroblasts.Method: total RNA was extracted, using Trizol from strains of fibroblasts derived from IPF patients and healthy controls, and were used to determine the levels of transcripts of ADAR 1, 2, 3, miR-21, miR-155 and Let-7d by real time PCR, Taqman probes were used for real time PCR. Monoclonal antibodies were used to determine the subcellular localization of ADAR 1,2 and 3 in lung fibroblasts. Results: ADAR 1 mRNA is decresead in IPF fibroblasts compared with controls. miR-21 is upregulated in IPF fibroblasts compared with controls. Let-7d is downregulated in IPF fibroblasts. ADAR1 is mainly localized in nucleolus and colocalizes with ADAR2 in control fibroblasts.

Effect of Omega 3 Fatty Acids in Diabetic Female Rats.

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Type 2 diabetes (T2D) frequently results from progressive failure of pancreatic beta cell function in chronic insulin resistance. Several clinical, experimental, and epidemiologic studies have confirmed that the intake of omega-3 polyunsaturated fatty acids from fish oil exerts a favorable effect on T2D development and progression. This protective effect is believed to be mediated by changes in plasma lipids, particularly triacylglycerols. It has been suggested moreover that enrichment of cell membranes with omega-3 fatty acids may lead to improved peripheral insulin action. Increased interest in using omega-3 fatty acids led us to examine their metabolic effects in female Wistar rats with T2D. There are many reports in males, but little on female diabetic rats. The primary aim of this study was to evaluate the effect of omega-3 fatty acid on lipid and carbohydrates metabolism in a T2D model in female rats. Methods: 48 hours female newborn rats were induced to T2D by a unique intraperitoneal injection of streptozotocin (STZ) of 135 mg/kg of body weight in 50 µl of citrate buffer. It is produced toxicity in pancreatic β cells and consequently hyperglycemia. The control groups were injected only with 50 µl of citrate buffer. After weaning, from STZ groups one was supplemented with flax seed oil (125 mg/kg body weight daily) (STZ-ω3) and the other one not (STZ). The same criterion was applied for control groups (C-ω3 and C). Periodical measurements of blood glucose concentration, cholesterol, triglycerides, and glucose tolerance curve, were taken as the indicative parameters of the metabolic alterations. Fatty acids supplement was analyzed by gas chromatography. Results: Blood glucose was recorded every week, and at the fourth month glycaemia was 106 mg/dl for C group, 117 mg/dl for C-ω3, 145 mg/dl for STZ, and 106 mg/dl for STZ-ω3. Induced hyperglycemia was not so high, and at this time, it was apparently controlled by omega-3, but not for so long. In such conditions, it is clear that female lipid metabolism was not altered significantly, except in control group with omega-3 (C-ω3). Cholesterol (mg/dl): 178 (C), 303 (C-ω3), 160 (STZ), and 151 (STZ-ω3). Triglycerides (mg/dl): 165 (C), 173 (C-ω3), 170 (STZ), and 173 (STZ-ω3). The STZ group has a lower growth rate and difficulties controlling blood glycaemia (according to the glucose tolerance curves). In this work, it will be shown that omega-3 fatty acids had only limited beneficial effects on controlling diabetes in female Wistar rats.

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Effect of plant lectins of *Ruta graveolens* on breast cancer cells

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The lectins are proteins present in all living organism that may link of specific and reversible form at carbohydrates. In the plants these proteins involved in the interactions among nitrogen-fixing bacteria with root, present mitogénico activity, protective effect against pathogenic action of microorganism. On the other hand the plant lectins have cashed very important due of its use in the detection of malignant transformations in cells generating a preferential agglutination, citotoxic and antitumor in cancer cells.

This study had as main objective determine the effect of the plant lectins of *Ruta graveolens* on breast cancer cells. For it is led held the salting out, affinity chromatography, partial characterization of proteins type lectin, agglutination testing, citotoxicity testing in MCF-7 breast cancer cells, evaluation of the activation of Akt and NF-kB in MCF-7 cells and evaluation of adjuvant effect of lectins in MCF.7 cells treated with tamoxifen.

The results obtained in this study demonstrate greater obtaining of lectins extracted by the method of Tris-HCl in compared with the salt extraction with NaCl; however these proteins show differences in their structure and biological activity. Likewise were observed differences in the citotoxic effect and activation of Akt and NF-kB given by the plant lectins of *Ruta graveolens* obtained with both methods of extraction.

Keywords: Plant lectins, breast cancer and NF-kB

Treatment with Pirfenidone produces an important reduction in necroinflammation score, fibrosis index and increased gene expression of CB2 in patients with liver cirrhosis

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Background and Aims: Hepatitis C virus (HCV) chronic infection stimulates liver inflammation and fibrogénesis and increases serum levels of TGF-α, IL-6, TNF-α. Cannabinoids receptors (CB1 and CB2) regulate fibrosis progression in patients with chronic hepatitis C (CHC). Pirfenidone (PFD) is an anti-fibrogenic and anti-inflammatory drug. The aim of this study was to assess if treatment for 2 years with PFD improves necroinflammation score and fibrosis index through diminish of TGF-β1, IL-6, TNF-α, CB1 and increased CB2 gene expression.

Methods: 28 CHC patients received Pirfenidone (1200 mg/day) for 24 months. Liver biopsies and serum samples were obtained at the beginning and at the end of treatment. In liver, CB1 and CB2 gene expression were evaluated by Real Time-PCR, along with necroinflammation and fibrosis using Modified histological activity index (HAI) of Ishak. TGF-β, IL-6 and TNF-α serum levels were measured using ELISA. HCV genotype was established and viral load was measured every 6 months, quality of life was assessed using SF36 questionnaires.

Results: At the end of 24 months of treatment, necroinflamation score was reduced an average of 3 points in 70% of patients ($p<0.004$) and fibrosis decreased 2 points-average in 55% of patients ($p<0.02$). TGF-β and IL-6 serum levels decreased significantly in 73% and 91% of patients ($p<0.05$), respectively. TNF-α diminish in 82% of patients ($p>0.05$). ALT levels showed a tendency to normalize in 81% of patients and quality of life was improved in 100% of patients. CB2 mRNA increased (85.71%), while CB1 diminished in 28.57% of them.

Conclusions: The treatment for 2 years with PFD reduced inflammation and fibrosis and improved liver function in patients with CHC through diminution of TGF-β, IL-6, TNF-α and CB2 mRNA increase.

Development of anin vitro experimental modelforhumanamniochorionrupture

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Premature rupture of amniochorion (ACM) is one of the most common obstetric complications and so far lacks preventive and therapeutic measures for its management. The characterization of the cellular and molecular mechanisms leading to rupture of the membranes isthe subject of study in our lab. Nevertheless, now it is possible to highlight the role that inflammatory cells have when they migrate into the amniochorion environment and act as signals to begin connective tissue degradation of these structures.

The aim of the presentstudy was to developan in vitromodelof the ACMrupture event that emulatestheactual rupture sequence.

Methods: Full-thickness human ACMs were obtained after delivery by elective cesarean section from women at 37-40 wks of gestation, with no evidence of active labor. The membranes were cultured in a two compartment experimental model in which the upper compartment was delimited by the amnion and the lower chamber by the choriodecidual membrane. First these membranes were co-incubated with different types of enzymes to evaluate their timing degradation capacity. After this process, other membranes were co-incubated with leukocytes isolated from the choriodecidualcompartment of ACM with labor, without labor and with infection. The ACMs were submitted to a mechanical stimulus during the whole period of incubation by an eppendorf tube with 1ml of solution. Control ACM membranes were used and the cellular stimulus were not applied. The ACMs were observed during 12, 24 and 48 hours after the stimulus until they were broken. We quantified active matrix metalloproteinase (MMP-9) in every environment where the membranes were embeddedandqualitative results of the membrane structures were shown.

Results: With this model we have shown that the treatment used resultsin the rupture of the membranes after 48 hours of culture with the entrance of the eppendorf tube to the inferior compartment. We observed this event only in the ACMs cultivated in presence of leukocytes isolated from the choriodecidualcompartment of ACMs with labor and with infection. Also active MMP-9 was found in these cultured media.

Conclusions: It was possible to develop an in vitro model simulating sequentially the series of events that change the homeostasis of connective tissue and show that the secretion of leukocytes isolated from the membranes with labor and infection have mediators that activate MMP-9.

Transcriptomic profiling of adipose tissue in response to ACET/moxibustion treatment in obese women

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Obesity is a chronic and mild systemic inflammatory condition that affects both developed and developing countries worldwide. It is characterized by an increased adipose tissue mass, which is associated to the deregulation of adipokines involved in the regulation of homeostasis, feeding, thermogenesis, inflammation and insulin resistance, among others. Complementary and alternative medicine represents an effective therapeutic option to aid with weight loss. Previously, we showed that acupuncture catgut embedding therapy (ACET) with moxibustion was able to reduce body weight and reverse insulin resistance in obese women. Here, we aimed to evidence changes in adipokines synthesis and gene expression in adipose tissue that could explain the effects of ACET-moxibustion.

Two groups of obese women were treated with ACET-moxibustion (CGM group) or sham acupuncture as control. Leptin, adiponectin, TNF-alpha and resistinwere quantified by ELISA in blood samples. Gene expression in adipose tissue was determined by microarray assays.Surprisingly, results showed that loss of body weight after ACET-moxibustion treatment was not associated to changes in circulating adipokines levels. Interestingly, transcripcional profiling of adipose tissue revealed that 249 genes were modulated, included 154 upregulated genes (FC ≥ 1.5) and 95 downregulated genes (FC ≤ -1.5). In agreement with biochemical data, adipokines genes were not modulated. The analysis of KEGG pathways revealed that modulated genes are involved in neurologic pathwaysand metabolism and endocrine function, which could help to understand the molecular mechanisms that lead to body weight reduction in response to ACET-moxibustiontreatment.

The effect of aqueous extracts of *Hibiscus sabdariffa* L. and NSAIDs on the expression of circulating cytokines in brewer's yeast-induced fever. Adrián Gómez Baltazar[1], Luz Torner Aguilar[2], Martha Eva Viveros Sandoval[3], Víctor Manuel Farías Rodríguez[4]and Bertha Fenton Navarro*[1]

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Fever is a common medical sign characterized by an elevationof temperature above the normal range of 36.5 -37.5°C. Several cytokines, including IL-1β, IL-6 and TNF-α were also demonstratedto have pyretic effects and can be produced in the periphery as well as in the CNS. Cytokines induce PGE_2 release in the brain from blood brain barrier endothelial cell andcan directly modulate fever neurons.*Hibiscus sabdariffa*L. has been popularly used as beverage and many pharmacological activities are reported, only few pharmacological studies on the antipyretic action of this plant have been undertaken despite the claims of its effectiveness in relief of pyrexia in folkloric medicines. **Objective:**Compare the effect of aqueous extracts of *Hibiscus sabdariffa* L. and NSAIDs on the expression of circulating cytokines (IL-1β, TNF-α, and IL-6) in brewer's yeast-induced fever.**Materials and methods:**The antipyretic activity of aqueous extract of *Hibiscus sabdariffa* L. was evaluated in brewer's yeast-induced fever in rats.The rectal temperature of each rat were measured using an OMRONthermometer in different timesafter the treatment administration. Treatment groups wereA. Aqueousextract (AE), B. Pigments (P) and C. Protein fraction (PF) and standard drugs D. Paracetamol, E. Nimesulide and F. Saline solution (SS). The concentrations of circulating cytokines were measuredby quantitative sandwich ELISA. **Results:** The aqueous extracts exhibit antipyretic activity, CE remove fever at 90 minutes, PF at60 minutes and P at30 minutes in the same way that the groups treated with paracetamol and nimesulide, with the advantage that the extracts did not cause hypothermia. The expression of IL-1β in both the extracts and the NSAIDs was below of the values of the control group treated with SS in which we observed increased expression. While TNF-α differences between groups was observed only up to 180 minutes where the group of PF has higher values than the rest of the groups. Finally, IL-6 concentrations were increased at 60 min in P and nimusulide, with SS increase at 90 and both P group and crude extract at 120 minutes. **Conclusion:**The effect of extracts of *Hibiscus sabdariffa* L.in the antipyretic activity and expression levels of circulating cytokines is very similar, inhibiting the expression of IL-1b and TNF but not IL-6.

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DNA MICROARRAYS MAKING FOR IDENTIFICATION OF GENETICALLY MODIFIED ORGANISMS.

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Making DNA Microarray in México for over 10 years has led to DNA Microarrays Unit, of the Universidad Nacional Autónoma de México, has disigned strategies for the simultaneous detection of pathogens in food, livestock and meat products. Currently the Unit is generating a chip microarrays prototype recognition of the genetically modified organisms (GMOs) justified by the fact that Corn and Soybean production in the World is about 60%. Also in late 2010, 87 transgenic events added the large number of samples that the competent authorities have to assess and analyze requiered methods for mass detection as in the case of DNA Microarrays which allow the simultaneous análisis of thousans of samples. With the chip being designed, is to have a tool that can identify events authorized of the seeds or products derived from these GMOs, in order to speedy up the identification process. The tool does not replace existing methods rather expedite aims to assist and reliable análisis required to ensure food quality and safety thereof. The chip must be able to identify any unauthorized event, the organismo(s), promotors, enhancers, transcriptional

CDP-choline AMELIORATES HEART REPERFUSION DAMAGE STRENGTHEN BY HYPERTHYROIDISM

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Thyroid hormone has a remarkable influence on heart and cardiovascular system. Available data indicate that hyperthyroid state is associated with an increased heart rate, ventricular arrhythmias, and high blood pressure, in addition to a decrease in vascular peripheral resistance (Panagoulis et al. 2008; Mazza et al., 2011; Danzi and Klein, 2012). Hyperthyroidism improves heart metabolism whose characteristic is increased oxygen consumption due to an elevated induction of gene expression of enzymes of the respiratory chain by T3 (Virbasius and Scarpulla, 1990; Luciakova and Nelson, 1992). This latter is associated with an amplified generation of reactive oxygen-derived species (ROS) that leads to a significant heart tachycardia and arrhythmias (Anjo et al., 2013). Moreover, hyperthyroidism switches the heart vulnerable, to a highest degree, to undergo severe tissue injury after reperfusion following an ischemic period (Masullo et al. 2000; Pavón et al.
2009). Reperfusion must be applied as soon as possible; reoxygenation bringing about through the implant of intracoronary stents or coronary by-pass has been successfully used to allow blood reperfusion. However, such an event is responsible of additional myocardium damage; it causes severe cell injury due to the increased production of ROS. Oxidative stress is one of the important promoters involved in mechanism of cell Ca^{2+} overload, which contributes significantly to the myocardial insult (Weiss et al. 2003; Peng and Jou 2010). It is recognized that during myocardial reperfusion damage, after a period of ischemia, opening of the mitochondrial nonspecific pore occurs (Arteaga et al. 1992; Griffiths and Halestrap 1993; Li et al. 2014). This process, called permeability transition, lies beneath heart reperfusion injury (Hernández-Esquivel et al. 2014), and is exacerbated in hyperthyroidism (Pavón et al. 2009). With the purpose to circumvent the damaging effect of T3 on heart function several chemicals have been used, among them β-blockers (Fumarola et al. 2010), vitamin E (Venditti et al. 2011), inhibitors of Ca^{2+} channels (Kelestimur and Asku, 1996), and octylguanidine, this latter an inhibitor of membrane permeability transition (Pavón et al. 2009). This work was aimed to explore the possibility that the nucleotide CDP-choline (citicoline) may protect heart function against the deleterious effect of reperfusion in hyperthyroid rats. The neuroprotective action of citicoline has been well documented. It has been reported that this precursor of phospholipids has been used as a memory-enhancer drug, in acute ischemic stroke, Alzheimer's disease, and other sort of neurological diseases (Conant and Schaus 2004; Secades et al. 2006; Turkkkan et al. 2010). Recently we found that citicoline avoid heart damage occurred after ischemia/reperfusion processes (Hernández-Esquivel et al. 2014). The present work shows that citicoline protects from the vulnerability of hyperthyroid heart to undergo injury, evidenced by reperfusion arrhythmias and the incidence of ventricular tachycardia. As is also shown citicoline inhibits permeability transition in hyperthyroid mitochondria, preserves oxidative phosphorylation, the activity of the mitochondrial enzyme *cis*-aconitase, as well as the activity of the enzyme superoxide dismutase, and avoids the oxidative disruption of mitochondrial DNA and the release of cytokines.

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Inhibitory Activity of *ReishiMedicinal Mushroom, Ganodermalucidum*on Transformed Cells by Human Papillomavirus.

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ABSTRACT: In this study, we investigated the effects of the aqueous extracts of Lingzhi or Reishi medicinal mushroom, *Ganodermalucidum*, obtained from three localities (China; and Morelos and Michoacan, Mexico) on cervical cells transformed by human papillomavirus (HeLa and SiHa) and C-33A cancer cells. The cells were plated in DMEM medium supplemented, and were incubated in the presence of different concentrations of *G. lucidum*for 24 h. Cell proliferation was determined by MTT colorimetric assay and viability by trypan blue assay. Inhibitory dose was determined (IC_{50}) of the three different extracts of *G. lucidum*in the culture cell lines mentioned above. The apoptosis process was confirmed by nuclear DNA fragmentation and the cell cycle was determined by flow cytometry. The results showed that aqueous extracts *G. lucidum*obtained from three localities produced inhibition in the proliferation of VPH transformed cells; they also induced apoptosis and cell cycle arrest in HeLa, SiHa, and C-33A cancer cells. Therefore, it was found that aqueous extracts *G. lucidum*obtained from three different locations produced inhibitory effect on cancer cells and may have a potential therapeutic use for the prevention and treatment of this disease.

Effects of the direct factor Xa inhibitor rivaroxaban on platelet function in Mexican patients with thrombosis

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Introduction. Rivaroxaban (RRB) is an anticoagulant drug that directly inhibits activated factor X (FXa) in the plasma phase of hemostasis. The conversion of inactivated factor X to FXa occurs on membrane phospholipids of activated platelets and allows the generation of thrombin. Considering that FXa has antiplatelet effects and RRB has shown to decrease platelets activation in basic research studies, it is of our interest to know whether this anticoagulant therapy may affect platelets function in patients with thrombosis. Our results will contribute mainly to the knowledge of the RRB security as a new anticoagulant drug for Mexican patients with thrombosis diseases.

Material and methods. In a prospective, longitudinal, controlled, non-randomized study, we are analyzing the platelet function in patients diagnosed with thrombosis (19 women and 11 men). All selected patient were between 18 and 40 years old with an average age = 32 years. Platelet function was assessed by platelet aggregation induced with three agonists: adenosine diphosphate (ADP; 10µM), epinephrine (EPI; 10µM) and arachidonic acid (AA, 0.5 mM). For this test we used platelet-rich plasma to a final concentration of $200x10^3$ platelets/µl. Patients were divided into three groups depending on the dose of anticoagulant. Group 1: RRB 10 mg/day; group 2: RRB 15 mg/day and group 3: RRB 20 mg/day. We excluded patients with thrombocytopenia and those ones who consumed any drug that interferes with the activity of platelets in the last 15 days. Platelet aggregation was performed before and one month after the uninterrupted anticoagulant therapy.

Results. So far we have already analyzed platelets function from 30 patients (10 patients for each study group). We observed no changes suggestive of altered platelet function. The results of the baseline platelet aggregation and post-treatment were similar for the three agonists irrespectively of the dosage used. A slight increase in platelet aggregation induced by AA for group 1 was seen (74 to 84%). However, the increase was not significant. All results are shown in the following table.

	Basal			Post-TX		
Groups	AA (%)	ADP (%)	EPI (%)	AA (%)	ADP (%)	EPI (%)
RRB 10 mg/day	74 ±10	70 ±16	67 ±34	84 ±11	69 ±13	60 ±31
RRB 15 mg/day	70 ±14	64 ±20	50 ±35	70 ±20	61 ±18	51 ±34
RRB 20 mg/day	70 ±21	65 ±16	66 ±20	73 ±15	63 ±11	63 ±21

Results are expressed as mean ± standard deviation. The analysis of the results before and after anticoagulant therapy was done with Wilcoxon´s test. *: $p < 0.05$ between baseline and post-TX determination.

Preliminary conclusions. Contrary to that observed in basic research studies, our results suggest that inhibition of FXa by RRB did not interfere with platelet function in patients with thrombosis irrespectively of the dosage used.

INSULIN SENSITIVITY IS INVERSELY RELATED TO CELLULAR ENERGY STATUS, AS REVEALED BY BIOTIN DEPRIVATION. Hernández-Vázquez, Alain de J.; Salvador-Adriano, Ana; Vargas-Chávez, Sonia; Ortega-Cuellar, Daniel; Sánchez-Salvador, Alan; Tovar, Armando R. y **Velázquez-Arellano, Antonio.**Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas, UNAM. Av. De la IMAN #1 Col. Insurgentes-Cuicuilco, C.P.04530 velare@unam.mx

We have reported an early decrease of glycemia in rats fed a biotin-deficient diet, with reduced cellular ATP levels and suggesting increased insulin sensitivity. Here we show that biotin deprived rats are more tolerant to glucose as shown by both oral and intraperitoneal glucose tolerance tests, during which insulin plasma levels were significantly diminished in deficient rats compared to controls. Biotin-deficient rats had lower blood glucose concentrations during intraperitoneal insulin sensitivity tests than controls. Furthermore, more glucose was infused to maintain euglycemia in the biotin deficient rats during hyperinsulinemic euglycemic clamps studies. These results demonstrate augmented sensitivity to insulin in biotin-deprived rats. They are most likely consequence of an insulin-independent effect of AMPK activation on GLUT4 membrane translocation with increased glucose uptake. In biotin deficient cultured L6 muscle cells, there was increased phosphorylation of the energy sensor AMPK. We have now confirmed the augmented AMPK activation in both biotin-deprived in vivo muscle and in cultured muscle cells. In these cells, glucose uptake is increased by AMPK activation by AICAR, and is diminished by its knockdown by the specific siRNAs directed against its a1 and a2 catalytic subunits; all of these effects being largely independent of the activity of the insulin signaling pathway that was inhibited with Wortmannin. The enhanced insulin sensitivity in biotin deficiency likely has adaptive value for organisms due to the hormone promotion of uptake and utilization not only of glucose, but other nutrients such as branched-chain amino acids, whose deficiency has been reported to increase insulin tolerance.

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Development and evaluation of a novel panel of tumor-associated antigens with applications in breast cancer diagnosis.

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Breast cancer is the most frequently diagnosed cancer and the leading cause of death in women worldwide accounting for 23% (1.38 million) of the total new cases and 14%(485,400) of the total cancer deaths. In Mexico, breast cancer is a public health problem, and the reduction in mortality should rely on early tumor detection and novel therapeutic strategies. Since three decades ago, it is well known that breast cancer patients produce serum antigens derived from immunogenic tumor proteins dubbed as tumor-associated antigens (AAs). Notably, these antigens are absent in healthy people, thus they are considered as excellent biomarkers in cancer. However, the number of AATs validated in breast cancer is still scarce.

In this study, we evaluated an enzyme-linked immunosorbent assay (ELISA) for the immunodetection of a panel of 12 potential AAs represented by p53, CEA, c-Myc, ciclin-B1, PRDX-6, Nm23, eIFE-5A, GLIO-1, DJ1, Hsp27, Hsp70, and Ki67 tumor proteins. Some of these proteins have been established as potential AAs in other types of cancer, and the others were previously identified in our laboratory as deregulated in breast tumors from Mexican patients. 100 serum samples from breast cancer patients (clinical stage III-IV) and 50 serum samples from healthy women were obtained. Results from ELISA assays showed that 23% of patients present auto-antibodies against at least one of the tested AAs. In addition, we detected auto-antibodies against Hsp27, CEA, eIF5A and glioxalase-1 in three healthy women. We also tested serum reactivity against mixtures of three AATs represented by Hsp27/Nm23/c-Myc, Hsp70/DJ1/GLIO-1 and p53/CEA/PRDX-6. Results showed the presence of serum anti-antibodies raised against these three panels of AAts in 3.3%, 10% and 10% of breast cancer patients, respectively. These AATs effectively identify the breast cancer patients from healthy group. Now, we are testing additional panels of 3 to 5 AATs and analyzing serum samples from breast cancer patients in early clinical stages. In conclusion, our data suggested that Hsp27/Nm23/c-Myc, Hsp70/DJ1/GLIO-1 and p53/CEA/PRDX-6 might represent novel serum AATs useful for the detection of breast cancer.

Spoligotyping of resistant *M. tuberculosis* isolates in Jalisco México

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Tuberculosis is a disease causes by the bacillus M. tuberculosis.It is disseminated by air and it is one of the leading causes of deaths worldwide. One of the biggest concerns in TB control is the dispersion of strains resistance to first line antibiotics, this could be avoided if the circulating strain dynamics of transmission and pathogenesis of tuberculosis are better known. A variety of methods to visualize polymorphisms in *M. tuberculosis*havebeen described, RFLP hasbeen considered the gold standard since 1993.However,RFLP has low sensitivity and is hard tointerpret.Others techniques such as spoligotypingdetect the direct repeat locus (DR).This method is cheap, fast and has high reproducibility. In México,especially in Jalisco, there arefew studies about dispersion and circulation of tuberculosis strains. To analyze the dynamics of transmission, detect suspicious outbreaks and distinguish between exogenous reinfection or endogenous reactivation in relapse, this study analyze the pattern of tuberculosis strains in Jalisco using spoligotypoing.The results will be worthwhileforstopping the dissemination of the disease in Mexico.

We analyzed65 clinical samples collected between April 2012 to July 2014 at the StateHealth Laboratory of Jalisco. Using the CTAB method, the DNA was isolated.Previously, drug susceptibility testing was completedusingVersaTREK.Then,using the spoligotypingmethod we identifythe different types of strains circulating in Jalisco, finally applying adendogram analysis(www.miru-vntr.com) we evaluated the evolutive distance and the similarity between the circulating strains.

Out of a total of 46 isolates tested by drug susceptibility29.23% were sensitive to the first line drugs, 9.23% were classified as MDR (multi drug resistant), 27.69% were monorresistant, 12.30%polyrresistant and the remaining 21.53% belonged to patients who received treatment for 6 months but do not became negative. For all samples the IS6110 sequence was amplified using PCR to confirm they belong to the *M. tuberculosis Complex*. The spoligotypingdetected 7 different types of strains linagesT1 (18,75%), H3 (7,81%), MANU (4.68%), X1 (3.12%), EAI5 and LAM1 (1.56%). It isinterestingthat the most predominant strain was from the orphan family (60%). This could be because of the lack of previous studies in Mexico the most relevant strains are undetermined and therefore orphan strains. Using dendogramanalysis, the strains weregrouped in 6 different clusters of spoligotype patterns. To better understand the dispersionpatternthe data was analyzed by a minimum spanning tree (www.miru-vntr.com),3different clusters are shown in Jalisco. Compared with previous studies in Mexico, our results show a lower diversity of lineages but a greater presence of orphan strains, these results could be important and novel because new tuberculosis the strains detected circulating in Jalisco have not been previously reported.

The analyzedtuberculosis strains showed a great diversity of linages and the presence of high orphan strains in Jalisco. An analysis of tuberculosis strains that are circulating will be useful to the health sector to establish programs to control and prevent tuberculosis strains especially those that have some resistance to first line antibiotics.

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Matrix Metalloproteinase (MMP)-28 increases growth rate and migration of lung alveolar epithelial cells and localizes in the nuclei of alveolar epithelial cells in Idiopathic Pulmonary Fibrosis.

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Matrix Metalloproteinases (MMPs) are a family of zinc-dependent enzymes that not only modify the extracellular matrix (ECM), but also play important roles in proliferation, apoptosis, migration, differentiation and angiogenesis. MMPs are secreted or membrane-bound enzymes, and recently some of them have been localized in mitochondria and even inside the nucleus.

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive and generally lethal disease of unknown etiology where the aberrant activated alveolar epithelial cells (AECs) induce the expansion and activation of the fibroblast population, along with excessive synthesis of ECM and destruction of the lung architecture. Microarray assay revealed that some MMPs are upregulated in IPF patients. One of them is MMP-28, the last member of the family, a protein that had not been studied in human lung. By immunohistochemistry of lung biopsies of IPF patients, we found that MMP-28 is expressed by the AECs, but interestingly it localized in the nuclei, while there was no positive staining in control tissues.

With this basis, we analyzed whether MMP-28 localizes in the nucleus of the human AECs A549 cell line. Western blot analysis of nuclear and cytoplasmic fractions showed that under certain conditions MMP-28 localizes in the nucleus of A549 cells. *In silico* analysis revealed a probable Nuclear Localization Signal (NLS) in MMP-28, which could represent a mechanism for getting inside the nucleus.

In order to study the function and localization of this protein, A549 cells were transfected with MMP-28-mycDDK, as well as the endogene expression was silenced with shRNA. A549 cells with shMMP28 showed decreased growth rate and decelerated wound closing ($p<0.01$), while cells overexpressing MMP-28 showed the opposite: faster growth rate and accelerated wound closing ($p<0.01$). We performed site-directed mutagenesis (K→Q) in the NLS to test the hypothesis, and future experiments include an approach to the probable nuclear function of MMP-28. This research will help to understand the role of MMP-28 in AECs in IPF.

Effect of nicotinamide on cell viability and secretion of plasminogen activators in invasive MDA-MB-231 breast cancer cells.

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The invasion of tissues by cancer cells involves the activity of extracellular proteases, like urokinase plasminogen activator (PLAU), that catalyzes the formation of plasmin, starting a proteolytic cascade that contributes to the breakdown of extracellular matrix (ECM), this enzyme is secreted to the extracellular medium and its overexpression correlates with aggressive tumors.Reactive oxygen species (ROS) may regulate the activity of transcription factors such as nuclear factor κβ (NF-κβ). Promoter region of PLAU contains an NF-κβ binding element, which is sensitive to redox changes, antioxidants, such as nicotinamidethrough its ability to scavenger ROS, can regulate the expression, secretion and activity of PLAU associated with the invasive ability of cancer cells.Our objective was to evaluate the effect of nicotinamide on cell viability, apoptosis and the secretion of PLAU in MDA-MB-231 breast cancer cells. Nicotinamide was used at 0.5, 1, 5, 10, 15, 20, 25 and 30 mM. The growth of cell population was evaluated by the MTT assay, apoptosis was measured using the Annexin V-FITC/PI double staining by flow cytometry and the plasminogen activator activity was evaluated in the conditioned medium using a two steps procedure, incubating first with human plasminogen10 μg/mL followed by the measurement of the activity of plasmin using an amydolitic assay. It was observed a doses dependent inhibition of population cell growth and of the plasminogen activator activity on the conditioned medium, together with doses dependent induction of apoptosis. Nicotinamide induces a cytotoxic effect on MDA-MB-231 breast cancer cells and inhibits the secretion of PLAU indicating the reduction of invasive activity of these cells, probably through the reduction of ROS. Supported by PAPIT, DGAPA, UNAM, grant IN223014.

Increased autophagic flux in A549 lung epithelial cells after tunicamycin treatment.

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Autophagy and senescence are two distinct cellular responses to stress. Cellular senescence is a stable form of cell cycle arrest that limits the proliferation of damaged cells. On the other hand, autophagy is an intracellular degradative system that cells use to deliver cytoplasmic substrates to lysosomes for degradation. Genetic inhibition of autophagy induces degenerative changes in mammalian tissues that resemble those associated with aging, and normal and pathological aging are often associated with a reduced autophagic potential. Recently, alterations in both pathways has been related to idiopathic pulmonary fibrosis. Idiopathic pulmonary fibrosis is a chronic fibrotic lung disease of unknown cause that occurs in adults and has a poor prognosis. A growing body of evidence indicates that aberrant activation of alveolar epithelial cells and fibroblasts in an aging lung plays a critical role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). In order to explore how senescence influences autophagic flux and lung fibrosis, we treated lung epithelial cells A549 with the endoplasmic reticulum stressor tunicamycin and we have measured the level of endoplasmic reticulum (ER) stress and autophagy markers. Tunicamycin significantly increased the percentage of senescent A549 cells as measured by an increase in staining for β-galactosidase, along with a decrease in expression of PCNA protein. Paradoxically, the level of p21 and XBP1 decrease after tunicamycin treatment. We also measured the conversion of LC3 from LC3-I (free form) to LC3-II (lipidated form) in A549 cells by immunoblot and found that LC3B-II levels were remarkably increased at 24 hours post-tunicamycin treatment compared to vehicle control cells. However, p62 level did not increase after tunicamycin treatment in those cells. By immunofluorescence, we observed very few autophagosomes in A549 cells in basal conditions or after vehicle treatment. However, after 24h of tunicamycin treatment, the number of LC3-positive puncta per cell significantly increased. These data indicates that the ER stress induces senescence and the autophagic activity in A549 cells. We next examined the behavior of lung fibroblast stimulated with medium from tunicamycin-treated and control A549 cells, and we found that LC3B-II levels were significantly reduced after the stimulation with the medium form tunicamycin-treated A549 cells, compared to untreated fibroblast. These data indicates that damaged or stressed lung epithelial cells could regulate the autophagic activity in lung fibroblasts.

Omega-3 Fatty acids on Diabetic Pregnant Rats: Effects on Placental Mitochondrial Function and Fatty Acids Composition.

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During the pregnancy, the mother adapts her metabolism for supporting the continuous draining of substrates by the fetus. Nutritional status of the mother during gestation is related to the fetal growth. The maternal diabetes is characterized by an increased placental transport of glucose and other nutrients from the mother to the fetus, resulting in macrosomia, which is strongly associated with fetal death, prematurity, birth trauma, and respiratory distress syndrome. It has been shown that polyunsaturated fatty acids (PUFA) are essential for normal growth and development of the fetus, but an excess of PUFA in dietary may enhance lipoperoxidation and reduce antioxidant capacity. Omega-3 fatty acids are PUFA and currently they are highly recommended for pregnant women but also for controlling diabetes. In this work we are interested in studying the effect of dietary omega-3 fatty acids to diabetic female rats previous to pregnancy and during gestation, not only on physiological parameters, but also on placental mitochondrial function and fatty acids composition.

Type 2 diabetes was developed on 48 hours-old female wistar rats by intraperitoneal injection of streptozotocin (STZ) in citrate buffer, and control group (C) injected with citrate buffer only. After weaning, from STZ groups one was supplemented with flax seed oil (125 mg/kg body weight daily) (STZ-omega 3) and the other one not (STZ). The same criterion was applied for control groups (C-omega 3 and C). Fatty acids supplement was analyzed by gas chromatography. Periodical measurements of blood glucose concentration, cholesterol, triglycerides, and glucose tolerance curve, were taken as the indicative parameters of the metabolic condition. Rats were kept at least five months to let the diabetes to progress and then mated with normal males. On day 19 of pregnancy, the animals from each group were killed. Placental and liver mitochondrial activity was measured with a Clark type electrode and fatty acids composition was determined by gas chromatography.

Changes were found in the number and size of fetuses. Diabetic rats had less fetuses and bigger, and heavier placentas. Omega-3 supplement had partial beneficial effect increasing number of fetuses but never the same as control group. There were differences in mitochondrial activity between groups and several small changes in fatty acid composition, but the unsaturated to saturated (U/S) fatty acids index shows that the potential membrane fluidity of mitochondria could be lowered in the six-months-old diabetic rats, which is different to young diabetic rats. In conclusion, there were not significant beneficial effects of omega-3 fatty acids on diabetic rats. Thus, since benefit to risks of modifying maternal fat intake in pregnancy are not yet completely understood, additional studies are needed before recommending omega-3 intake in pregnancy. CONACYT postdoctoral Fellowship to MCFG; PAPIIT IN216314-3.

Truncated receptor type II TGF-β (TβRIIΔcyt) decreases hepatic fibrosis and steatosis modulating gene expression of TGF-β, Col-1, PAI-1 and Cannabinoids receptors.

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Introduction: Transforming growth factor β (TGF-β) is the main profibrogenic cytokine; consequently strategies to block its signal pathway have been developed. Gene therapy using a truncated type II receptor for TGF-β (TβRII□cyt) had showed to reduce hepatic fibrosis; but its effect on the expression of cannabinoid receptors (CB1 and CB2) which participate in the progression of liver cirrhosis and steatosis has not been elucidated.

Objective: Evaluate Ad-TβRIIΔcyt treatment on fibrosis, steatosis and gene expression of cannabinoid receptors and profibrogenic molecules in the liver.

Material and Methods: Cirrhosis was induced administrating CCl_4 for 8 weeks. Cirrhotic rats were divided into groups (n=6), control, therapeutic gene (Ad-TβRIIΔcyt) and irrelevant gene (Ad-GFP). At 4th week of CCl_4 intoxication, $2x10^{10}$ pi/kg of Ad-TβRIIΔcyt or Ad-GFP was administrated via iliac vein. Animals were sacrificed at day 2, 3 and 28 after treatment. Fibrosis index, steatosis, hydroxiproline content, gene expression of Col-1, TGF-β, PAI-1, CB1 and CB2 was analyzed, as well as, ALT and AST serum levels.

Results: Treatment with Ad-TβRIIΔcyt decreased at day 28; 35% of fibrotic tissue and 42% of liver steatosis ($p<0.001$); also suppressed the expression of profibrogenic molecules: TGF-β (11-fold), Col-1A(I) (5-fold) and PAI-1 (19-fold at day 3) compared to controls ($p<0.05$). At day 2, mRNA levels of CB1 (profibrogenic) decreased, just as, CB2 mRNA increased (anti-fibrogenic); although, CBs changes are not statistically significant. Hydroxiproline content decreased 3 fold ($p<0.01$); AST 22% and ALT 37% ($p<0.05$) at day 28, in the Ad-TβRIIΔcyt group compared to control group.

Conclusion: Expression of TGF-□truncated receptor II prevents fibrosis progression and improves liver function by lowering gene expression of profibrogenic molecules (TGF-β, Col-1, PAI and CB1). Nevertheless, cannabinoids receptor expression is not significantly affected; a reduction in steatosis is also observed when Ad-TβRII□cyt therapy was employed in this experimental model of cirrhosis.

Antioxidant effect of Stevia rebaudiana Bertoni in heart of rats feed with a hyper-glycemic/hyper-calorie diet

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Metabolic syndrome (SM) is a multifactorial disorder that involves alimentation and genetic factors, which is characterized by increases in glycaemia, hyperinsulinemia, dyslipidemias, and has been related with the development of diabetes mellitus type 2. Stevia rebaudiana Bertoni (SrB) is used in the treatment of SM, because this plant has shown hypoglycemic and antioxidant properties, and also regulates the metabolism of carbohydrate and lipids.

The main objective of this work was investigated if the extract of SrB has antioxidant and metabolic beneficial effects in rats in a model of SM induced by a hypercaloric diet. Rats were fed by 30 and 60 days with a normal-calorie diet (n=10), a hyper-glycemic/hyper-calorie diet (n=10), and two groups of rats with the same diets, but administered with an extract of SrB (i.p. 20U).

In each animal were determined glucose tolerance, insulin response, and the plasma concentration of triglycerides and HDL-C. In the heart of each animal were determined the levels of glycogen and triglycerides, as well as, the redox status by the determination of nitrites, malondialdehyde, glutathione, ROS, and the enzymes catalase and superoxide dismutase. In addition, heart tissue was evaluated with staining of hematoxylin/eosin, PAS and Masson's trichrome.

Results obtained showed that rats administered with SrB extract decrease the glucose in plasma, but had an increase in the levels of insulin, animals also shown an increase of triglycerides and a decrease in HDL-C. In the heart of the rats treated with SrB was observed a decrease of triglycerides, but anincrease of glycogen levels, the extract had an antioxidant effect, which was reflected by the decrease of nitrites, ROS, malondialdehyde, glutathione, and the activity of CAT. Histological studies indicate heart structural changes and an increase of collagen deposition.

Taken together, these results show that SrB has hypoglycemic effects, probably due to the hyperinsulinemia status present in the rats, which conditions dyslipidemia in the animals. In heart SrB shown antioxidant beneficial properties, although does not protects the histologic integrity and the metabolic changes of triglycerides and glycogen.

Role of R-Spondin2 in the pathogenesis of idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and lethal lung disorder of unknown etiology. IPF is characterized fibroblast proliferation and excessive extracellular matrix accumulation with the subsequent destruction of the lung parenchyma. The R-spondins family (RSPO) represents a recently described group of secretory proteins with essential activities in development. From this family, RSPO2 is expressed primarily in the lungs and mutations of this protein cause pulmonary hypoplasia and severe defects in the respiratory tract. Three proteins of the G protein coupled family (LGR4, LGR5 and LGR6) have been identified as possible receptors. Interestingly, R-spondins, including RSPO2, enhance the canonical Wnt signaling pathway resulting in activation of β-catenin. Wnt has been shown to interact with the TGFβ pathway, a critical route in the pathogenesis of IPF. To date, there are no studies examining a possible role of RSPO2 on fibrotic lung diseases.

In this study we first examined by qPCR the expression of RSPO2 and its receptor LGR6 in IPF and normal lungs. Our results showed that both RSPO2 ($2^{-\Delta CT}$ 1037.8^{-5} $\pm 1539.1^{-5}$ vs 7.8071^{-5} $\pm 4.56715^{-5}$) and LGR6 ($2^{-\Delta CT}$ 1.2^{-2} $\pm$ 1.6^{-2} vs 1.2^{-4} $\pm$ 9.9^{-6}) are up-regulated in IPF compared with normal lungs. These observations were confirmed at the protein level by immunohistochemistry where we found that both molecules are localized primarily in fibroblast and epithelial cells. RSPO2 gene expression was also measured in fibroblasts derived from IPF and normal lungs. We found an increased expression in cells from IPF ($2^{-\Delta CT}$ 2.59^{-5} $\pm$ 3.6^{-6} vs 1.39^{-5} $\pm$ 1.35^{-6}). Then, we explore some functional effects or RSPO2 on human lung fibroblasts and alveolar epithelial cells. Treatment of fibroblasts with RSPO2 induced cell proliferation as measured by WST-1. In epithelial cells, we found that RSPO2, Wnt3a or Wnt3a/RSPO2 do not affect basal apoptosis. We also examined the ability of Wnt3a and RSPO2 to protect to cell death induced with TNFα/IFNγ. The combination of TNFα/IFNγ increased apoptosis to 27.95±8.41%. This effect was significantly reduced by treatment with Wnt3a which decreased apoptosis to near basal levels (16.20%±0.71% $p<0.05$). RSPO2 alone showed a not significant trend while the combination of Wnt3a/RSPO2 strongly attenuates the apoptotic effect of TNFα/IFNγ. Our findings demonstrate by the first time that RSPO2 and the receptor LGR6 are up-regulated in IPF although their role in the pathogenesis of this disease remains uncertain.

Transforming Growth Factor beta induces downregulation of DNA methyltransferase 1 and upregulation of ten-eleven-translocation 3 in human lung fibroblasts.

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Idiopathic pulmonary fibrosis (IPF) is a complex disease of unknown etiology influenced by both genetic and environmental factors. Environmental factors affect gene expression without changes in the DNA sequence, which is the field of epigenetics. Recent studies have shown that several epigenetic mechanisms influence the initiation and progression of IPF including DNA methylation, histone modifications and micro RNA dysregulation.

Transforming Growth Factor beta (TGF-β) plays a major role in the physiopathology of IPF inducing fibroblast to myofibroblasts differentiation and the secretion of extracellular matrix molecules. However, its possible role over the methylation machinery has not been explored.

In this study we explored the effect of TGF-b on the expression of the three DNA methyltransferases (DNMT1, 2, and 3). Human lung fibroblasts were treated with TGF-b for 24 and 120 hours and Western Blot analysis showed a significant decrease in DNMT1 protein at both periods.

In a parallel experiment, microarray analysis of human lung fibroblasts stimulated with TGF-b for 24 hours showed that 528 genes were up-regulated while at 120 hours 467 genes were increased. From these genes, 325 were shared at both periods. On the other hand, 656 genes were downregulated at 24h while at 120 hours 692 genes were decreased. From these genes 446 were shared in the two time periods. These results show that there are a number of differentially expressed genes depending on the duration of the TGF-b treatment.

Interestingly, one of the increased genes in both conditions (24 and 120 hours), is ten-eleven-translocation 3 (TET3), a gene belonging to a recently discovered family protein proposed as part of a multistep DNA demethylation process. This result was confirmed by real time PCR.

Our findings indicate that TGF-b may modify the DNA methylation machinery, decreasing the expression of DNMT1 and increasing the expression of TET3. We are now exploring if this epigenetic effects of TGF beta influence the expression of profibrotic genes.

Effects ofavocado oilon lipid profile, kidney mitochondrial functionand proteinuriaintype 2diabetes

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Diabetes is characterizedbyimpaired metabolismofcarbohydratesandlipidsleadingto the developmentofa variety of complications. Besides, there is increasingly evidencethat oxidativestressis involved inthe pathogenesisof diabetic nephropathy, which is the most devastatingandexpensive complicationin diabetic patientsworldwide.Diabetesis also associatedwith alterationsin mitochondrialfunction thatresult in increased formationof reactiveoxygen species (ROS) due to defective function of complex III, which in turn activates signaling pathways leading to kidney failure. We have previously demonstrated that avocado oil supplementation prevents the impairment in complex III activity induced by type I diabetesanddecreases the formationof ROS.However, the effect of avocado oil on renal function, dyslipidemia and mitochondrial function in type II diabetesis unclear.Thus,theobjective of this studywas to determinethe effect ofavocado oilon lipid profile, proteinuriaand mitochondrialfunctionfrom kidneys of Goto-Kakizaki (GK) rats, a rodent model ofType 2diabetes. GK rats exhibited an increment of 70% in the levels of serum LDL that was prevented by the treatment during 6 months with avocado oil. At 3 months,a 55% increase inproteinuriawas observed in GK ratsand this effect was prevented by avocado oil administration;however,at 6 months thiswas similar in GK rats independently of avocado oil supplementation. Regarding to mitochondrial function, mitochondria from GK rats showed a ~50% decreaseinrespiratory control ratio (RCR) at 3 and6months. In both casesthe treatmentwithavocado oilincreased in 16%and 182.2% the RCRvaluescomparedto the GK rats. Mitochondrial membrane potentialdecreased 70%in the GKafter at 3 months and this effect was not preventedby the treatment withavocado oil,whileat 6 months,no differenceswere observed inthis parameter betweenthe different groups. Together, these results suggest that avocado oil improves dyslipidemia intype II diabetes and delays theappearance ofproteinuriain association with improved kidney mitochondrial function.

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Generation of anaptasensorbased on gold nanoparticlesfor detection of HPV-16 L1 VLPs.

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Aptamers are in vitro selected single-stranded nucleic acid molecules that bind a broad range of targets with high affinity and specificity. These properties make aptamers the ideal candidates for molecular recognition on biosensors.Biosensorsare capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which physically interacts with a transducer element(i.e. gold nanoparticles (GNPs)). GNPs are less than 100nm andshow a red color when dispersed in solution, but exhibit a transition to a blue color when in aggregated by high salt concentration. GNPs also exhibit the ability to interact with nucleic acids by electrostatic forces.
Cervical cancer is associated to persistent infection with high risk human papillomavirus (HPV) especially types 16 and 18.Highly sensitive methods are needed to diagnose the persistent infection. Smaller and cheaper devices are highly sought for replacing expensive andtime-consuming laboratory analyses, thus improvingearly detection and adequate treatment selection for cervical cancer. The HPV capsid is mainly composed of the viral L1 protein that can self-assemble into virus-like particles (VLPs)that are structurally and immunologically similar to the infectious virions.In our laboratory we selected an RNA aptamerthatspecifically recognizes HPV16 L1 VLPsproduced in baculovirus.
Our main objective is to investigate whether this aptamer can be used in combination with GNPs to design and construct an aptasensorto detect VLPs. GNPs were treated with aptamerRNA toprevent their aggregation in the presence of salt. A switch in color from red to purple triggered bydesorption of the aptamer from the GNPs was expected as a result of the aptamer–VLPs interaction.
An aqueous solution ofmonodisperse quasi-spherical GNPs was obtained by a modified Turkevitchmethod. During preparation,the expected development of a dark cherry red color was observed suggesting a GNPs size of approximately 20 nm later confirmed by UV–visiblespectrophotometry and transmission electron microscopy. Initial aggregation assays withincreasingNaCl concentrations established the minimal concentration necessary to aggregate the GNPs (0.5 mM). Additional aggregation assays optimizing the aptamer concentration of aptamer required toprevent the aggregation of GNPs were also performed showing thatan aptamer concentration of 9 nMis enough to prevent GNPs aggregation at high salt concentrations. Although still preliminary, the presentdata suggest that our GNP-aptamer aptasensor has the potential to becomea diagnostic tool for HPV infection and cervical cancer.

Cecropine, an antibacterian peptide and its effects over heart function

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Introduction – Cecropin (Ccrp) is an antimicrobial peptide produced by insects in response to microbial infections. This peptide is able to affect bacterial membrane permeability, and diminishes hemolytic activity and degranulation in mast cell. We have recently observed that Ccrp has harmful effects on isolated cardiac mitochondria and also a pro-oxidative activity. It has also showed cytotoxic activity against cancer cells. This last characteristic is under intensive investigation; therefore it is fundamental determine if the Ccrp is not producing cardio toxicity. **Purpose** – In an effort to understand if this antibacterial peptide could influence heart function, Ccrp was administrated in isolated rat-heart (Langendorff) and *in vivo* to evaluate cardiac frequency and systolic function of left ventricle.**Methods** –Toxicity of different concentrations of Ccrp (i.e. 50, 100, 200 µg/ml) in breast cancer cells ZR7530 was evaluated by MTT at 48 hours. In isolated hearths two doses of Ccrp of400 and 1600 ng were tested. For Langendorff model, rats were euthanized with pentobarbital (60 mg/kg weight i.p.), heart was excised and by the ascending aorta connected to a system of retrograde perfusion, with Krebs solution through all the experimental time. After an adaptation period (30 min), the Ccrp was administrated. The Ccrp was recirculated by one hour, and then hearts were washed with fresh solution for 30 min. Records were collected at 10 and 30 min. The mechanical performance and the vascular resistance were also obtained.The echocardiographic evaluation was performed in live animals by 30 minutes, followed by the cardiac frequency and systolic function of left ventricle. In both models, cardiac tissue samples were collected; the content of reactive oxygen species (ROS), superoxide dismutase activity (SOD) and pro-inflammatory cytokines were measured. In the perfusion solution, the levels of nitrites were also evaluated.**Results** – A significant decrease in toxicity of ZR7530 was observed with 200 µl/ml of Ccrp. Besides Ccrpat 400 ng produced a minor diminution in the mechanical performance in heart, but there was a significant release of ROS and pro-inflammatory cytokines. A significant reduction in nitrites level was observed in 1600 ng dose.In the complete animal, the Ccrp did not produce changes in the echocardiography, in the systolic, diastolic diameters nor systolic function. A non-significant diminution in cardiac frequency was found for 1600 ng dose.**Conclusion** –The Ccrp concentrations used in this work did not shown evidence of neither mechanical alterations nor biochemical in heart; however, they had a toxic effect on cancer cells. Further studies are required to understand the function of these molecules as anti-cancer drug that could not have deleterious effect on the heart.

Relevance of Notchcell signaling pathway in cellular subpopulation with ALDH high activity enriched in cancer stem cells derived from cervical cell lines

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Cancer stem cells (CSC) are proposed as the source of heterogeneity, initiation and maintenance of a tumor (1, 2). CSCs are a small proportion within the tumor with stemness properties such as a) self-renewal, which is defined as the ability to form new stem cells with the same potential of proliferation, expansion and differentiation; b) differentiation, which is the ability to result in a heterogeneous cell progeny diversify and specialized according to hierarchical process (2).

Maintaining both stem cells and cancer stem cells as an important process mediated by different pathways such as Notch. CSCs are able to isolated due to their surface markers (phenotype) and also by the determination of aldehyde dehydrogenase (ALDH) activity, which it has been used to isolated CSC from several tumors (3, 4, 5, 6 and 7).

Since, Notch cell signaling pathways is crucial for self-renewal, the aim of this study is to evaluate the activation of proteins involved in the Notch pathway in enriched stem cell population isolated by their ALDH activity from different cervical cancer cell lines. As results, we enriched the CSC populations by the sphere formation culture assay. Using this culture, we evaluated the presence of key proteinsof Notch cell pathway and we found a direct correlation between the expression of Notch-1 protein and activation of target genes such as Hes-1 and c-myc.

In conclusionNotch-1 has an important role in the maintenance of cancer stem cells compared to other Notch ligands.

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S-allylcysteine in diabeticnephropathy in rat

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Thekidneyis a regulatorybodytoremovethebodytheendproducts of themetabolism of cells, regulatebloodpressure and retainsubstancesessentialforthesurvival of individuals. Acute renal failure (ARF) is a conditioncharacterizedbyanabrupt decline in renal function, consequentlyresulting in theaccumulation of nitrogenwasteproducts in theblood, such as urea nitrogen and creatinine.

Diabetes mellitus (DM) is a group of conditionscharacterizedbyvaryingdegrees of insulinresistance (IR), reducedsecretion of the hormone and increasedamount of glucose in blood. Severalflaws in theaction, orbothfunctionssecretion of insulinoriginatehyperglycemia in DM. Diabeticnephropathyisone of themostfrequentcomplications of DM, and IR isusuallyattributedtometabolicconsequences of abnormalglucoseregulation.

Amongthemodelsto induce DM in rodents are foundusingchemicalagentssuch as streptozotocin (STZ). Thiscompoundactsspecificallyondiabetogenicdosepancreatic β cells. A highfatdiet causes obesity in rats and IR.

It has beenshownthatagedgarlicextract (AGE) improvescholesterolprofile and decreasestriglycerides. The S-allyl-cysteine (SAC) isone of thesulfurcompounds in garlic, has antioxidantactivity, reno protectiveeffect, amongothers.

In thisstudytheantioxidantcapacity of the SAC wasinvestigatedtodecreasethe renal damagecausedby DM, establishing a modelwithpancreaticdamageby STZ in maleWistarratswithhigh-caloriediet (high in fat and carbohydrates) and theability of SAC to be evaluated reduce damage in thismodel.

A model of diabetes associatedwithhighcaloriediet, in whichpolyuria, polyphagia, polydipsia, hyperglycemia, hypercholesterolemia, hyperlipidemia, and renal injury (increasedcreatinine and decreasedcreatinineclearance) wasobservedwasobtained.

The SAC helpsto reduce levels of glucose, cholesterol and triglycerides; avoidingchangingtheactivity of antioxidantenzymes and partiallypreventedthefunctional and structural renal damage.

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Evaluation of the cardioprotective effect of Citicolione and Erythropoietin against Doxorrubicin-induced cell death.

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Doxorubicin is an anthracycline that is used for the treatment of various neoplasias. Unfortunately, the use of Doxorubicin for the treatment of cervical cancer has been hampered by the strong cardiotoxicity showed in patients. In a previous work we showed that Citicoline is able to protect cardiac myocytes from hypoxia-induced cell death. Besides, it is known that Erythropoietin is also a potential cardioprotective agent.

The aim of the present study is to evaluate whether Citicoline and Erythropietin are able to protect cardiac myocytes from death induced by concentrations of Doxorubicin that are toxic for cervical cancer cells.

Two cell lines were used, HeLa which is a cervical cáncer-derived cell line, and H9C2 a newborncardiocytes-derived cell line. The Lethal Dose, 50% (LD_{50}) of Doxorubicin was first determined in 30,000 HeLa cells cultured in 96-well plates. After 2 hr incubation increasing doses of Doxorubicin (1-10 µg/ml) were added. Cell viability was measured by the MTT assay. The calculated LD_{50} for Doxorubicin was 5.4 µg/ml at 24 hr. Next, we evaluated the effect of the calculated LD_{50} on the viability of H9C2 cells at 24, 48, 72, and 96hr. The results showed that incubation of H9C2 cells for 24 hr with Doxorubicin induces the death of almost 50% of cardiac cells. The potential effect of Citicoline and Erythropoietin was evaluated by pre-incubating the cells with increasing doses of Citicoline (1-75 mg/ml) or Erythropoietin (0-125 U/ml), for 2 hr. The calculated LD_{50} was then added, and the cell viability was evaluated by the MTT assay. The results showed a significant protective effect of Citicoline against the Doxorubicin-induced death of H9C2 cells. In contrast, Erythropoietin was not able to inhibit Doxorubicin-induced death. Our data suggest that Citicoline might be a supporting, cardioprotective agent for cervical cancer patients receiving a treatment with Doxorubicin.

Characterization of an intrauterine microenvironment conditioned by choriodeciduals cells around the on set of human labor.

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BACKGROUND

During human labor choriodecidua interface is enriched by chemokines, pro-inflammatory cytokines, prostaglandins and matrix metalloproteinases. The rise of these mediators has been associated with some events of human labor such as myometrium contractions, cervix ripening and the rupture of fetal membranes. Cellular sources of these mediators have not been clearly identified.

OBJECTIVE

Analyze the *in vitro* secretion of mediators associated with the induction of labor secreted by choriodecidual leukocytes from term pregnancies.

METHODS

A non-enzymatic method was developed to obtain leukocytes from the choriodecidual interface of term fetal membranes. Subpopulations of choriodecidual leukocytes were characterized by flow cytometry. Isolated choriodecidual leukocytes were cultured for 24, 48 and 72 hours. Cell supernatants were collected and analyzed by gelatin zymography to asess gelatinolytic activity of MMP-9 and 20 cytokines/chemokines related to inflammatory events. These were evaluated by the Multiplex assay.

RESULTS

T lymphocytes, monocytes and Natural Killer cells represent the mayor subpopulations of choriodecidual leukocytes. Choriodecidual leukocytes secrete IL-2, INFγ, IL-6, TNFα, IL-1β, IL-8, IL-10, IP-10, MCP-1, MIP-1α y MIP-1β. Choriodecidual leukocytes secreted large amounts of MMP-9 in pro-enzyme form; active forms were observed after 24 hours of culture.

DISCUSSION

Infiltrated leukocytes into intrauterine space are enriched by T lymphocytes at term gestation. Choriodecidual leukocytes have functional characteristics that include secretion and activation of MMP-9, secretion of cytokines and chemokines. These mediators are involved in a complex signaling pathway that induce simultaneous changes in different tissues such as myometrium and fetal membranes. Findings suggest that the specific leukocyte recruitment may play a roll in triggering human term labor.

Screening of the antibacterial, cytotoxicity, antioxidant activities and chemical composition of *Plumbago auriculata* roots.

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Plumbago auriculata belong to the family of *Plumbaginaceae*, The root of *P. indica* has been reported to use as antifungal, antibacterial, antiparasitic and antifertility activities. *P. zeylanica* is a popular medicinal herb used as a remedy for skin diseases, infections and intestinal worms in Asia, in West Africa the root have been used to treat gonorrhea, syphilis, tuberculosis, rheumatic pain, swelling and wounds. It has been reported its strong antioxidant activity and antimicrobial activity (Sulma *et al*., 2013). There are no reports on the chemical composition and biological activity of *Plumbago auriculata* growing in Mexico, so the present work reports the antibacterial, cytotoxicity, antioxidant activities and chemical composition of the roots of Plumbago *auriculata*.

The antimicrobial activities of the ethanolic extract of roots from old and young *P. auriculata* plants were evaluated against Escherichia *coli*, *Salmonella typhimurium*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus.* In the present study, the ethanolic extracts of the old roots exhibited potent brine shrimp lethality LC_{50} 250 µg/ml. The ethanolic extracts of the young roots have shown the lowest cytotoxicity with LC_{50} values >1000µg/ml.

Antioxidant property was measure using DPPH, ABTS and FRAPS assays. The results were express as Trolox equivalent antioxidant capacity (TEAC), the ethanolic extract of the old roots present 974.63 µM Trolox/ml and the ethanolic extract of the young roots present 563.86 µM Trolox/ml. The phytochemical screening identified 5-hydroxy-2--1,4-naphtoquinone, best known as plumbagin, palmitic acid, linoleic acid, stigmast-5-en-3-ol and neoisoshinanolone as the major compounds of the ethanolic extracts obtained from the roots of old and young plants. The results indicated that the ethanolic extracts of *P. auriculata* roots possess significant antibacterial and antioxidant activities and low toxicity, implying that this plant can be used as a potential source of natural antioxidants.

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Looking for new antimalarials. Virtual screening into the glycolytic enzyme phosphoglyceratemutase 1 from *Plasmodium falciparum*.

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Introduction. Malaria, caused by the parasite *Plasmodium falciparum*, has a prevalence of 250 million clinical cases and a lethal rate of 1 million people per year. In view of the parasite has developed resistance to drugs already established as the treatment, it is necessary to develop new drugs that can resolve this problem.In this regard, the parasite is dependent on glycolysis as the sole source of energy, enzymes from this pathway, such as phosphoglyceratemutase(PGAM), which catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate, are considered asan excellent target for the search of new inhibitors that can servein the development of new drugs against malaria.
Methods. Virtual screening was made using the crystal structure of PGAM1 from *P. falciparum* (PfPGAM1, PDB: 1XQ9), and the “Fragment” library of small molecules from Chembridge. The search of potential inhibitors was performed with MOE (www.chemcomp.com) and Glide (www.schrodinger.com) software, having the catalytic site as target.
Results. From the 7677 molecules docked, compounds 1901, 3383, and 1708 were the best matched among MOE and Glide. According to an extra precision docking, the binding energy from these molecules was -3.898, -4.439, and -3.983 Kcal/mol, respectively. Hydrogen bond and cation-π interactions were formed between these molecules and some residues at the catalytic site. Additionally, a predicted drug likeness score of -1.37, -1.45 and 0.84 for 1901, 3833, and 1708 was obtained, respectively; indicating that these molecules could be considered as potential drugs.
Conclusions. The three compounds found trough virtual screening, have the potential to inhibit PGAM1 and could be used as hits to obtain new antimalarial drugs.

Regulation of mitochondrial permeability transition by Sirt3-catalyzed cyclophilin D deacetylation and its relevance for ventricular dysfunction in heart and isolated cardiomyocytes.

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Background. Metabolicsyndrome (MS) can be defined as a group of signs that increases the risk of cardiovascular diseases (CVD). These signs include central obesity, hypertriglyceridemia and hypertension. We are interested in the mechanisms that trigger ventricular dysfunction in a MS murine model, as a way to understand how mitochondrial function fails in CVD. The sustained opening of the mitochondrial permeabilitytransitionpore (PTP) is a major event in the onset of irreversible myocardial injury. Several mitochondrial proteins modulate the PTP, including the cyclophilin D (CyD), the adenine nucleotide translocator (ANT) and the SIRTUINS. In this regard, SIRT-3 has emerged recently as a pivotal mediator of mitochondrial metabolism and PTP inductor in a knockout murine model. However, the precise role of SIRT-·3 in a pathophysiologic context remains indefinable.
Methods. Male Wistar rats with sucrose-induced MS were subjected to cardiac echocardiography and *ex-vivo*contraction measurements at 6 and 12 months. Respiratory activity, calcium retention capacity and deacetylation profile were investigated in isolated mitochondria. The expression of SIRT-3, ANT and CyD was evaluated by qPCRor western blot. In addition, isolated cardiomyocytes were exposed to ethanol for 48 hours to decrease SIRT-3 activity. Inhibition of ethanol metabolism by 4-methylpyrazole (4-MP), an inhibitor of alcohol dehydrogenase, was used to prevent the ethanol-decrease SIRT-3 activity.
Results. We observed differences in the E/A ratio(control 1.07±0.01 vs. MS 0.85±0.06, $p<0.029$) and the ventricular deceleration time(0.029±0.002 vs. 0.034±0.003,$p<0.04$)indicating an abnormallusitropism. No significant differences were found inrespiratory activity and respiratory control from isolated mitochondria. Nevertheless, calcium retention in MS mitochondria was reduced (0.83±0.03 vs 0.65±0.04 $Abs.min^{-1}$$p<0.004$)indicating premature PTP opening.Proneness in PTP opening was associated with lower expression (60% $p<0.001$) of SIRT-3.These changes correlated with the MS mitochondrial acetylation profile.The ethanol-enhanced acetylation of Cyp-D also increases the interaction of Cyp-D with the ANT, increasing the probability of PTP opening.
Conclusions. It appears that metabolic changes inherent to MS promote alterations in the expression of SIRT-3 and correlates with PTP opening sensibility.

Hypothyroidism decreases the immunolocalization of Farnesoid receptor (FXR) inpancreatic isletand liver cells of rabbits

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Thyroid hormone receptors (TRα and TRβ) are present in islet and liver cells. In adulthood,hypothyroidism affects the morphometry of islets and hepatocytes, regulating their proliferation. In both tissues, hypothyroidism increases the lipid droplet accumulation. In another hand, farnesoid X receptors (FXR) are nuclear receptors whose activation have been related to thesecretion of insulin, translocation of GLUT2, triglyceride accumulation, and proliferation in pancreas and liver. The possible regulation of FXR by thyroid hormones in these organs is yet unknown. Therefore the aim of this study was evaluate the effect of hypothyroidism on the immunolocalization of FXR in the islet and liver cells of female rabbits. METHODS. Twelve Chinchilla-breed virgin female adult rabbits were divided in control (n=6) and hypothyroid (n=6, methimazole, 0.02% in drinking water for 30 days). After of treatment, rabbits were sacrificed and their pancreas and liver were extracted and histology processed. Immunohistochemistry anti-FXR was used and the proportion of positive nuclei was quantified per islet or per microscopic field for liver. The islets were arbitrary classified in large, medium, and small. Student test, U-Mann-Whitney tests, ANOVA two way were using for comparing variables between groups. RESULTS. In comparison with controls, hypothyroid tissues showed a significant low percentage of immunoreactive anti-FXR nuclei. All size of isletswere affected. CONCLUSIONS. The present results suggest a possible action of thyroid hormones on the immunolocalization of FXR in islet cells and hepatocytes. This decrease in the presence of FXR could be related to the glucose metabolism, triglycerides accumulation, and the proliferation of these tissues.

Cardiovascular risk factors in childhood influence the number of Colony-Forming Units generated *in vitro* by circulating endothelial progenitor cells in peripheral blood.

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INTRODUCTION: Childhood obesity is associated with early endothelial and vascular dysfunction that might lead to cardiovascular disease. The number of Colony-Forming Units (CFU) generated *in vitro* by circulating endothelial progenitor cells (EPC) in peripheral blood has been proposed as a biomarker of endothelial function and cardiovascular disease risk in adults. There is a strong correlation between these CFU and the risk factors included in Framingham´s score, in healthy adults. The number of CFU decreases in adults with overweight and obesity, and some data indicates that this decrease correlates with high levels of triglycerides and low levels of HDL cholesterol. Recently, it was reported that children with familiar hypercholesterolemia had lower CFU than healthy ones. However, relationship between the amount of CFU as an indicator of endothelial dysfunction and other cardiovascular risk factors at an early age has not been demonstrated so far.
OBJECTIVE: Determine the relationship between the number of CFU generated *in vitro* by circulating EPC in peripheral bloodand cardiovascular risk factors in children.
METHODS: We performed a transversal study with 49 children (32 boys and 17 girls). We evaluated the body mass index (BMI) as well as the concentration of triglycerides, cholesterol and glucose in blood. We obtained information about the breastfeeding history and physical activity. In order to culture and quantify the number of CFU generated *in vitro* by the circulating progenitors cells in peripheral blood, we used the kit EndoCult Liquid Medium (STEMCELL Technologies).
RESULTS: The overweight-obesity, the high levels of cholesterol, triglycerides and glucose, as well as the absence of breastfeeding negatively affect the number of CFU in children. We did not find any relationship between the number of CFU and the amount of physical activity.
CONCLUSION: Cardiovascular disease risk factors such as overweight and obesity, dyslipidemia, hyperglycemia and the absence of breastfeeding are associated with a decrease in the number of CFU generated *in vitro* by circulatingendothelial progenitor cells in peripheral blood in childhood.

Preliminary analysis of pro-inflammatory and metabolic changes in school children with different proportions of body fat.

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Introduction: Obesity is a condition in which excess fatty tissue adversely affects health. The positive correlation of increased adipose tissue with metabolic changes associated with insulin resistance and increased concentrations of inflammatory mediators, suggest a complex of pathophysiological mechanisms leading to comorbidity obesity and early onset. It is unknown whether these changes are successive or simultaneous and/or synergize to produce damage. We have proposed to begin characterizing the sequence of these changes. Objective: To describe the simultaneous changes in the metabolic profile and concentrations of pro-inflammatory mediators in serum of school children classified by different proportions of body fat. Materials and Methods: We studied a pseudo-cohort of school children 6 to 11 years old. Adiposity was measured by body mass index [BMI], index size/waist [ICT] and waist circumference [CC]. Children were classified and analyzed into four groups: 1: normal weight, 2: overweight, 3: obese and 4: extremely obese. Serum samples were processed for quantification of glucose, triacylglycerols (Tg), total cholesterol (CT), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), acylcarnitines and amino acids by the Tandem Mass Spectrometry technique. The 21 pro-inflammatory cytokines panel was measured by the Multiplex technique. For the data analysis, the study population was classified into groups based on the composition of body fat using the aforementioned classifiers. We showed descriptive data in a preliminary analysis of the correlation. The differences were considered statistically significant with p values of <0.05. Results: 170 children of both genders aged 6-11 years were analyzed. The prevalence of pre-diabetes in the normal group was 24.4%, in the overweight group
44.4%, in the obese group 40% and in the extremely obese group 41.7%. The prevalence of high Tg in the normal group was 24.4%, in the overweight group 42.2%, in the obese group 65.7% and in the extremely obese group 50%. The highest prevalence of CT in all four groups was classified as borderline high. No high prevalence of LDL-C was found. The prevalence of low HDL in the normal group was 48.7%, in the overweight group 37.8%, in the obese group 37.1% and in the extremely obese group 16.7%. The amino acid glycine concentration (p=0.003) was higher in the group with normal weight. Valine(p=0.017) was higher in the obese group and arginine(p=0.010), leucine(p=0.031), tyrosine (p=0.008) and valine(p=0.000) were higher in the extremely obese group. With respect to the concentration of acylcarnitines, the medium chains C6(p=0.008), C8 (p=0.038) were lower in the overweight group and conversely the long chains C14(p=0.007), C16 (p=0.018), C18:1(p=0.049), C18: 1
OH (p=0.000) and C18:2(p=0.010) / C14:OH(p=0.000) and C18:1OH(p=0.000) were significantly higher in the obese and extremely obese groups respectively. When the overweight group of school children was compared to the obese group and the extremely obese group, we found that the concentration of acylcarnitines of medium and long chains C6(p=0.021), C8(p=0.031), C14(p=0.043), C1:OH(p=0.044), C18:1(p=0.036) and C18:1OH(p=0.009) were higher in the obese group. However long chain acylcarnitines C10:1(p=0.040) and C14:OH(p=0.024) showed a significant decrease in the extremely obese group. We found no significant differences between the values of the different cytokines in serum between groups except for IL-10(p=0.021) and MIP1α (p=0.014) that were significantly increased in the extremely obese group. Conclusion: We found evidence that with increased body fat composition in school children, there is progressive and significant metabolic changes, including greater amount of circulating glucose, Tg, changes in circulating amino acids and changes in acylcarnitines. Furthermore, we found no differences in circulating levels of pro-inflammatory cytokines, which could mean that this mechanism of injury appears later as a mediator for damage associated with childhood obesity.

The physiological role of the T-type Calcium Channels in cell lines bone cancer (Osteosarcoma).

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Osteosarcoma is the most common primary malignant bone tumor and is most frecuently in adolescents and children, in Mexico in 2010 an incidence of 300 patients with osteosarcoma was reported. This cancer is highly metastatic and would cause death of the patient in just a few months. To date there are not specific markers or drugs to this cancer, therefore, it is imperative to find a molecule highly related to any of the characteristics present in this type of cancer. It has been demonstrated that the T-type calcium channels are relating to various types of cancer, to name a few have prostate, colon, liver, breast cancer, glioblastoma, etc. T-type calcium channels belong to a family of transmembrane proteins which mediate calcium influx in response to membrane depolarization and regulate intracellular processes. In order to described the physiological role of the T-type Calcium Channels in cell lines involved in bone cancer (Saos-2 and SJSA-1) and in the cell line control (hFOB1.19). RESULTS. We describe the expression pattern of the $Ca_V3.3$ in cells lines through assays of confocal microscopy. Furthermore, we examined the ability to record the T-type currents in the cell lines involved in bone cancer.

Improvement of transduction with Ad-GFP vector mediated by siRNA- IFN-α in hepatic cells

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Introduction: Adenoviruses (Ad) are the most common vectors used in clinical trials for gene therapy. Ad have shown to have high tropism for liver being the ideal vector to delivery therapeutic genes to this organ. Interferon type 1 (α and β) play an important role in the elimination of adenovirus by the immune response of the organism. **Aim.** To evaluate the effect of IFNα inhibition by a small interfering RNA (siRNA) on rAd-GFP transduction and transgene expression in Huh7 hepatic cells. **Methods.** Huh7 cells were cultured in DMEM, 5% FBS at 37 °C and 5% CO_2, transfected with 70 nM of IFNα or irrelevant siRNA, incubated for six hours and then exposed to 1 x 10^9 vp/ml of rAd-GFP for 24 hrs. Expression of IFNα1 and TNF-α were determined by qRT-PCR. Cell transduction was analyzed by flow cytometry (FC) and qPCR and GFP protein by western blot. **Results.** 70 nM of IFNα1-siRNA inhibited 96% of IFNα1 gene expression ($p < 0.001$) and 65% of TNF-α ($p < 0.05$) compared to irrelevant-siRNA. Ad-GFP transduction measured by FC and q-PCR increased 39.2% and 27% respectively in IFNα1-siRNA treatment compared to control. GFP protein increased 50% when IFNα1-siRNA was used compared to control. **Conclusion.** Inhibition of IFNα mRNA with IFNα1-siRNA permits a higher transgene expression (GFP) indicating the crucial role of IFNα on adenovirus elimination in transduced cells. This strategy could be useful in clinical trials conducted for liver diseases, where adenovirus are used as vector for therapeutic genes; allowing an increased transgene expression leading to better results in the resolution liver diseases.

Intramuscular gene therapy using MMP8 gene modify profibrogenic gene expression in experimental liver fibrosis

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Background and aims. Liver cirrhosis is a potentially life-threatening disease caused by progressive displacement of functionalhepatocytes by fibrous tissue.Multiple protocols regarding liver fibrosis gene therapy using adenoviral vectors systemically delivered, have been reported. Unfortunately, a decrease in the efficiency of liver transduction and increase in proinflammatory cytokines have been exhibited when utilizing these delivering strategies. MMP-8 degrades preferentially collagen type I (collagen accounts for 60%–70% of the total collagen in fibroticlivers). We delivery MMP-8 gene in to the muscle, using an Adenovirus vector, protein is released systemically and is activated in the liver. Our aims was evaluate profibrogenic gene expression pattern and liver fibrosis prevention.

METHODS. Experimental liver fibrosis was induced in male Wistar rats by TTA administration for 7 weeks.Four groups were included: control (no fibrosis), TAA induced-cirrhosis (TAA), TAA+AdGFP (irrelevant gene) and TAA+AdMMP8 (therapeutic gene). At the beginning of the fifth week of TAA intoxication, administration of vectors in soleum muscle was accomplished. Sub-groups of rats (n=5) at the end of first, second and third week after vector administration were sacrificed. Percentage of fibrosis, liver function, gene expression of MMP8, proinflammatory genes (IL1-beta, TNF-alpha), profibrogenic genes (collagen $\alpha 1(I)$,CTGF and TGF-beta) and antifibrogenic genes (MMP1 and MMP9), were determined.

RESULTS. After 3 weeks of treatment: In the liver and serum, amount of MMP8 protein was sustained, fibrosis decreased up to 48%, proinflammatory genes expression was modified only at the end of the third week, profibrogenic gene expression decreased (Col $\alpha 1(I)$ 4 times, TGF-beta 3 times and CTGF 2 times), antifibrogenic genes expression increased (MMP9 2.8 times and MMP1 10 times). According to Knodell score, a clearly diminution of inflammatory cells infiltration in comparison with counterpart animals treated with AdGFP, could be appreciated.

CONCLUSIONS.A single dose of AdMMP8 in muscle is enough in order to obtain a stable liver MMP8 protein expression and activity during 21 days. Degradation of collagen in the liver modifies pro and anti-fibrogenic gene expression allowing a restoration of hepatic architecture.AdMMP8 delivered in muscle did not generate liver inflammation in fibrotic animals, MMP8 gene was expressed in muscle with satisfactory functions in the liver.

Antidiabetic drug design. Computational approaches for optimization of a new Protein Tyrosine Phosphatase 1B inhibitor

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Introduction. Diabetes affects over 371 million people worldwide and is expected to reach 552 million in 2030. The protein tyrosine phosphatase 1B (PTP1B), a negative regulator of the signaling cascade of insulin, is an excellent target for the search for a new therapy. Various compounds have been designed to inhibit the function of the PTP1B; however, despite the encouraging progress to date, it has not been possible to generate inhibitors of PTP1B with the appropriate combination of affinity, specificity and bioavailability. This work proposes the optimization, through a computational strategy, of thebenzimidazole carboxamide called PTSB6, which inhibits PTP1B with an I_{50} of 16 µM.

Methods. PTP1B (PDB: 2F71) as well as PTSB6 were prepared using Autodock Tools 1.5.6 software. Docking studies were made with AutoDock vina, Moe and Glide software. The best protein-ligand complex from each program was selected according to the highest binding energy reported. These results revealed the main scaffold and the positions susceptible of modifications in the inhibitor structure. Different types of substituents were proposed and the new molecules were summited to the same docking studies. Additionally, some molecular and pharmacological parameters were studied in the new molecules proposed and in the inhibitor PTSB6. Toxicity were obtained using ElectroShape Polypharmacology server, and for molecular parameters determination Qikpro, MedChem, Molsoft and FAFDrugs software were used. Finally, in the search of specificity, compounds were docked into T-Cell Protein Tyrosine Phosphatase(TCPTP), the closest human homologous of PTP1B.

Results. Through astructure-based drug designanalysis on the interaction between PTP1Band its inhibitor PTSB6, a series of 42 new molecules were proposed. Chemical substituents included sulfonic acids, sulfonamides, and carboxylic acids. Taking together all the data obtained, 12 new molecules including selectivity, potency and specificity with respect to PTP1B, were selected as potentialmore powerful inhibitors of the enzyme.

Conclusion. The discovery of selective PTP1B inhibitors with acceptable pharmacological properties has proven extremely difficult. In this work, 12 new potential PTP1B inhibitors were proposed taking into account selectivity, specificity and bioavailability. These compounds could serve in the search of a new antidiabetic drug.

Sorcin interacts with the mitochondrial calcium uniporter and inhibits calcium transport in cardiac mitochondria

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Sorcin is a cytosolic protein with EF-hand type, exerts negative inotropic effects in the heart via inactivation of L type calcium channels and ryanodine receptors (RyR2). Although sorcin modulates intracelular calcium handling and sorcin also localize in mitochondria, its functions on mitochondria are largely unknown. Here, we explore the potential interaction of sorcin with the recently cloned mitochondrial calcium uniporter (MCU).Using SDS-PAGE and western blot techniques, the incubation ofrecombinant sorcinwith rat heart mitoplasts resulted inits translocation to membranes in a calcium-dependent manner. The immunoprecipitation of sorcin, followed by SDS-PAGE and western blot analysis, revealed that the MCU is co-precipitated indicating a calcium-dependent interaction between these two entities. In adult cardiac myocytessorcintranslocates from cytosolic to membranes and associates with MCU, as shown by immunostaining experiments.In addition, using protein sequence analysis, we found three regions in the N terminus of MCU that are significantly similar to those segments in L type channel and RYR2 which interact with sorcin during calcium transients. To establish whether these regionsare instrumental in sorcin-MCU binding, three N-peptides of different length were syntezised. The interaction of the sorcin and MCU peptides was studied.In isolated rat heart mitochondria, sorcin inhibited the calcium uptake in nanomolar range, modulating respiratory activity, membrane potential, permeability transition and ATPsynthesis. In conclusion, a direct interaction of sorcin with MCU can modulate the calcium dynamic in mitochondria. This encourages for more investigation as sorcin could emerge as a novel regulator of MCU.

Differences in the expression O-glycosylated glycoproteins in Cervical Cancer Cell Lines with and without HPV detected by *Amaranthus leucocarpus* Lectin

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Introduction: The modifications in the *O*-glycosylation are a common event in cervical cancer (CaCu). Objective: The aim of this study was identify and purify glycoproteins O-glycosylated detected by *Amaranthus leucocarpus* lectin (ALL) in CaCu cells lines and compared with healthy cervical tissue. Methodology: cytochemical and flow cytometry and immunoprecipitation (IP) was performed with VPH positive cell lines CaLo, Caski, SiHa and VPH negative cell lines ViBo and C33A. Histochemistry, with ALL lectin, was realized in healthy and cervical tissue biopsies. Results: ALL lectin strongly stained the plasma membrane and cytoplasmic granules moderately recognized cytoskeletal structures in cells CaLo, Caski and SiHa, in contrast ViBo and C33A. In cells where ALL recognizes the plasma membrane, the lectin showed a strong interaction with cytoplasmic structures. Cervical epithelial and healthy cells showed mild recognition at the plasma membrane; specifically the intermediate layer and surface of the stratum basal layer showed no staining in the cytoplasm. For flow flow cytometry of cells were positive (%) and showed a mean fluorescence intensity (IMF) respectively: CaLo 94.2% (40,276), Caski 97.4% (66,405), SiHa 97.2% (36,434), ViBo 89.6% (26,536), C33A 99.2% (24,034). The fractions obtained from the IP showed bands like between 50 and 150 kDa in CaLo, Caski and SiHa Cells. ViBo and C33A Cells showed a common band between 25 and 37 kDa. Conclusion: The results showed changes in the location and intensity on expression of O-glycosidically linked glycans in CaCu cells lines that differ in healthy tissue. The glycoproteins purified by lectin ALL differ between HPV positive and negative cells. Our results suggesting the importance of sequence these glycoproteins and identify their participation in tumor progression and use as tumor markers or therapeutic targets.

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Antimalarial drug discovery.Molecular docking in the dimer interface of triosephosphate isomerase from*Plasmodium falciparum*.

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Introduction.*Plasmodium falciparum* causes Malaria, a disease which is responsible of around 1 million deaths per year worldwide, and more than 3 billion people live in areas at risk. Unsuccessful efforts to control and eradicate malaria are,in part, due to an increasing resistance to clinically used drugs. Therefore, there is an urgency to find new drugs for the treatment of this disease. An important metabolic characteristic is that *P. falciparum* depends on glycolysis as the unique ATP source for cellular work. Under this perspective, several groups have pointed the glycolytic enzyme triosephosphate isomerase (PfTIM) as a good target for antimalarial drug discovery.

Methods. A*hit-consensus* was performed through virtual screening using Glide and MOE software. To this end, the crystallographic structure of PfTIM (PDB ID: 3PSW) and the ChemBrigde small molecules database were used.Because the PfTIM is only active in its dimeric form, the dimer interface was selected as the target site for virtual screening. In order to gain more information, a flexible dockingprotocol using InducedFitprogram was applied to the three best common molecules.

Results. After analyzing and ordering by binding energy the output files from Glide and MOE, the top three common molecules were compounds 5403532, 5484635, and 5326476. Flexible docking studies showed a binding energy of -6.38, -3.51 and -4.24 Kcal/mol, respectively.These molecules made hydrogen bonds,cation-π,and π-πinteractions with residues at the dimer interface.Furthermore, a predicted drug likeness score of -0.37, -0.39, and 0.55 for 5403532, 5484635, and 5326476, respectively, supports that they could be considered as potential drugs.

Conclusions. The three compounds found by *hit-consensus* could potentially inhibit PfTIM, and serve as a starting point in the search of a new chemotherapy against Malaria.

Effects of histone deacetylase inhibitors on the development of bleomycin-induced pulmonary fibrosis.

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Lung fibrosis induced with bleomycinis characterized by activation, proliferation and differentiation of fibroblasts, with the subsequent abnormal deposit of extracellular matrix, which leads to the destruction of the pulmonary architecture and ultimately to death. There is sufficient evidence that the histone deacetylases (HDACs) play important roles in different pathological processes. However, the role of HDACs in the development and progression of pulmonary fibrosis remains unclear. We aim to investigate the effect of HDACs inhibitors on collagen deposition in bleomycin-treated mouse lungs and their involvement in fibroblasts proliferation and migration.
We developed an experimental model of pulmonary fibrosis in C57BL/6 micethat were instilledintratracheallywith a single dose of 8 U/kg of bleomycin. In the 2nd day after instillation, mice were treated with trichostatin A (TSA) or valproic acid (VPA) administeredeach two days in PBS/0.5% DMSO. Mice were euthanized 28 days after bleomycin instillation. Lung tissues were used for histological analysis, and collagen content was determined by hydroxyproline measure. Additionally, fibroblasts from bleomycin-treated lungswere isolated and proliferationand migration were analyzed by CyQuant and wound-healing assay, respectively.
Histology results showed a lower grade of lung damage and extension of fibrotic areas in those animals treated with TSA or VPA compared with bleomycin alone. Additionally, there was a reduction of lung collagen content; however this was only significant in mice treated with TSA ($p<0,01$). The proliferative feature of the fibroblasts isolated from lungs of mice treated with bleomycin was significantly decreased after 48 hrs of treatment with TSA ($p<0,01$) or VPA ($p<0,05$). Finally, the rate of wound closure of fibroblasts was decreased when the cells were treated with TSA.
These results suggest a putative protective role of TSA as shown in the murine model of pulmonary fibrosis and our findings in fibroblasts demonstrate a critical role of histone modifications in the characteristic signature of fibroproliferative-migratory feature of pulmonary fibrosis. However, future experiments concerning to fibroblasts transmigration and apoptosis are necessary to confirm this hypothesis.

Biological Studies of Metforminium Decavanadate, $(H_2Met)_3(V_{10}O_{28})\cdot 8H_2O$

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Metabolic Syndrome (SM) is defined like an environmental and genetic factors group with metabolic repercussions, this association promotes over risk to develop cardiovascular and metabolic pathologies emphasized diabetes mellitus type 2. SM prevalence in the world populations is esteemed over 50%; in Mexico affect more than 70% of the adult population and up to 30% of our children's. The studies majority showing seric environment and limited occasions the dynamic between tissues; in this context, the drugs like metformin can restore seric values normals, inferring that cellular dynamics of production, consumption and backup energy was improved, however trace minerals like a vanadium has shown similar effects. Into applied bioinorganic laboratory was synthesized and characterized a compound of metforminium decavanadate $[(H_2Met)_3(V_{10}O_{28})\bullet 8H_2O]$ that containing three metforminium dications bound to a decavanadate; both components posses regulation of metabolism and anti-diabetic properties, so that were used in Wistar rats which were induced by a high-caloric diet to deregulation of carbohydrates and lipids with a biochemical phenotype of metabolic syndrome. The experimental methodology consisted in the conformation of five groups of 20 rats each one, NC group (with normo-calorie diet), HC group (with hyper-calorie diet), HC+metf (with hyper-calorie diet plus metformin 0.12 M/kg daily), HC+$NaVO_3$ group (with hypercaloric diet plus 5uM $NaVO_3$, every 3 days) and HC+Metf-$V_{10}O_{28}$ group (with hypercaloric diet plus 2.5 uM of complex $[(H_2Met)_3(V_{10}O_{28})\bullet 8H_2O]$). All the groups HC showed deregulation of lipids and carbohydrates in plasma, even in different tissues increased concentrations of glycogen and triglycerides before of different treatments, and this time we call zero time. One month with treatments were evaluated: tolerance oral glucose, which showed almost regulation in metformin group, as well as the normalization in lipids in plasma, but in tissues although decreased glycogen of several organs, other ones shown increased and in all tissues increased triglycerides storage. The $NaVO_3$ group did not show glucose plasmatic improved, however, the plasma lipids decreased. The glycogen shown the same behavior than metformin and triglycerides even increased more than metformin. The metf-$V_{10}O_{28}$ showed some encouraging improvements in the different measurements. However in the second month of administration the metf-$V_{10}O_{28}$ showed a remarkable improved in all items unlike of groups administered with metfomin and $NaVO_3$ that no shown significant changes respect to one month administration. In conclusion the metf-$V_{10}O_{28}$ molecule administered by two months every 3 days, presented hypoglycemic characteristics and regulatory of lipids system in plasma and tissues.

Circadian study of metabotropic glutamate receptors type 5 in the liver

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Glutamate is one of the amino acid more abundant in the organism that acts as a neurotransmisor and as metabolic intermediate.In the nervous system, the glutamate can act through two types of membrane receptors: ionotropic (canals) or metabotropic (G proteins).However, in peripheral tissues, these receptorshave been little characterized.It isknown that an antagonist of mGluR5 protects the liver of cellular death under hypoxia or hepatotoxicity.Therefore,glutamate could have a role as a messenger in the physiology or pathology of the liver. Moreover, the circadian clock systemhas an important role in the regulation of homeostasis of the organisms. In this regard, restricted feeding schedules (RFS) in ratsinduces expression of a circadian clock, called feedingentrainableoscillator, which coordinates the physiology of various organs such as liver.

The objective of this study was to analyze the pattern of the mGluR5 presence in the liver in rats feeding ad libitum (AL) and under restricted feeding schedule (RFS). For this purpose, four experimental groups of rats (Wistar) maintained in cycles 12:12 light / dark were analyzed: 1) AL, free access to feed, 2) RFS, access to food for 2-h, daily for 3 weeks, 3) acute fasting during 21-h 4)acute fasting during 21-h and thenrefeeding for 2-h. The presence of mGluR5 was analyzed in liver homogenates obtained at intervals of 3 h during a 24-h period by Western blot and Immunohistochemistry,using antibodies against the isoforms a and b of mGluR5.

In liver homogenates, two variants of different molecular weight (~ 130 and 60 kDa) of mGluR5 were recognized. The average of the mGluR5 presence in liver tissue under RFS was higher compared with the AL group. This protein displayed a circadian rhythmicity only in AL group. Under fasting and fasting-refeeding conditions, the presence of mGluR5 were significantly lesser than AL condition.The presence of mGluR5 in liver is associated to the endothelial structures called sinusoids.

These results indicate that mGluR5 is present in the liver and is modified by different feeding conditions.Glutamate may have a role as a signaling molecule in addition to its role as a metabolite in liver physiology.

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Hypoglycemic effect of watercress (*Nasturtium officinale*) extracts on hyperglycemic rats.

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Abstract.

Diabetes mellitus is a group of illness or metabolic syndromes characterized for secondary hyperglycemia related to deficiency in the insulin secretion, it´s action or both. The pathogenesis of type 2 diabetes presents apoptosis and dysfunction of the β cells of pancreas, impairing it´s function and disturbing the glucose homeostasis. Besides the conventional treatment medicinal plants are used as alternative treatment due to significant hypoglycemic effects. **Objective:**Evaluate the hypoglycemic capacity of diverse extracts of watercress (*Nasturtium officinale*): aqueous extract, extract without pigments, alcoholic and acetonic pigments on hyperglycemic rats. **Materials and Methods:** Wistar male rats, analytical grade reagents and watercress extracts:aqueous extract (AE), extract without pigments (EWP) and alcoholic pigment extract (OHPE) or acetonic pigments extract (APE)). The hyperglycemia was induced by an IP injection of alloxan (200mg/kg). Minimum glucose levelsof 180mg/dL were considered hypeglycemic. Groups: Control: physiological solution, NPH insulin (4UI/kg), AE (900mg/kg), EWP (900mg/kg), APE (0.4mg/kg) in fasting glucose and glucose tolerance curves on healthy and hyperglycemic rats. **Results:**Healthy rats with fasting didn´t show variations in blood glucose levels when watercress extracts were administrated. While in the hyperglycemic rats the levels fall 23.8% with AE and 29.9% with EWP. Hyperglycemic rats in glucose tolerance curve with AE showed a 93.9% fall, and with EWP fall 20%, acetonic pigments didn´t have effect. **Conclusion:** The AE and the EWP of watercress show hypoglycemic activity.

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"T cells glycosylation pathway regulated by STAT-6 in a model of STAT-6$^{-/-}$ *knockout* mice"

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Abstract

STAT-6 is a transcription factor that is essential for the IL-4 signaling pathway and the development of CD4$^+$ T cells TH2 type. It has been reported that STAT-6/GATA-3affect the expression of fucosyltransferase and sialyltransferases, and recent studies have showed that theseglycosyltransferases play a key role in the function and susceptibility to apoptosis of TH2 cells. These data suggestthat STAT-6 could regulate several pathways of glycosylation that are essential for TH function. However, little is known aboutwhich glycosylation pathways are regulated by this transcription factor. The aim of this work is the identification of glycosylation pathways regulated by STAT-6 in T cells and their participation in the immune response, by evaluating the surface glycosylation pattern and glycosyltransferase gene expression usinglectins, flow cytometry and RT-PCRanalysis in a model of polyclonal activation and asthma in BALB/c STAT-6$^{-/-}$*knockout* mice. The asthma pathogenesis is mediated by TH2 cells and it is well documented the involvement of STAT-6 in this disease. The results showed that mice deficient for STAT-6 have 45% (±15.6) CD4 PHA$^+$ vs 13.8% (±4.1) by WT mice and 25.6% (±6.3) of CD8 PHA$^+$ vs 69.8% (±9.9) in WT mice. The polyclonal stimulation with anti-CD3 and anti-CD28 showed that STAT-6 deficient mice vs WT can significantly increase the ligands of lectins such as Con A, PNA, SNA, MAA and PHA for CD4$^+$ and Con A, PNA, SNA, MAA, PHA and ECL for CD8$^+$ T cells. In the asthma model we observed a reduced number of infiltrating cells in the lung tissue and an inverse result to that shown in the polyclonal activation model, the expression of ligands lectin on WT mice increases but only in tendentious manner. These results suggest an important regulatory role of PHA ligands by STAT-6 in WT mice, and a mechanism fine for regulation oflectins ligands such as sialic acids, lactosaminic residues, galactoseand mannose in the N- and O-glycans of surface from T cells by STAT-6.

Isolation and Characterization of Aptamers for Human Papilloma Virus Proteins Using Superficial Plasmon Resonance

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Human papilloma viruses (HPV)are small non-envelopedDNA viruses considered the main causative agent of cervical cancer, a common cancer in women worldwide. Most cases of cervical cancer have been associated withthe high risk HPV type 16 (HPV16). The integration of HPV DNA to the host genome is considered a key step towards malignant transformation of the infected cell. Integration disrupts expression of the viral transcriptional regulator E2 allowing the constitutive expression of the HPV transforming proteins E6 and E7 which are necessary to produce a transformed phenotype.

Aptamers are single-stranded oligonucleotides with defined tridimensional structure that bind with high affinity and specificity to a diverse range of molecular targets and have become powerful analytical, diagnostic or therapeutic tools.Aptamers are isolated from random sequence oligonucleotide libraries using the SELEX (Systematic Evolution Ligands by EXponential enrichment) method. The objective of our work is to isolate aptamers against the HPV16 E2 protein to provide a molecular tool for diagnosis of cervical cancer.

Several RNA aptamers were isolated by SELEX using the full-length HPV16 E2 protein. Nevertheless, they lacked specificityand thus we had the need to modify the SELEX protocol to increasespecificity and affinity through the use of state-of-the-art binding analysis technologies such as superficialplasmon resonance(SPR). Additionally, because the native affinity of E2 for nucleic acids, weproduced and purifiedthe HPV16 E2 amino-terminal domain(lacking DNA-binding activity) fused with GST (GSTE2NT-HPV16) to further refine the SELEX protocol. Initially, an anti-GST antibody was immobilized on the SPR chip surface to easily captureseveralGST-tagged proteins on the same SPR chip. The GSTE7-HPV16 and a RNA aptamer for E7 were used as specificity control. A RNA pool was transcribed from a randomized oligonucleotide library and sorted through the immobilized GST-proteins on the SPR chip, but we wereunable to record and PCR-amplify RNA-protein binding complexes probably due to added efficiency issues through the protocol. Thus, to improve efficiency,we successfully immobilizedthe purified GSTE2NT protein and several controls directly on the SPR chip.

Role of TMPRSS4 in idiopathic pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and lethal lung disease with a median survival of less than 3–5 years following diagnosis. IPF is characterized by the expansion of the fibroblast population and excessive extracellular matrix accumulation with the subsequent destruction of the lung architecture. The diagnosis is based on clinical and radiographic examination but often a biopsy and histopathological evaluation is required for differential diagnosis.

TMPRSS4 is a novel type II transmembrane serine protease found at the cell surface. Although their biological functions are unknown it has been observed that is highly expressed in pancreatic, colon and gastric cancer tissues . In this context, it has been suggested that TMPRSS4 promotes migration and facilitates the epithelial to mesenchymal transition (EMT), two critical processes in the pathogenesis of IPF. Thus, we hypothesized that overexpression of TMPRSS4 in the lung could promote the initiation and/or progression of IPF. To date there are no published work on fibrotic lung diseases and TMPRSS4.

We first examined the lung expression and localization of TMPRSS4 in IPF and normal lungs by qPCR and immunohistochemistry. Our results showed that this protease is not expressed in normal lungs while variable amounts of the mRNA were found in IPF lungs ($2^{-\Delta CT}$ $1.1x10^{-2}$ $\pm$ $9.4x10^{-5}$) and primarily expressed by epithelial and mast cells. Then we explored the role of this protease in an experimental model of lung fibrosis. TMPRSS4 haplodeficient and wild type mice received an intratracheal instillation of bleomycin and were studied at 28 days post-instillation. We found that the lung fibrotic response, measured by hydroxyproline (110.7 $\pm$ 13.8 versus 178.9 $\pm$ 12.4 μg/lung; $p<0.05$) and morphology (40% versus 15% extent of the lesions) was significantly attenuated in the haplodeficient mice. Our findings indicate that TMPRSS4 may play a role in the pathogenesis of IPF and other fibrotic lung disorders.

Maternal Separation and Post Weaning Social Isolation Differentially Affect Stress and Metabolic Vulnerability in Adult Rats.

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Elevated Glucocorticoid levels (GC) increase metabolic and cardiovascular risk. Maternal separation (MS) is an early life stress rodent model that programs Hypothalamic-Pituitary-Adrenal Axis (HPA) activity, increases basal GC levels, depressive like behavior and further adult stress vulnerability; however, weatherMS interacts with psychosocial stressors, such as post weaning social isolation, to increase metabolic vulnerability later in life is rather unknown. In here we evaluated the effect of periodic MS and post weaning social isolation on stress and metabolic vulnerability in two month old male spraguedawley rats. Litterswere subjected to 3 hours of dailyMS from postnatal day one to 14 (32°C in an independent room from the colony) or remained as undisturbed controls (CONT). Animals were weaned at postnatal day (PND) 21 and randomly assigned to social isolation (SI) or standard (STD) housing until adulthood. At PND60 animals were tested on the forced swim test and underwent a jugular vein catheterization procedure. After a one week recovery time, we evaluated basal and stress induced (2min cold swim) Corticosterone levels and the glucose tolerance test. Both MS and SI caused a passive coping strategy in the forced swimming test; however, statistical analysis revealed that, while SI effect on stress reactivity was stronger than MS(ANOVA $F_{1,147}$: 6.6p = 0.01), only maternally separated groups showed metabolic alterations in the glucose tolerance test(ANOVA $F_{1,189}$: 7.7p = 0.008). Triglyceride, Cholesterol and epididymal fat deposition were unchanged across treatments, although MS + SI group showed a 9.1 ± 1.8%(ANOVA AXB $F_{1,62}$: 7.9p = 0.001)increase in total body weight. In conclusion, our results indicate that MS and SI differentially affect stress and metabolic vulnerability without further interaction.

Searching potential inhibitors of acetohydroxyacid synthase from methicillin resistant *Staphylococcus aureus* through virtual screening.

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Introduction. Nosocomial infections (NI) account for one of the most important health issues in hospitals and carry a high morbidity, mortality and economic cost. The prevalence of nosocomial infections in Mexico is 21%. In recent years Methicillin-resistant *Staphylococcus aureus* (MRSA) have become the second pathogen reported in Mexico and the first one worldwide.The appearance of new resistant strains has created serious therapeutical problems. Therefore, there is an urgency to find new drugs against MRSA. In this context, the acetohydroxyacid synthase (AHAS) is a key enzyme because it catalyzes the first common step in the Branched Chain Amino Acid biosynthetic pathway.Therefore, AHAS from *Staphylococcus aureus* (SaAHAS) is a promising target for new drugs against MRSA.
Methods. The three dimensional (3D) structure of SaAHAS is still unavailable. Therefore, a homology modeling procedure was applied to build the 3D structure of SaAHAS. The generated model was validated by PROCHECK and Qmean score. The subset "Drug like" from ZINC data base was selected for virtual screening, this subset contains over 15 million compounds. The docking was performed using Glide (www.shrodinger.com) and MOE (www.chemcomp.com) software. The three common compounds between the two programs were selected for induced fit studies with Inducedfit program (www.shrodinger.com).
Results. The three common molecules were ZINC39729580, ZINC15768417 and ZINC08536413 with a binding energy, according to induced fit, of -8.161, -6.474, and -6.058 kcal/mol respectively. The first molecule made hydrogen bonds with Arg287 and Thr245, while the second molecule formed hydrogen bonds with Asp289, Arg287, Leu247 and Thr245. The third molecule made hydrogen bonds with Asp289, Arg287 and 266; being the residue Arg287 a common interaction.Taking into account the drug likeness score obtained by these compounds, theycould be considered potential drugs.
Conclusions. These molecules could be used as a guide for the design of inhibitors that could serve to obtain new drugs against MRSA.

Comparación del efecto neuroprotector de la silimarina y la silibina en un modelo murino de la enfermedad de Parkinson

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Parkinson's disease (PD) is the second most common neurodegenerative disease and is a growing public health problem in Mexico, since it is one of the leading causes of outpatient care in the third level. Is characterized by a chronic, progressive and disabling caused by the specific loss of dopaminergic neurons of the substantia nigra pars compact and thereby suffering a deficiency of dopamine. At present, L-DOPA is one of best drugs to treat PD. However, most patients are resistant to L-DOPA after few years of treatment, so it is necessary to find new therapeutic alternatives as may be silymarin.

Silymarin is a mixture of flavonoids (silybin, isosilybin, silydianin and silychristin) extracted from *Silybum marianum* plant and possesses anti- oxidant, anti-inflammatory and neuroprotective effects in the brain, as it inhibits the activation of microglia, decreases the tumor necrosis factor alpha and nitric oxide levels, and restores reduced glutathione levels after administration. One of the most abundant components of silymarin is silybin, with about 70%. Previously, in our laboratory we has demonstrated that silymarin has a neuroprotective effect when it is administered intraperitoneally in mice treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) —this toxin induces a selective loss of dopaminergic neurons of the substantia nigra with a depletion of striatal dopamine producing an histopathological syndrome similar to PD— as it maintains about 69% of the dopamine levels in animals intoxicated, also decreasing apoptotic cell death and preserves dopaminergic substantia nigra neurons. One of our goals is to know which one or ones are the active silymarin components. Our results so far indicate that, at least, silybin (measured by high resolution liquid chromatography) can reach the brain of MPTP intoxicated mice, so it is necessary the identification of these components through spectroscopy of absorption and fluorescence studies. Furthermore, administration of oral silybin preserves dopamine levels about 82% indicating that a large part of the neuroprotective effect of silymarin is due to silybin.

To date our studies indicate that silymarin can be used as a potential drug for the PD treatment, although more studies are necessary to improve the absorption of silymarincomponents orally and their full identification before passing clinical trials.

Comparison of the hypoglycemic activity in two varieties of *Averrhoa carambola* tested in streptozotocin-induced type 2 diabetic rats

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Averrhoa carambola L., is a woody, perennial tropical plant belonging to the Oxalidaceae family that is native of India and Southeast Asia. It was introduced into Mexico at the beginning of the 18th century, and it is now distributed in tropical and subtropical regions, mainly in the states of Colima, Chiapas, Guerrero, Michoacán, Morelos, Nayarit, Sinaloa, Jalisco, Tabasco and Veracruz. The fruit is used empirically as a coadjutant in the treatment of several illnesses, mainly diabetes, one of the most important public health issues world-wide, due to its persistent increase in the last years, most importantly in developing countries, such as Mexico. The main objective of this study was to compare the hypoglycemic activity in two varieties of *Averrhoa carambola* using Winstar rats as an experimental model, which were subjected to streptozotocin-induced type 2 diabetes using a dosage of 60 mg/ kg of body weight (BW). Four experimental groups were formed with a total of 23 rats, including positive and negative controls, in addition to the treatments with the Arkin and Golden Star fruit varieties. The fruits, 200 mg of lyophilized ripened fruit per kg of BW dissolved in a final volume of 500 µL, were fed orally to the experimental rats for 30 days, using an aesophagic catheter. Blood glucose levels were determined with a glucometer in samples taken from the caudal vein every 5 days, for a month´s duration, whereas the final blood glucose determination was obtained in samples obtained by retro-orbital bleeding via the glucose-oxidase based enzymatic method. Blood glucose levels were lower than controls (478.75 ± 30.54 for Arkin and 450 ± 44.35 for Golden Star) in the groups of experimental rats fed with star fruit during 30 days. Both the Arkin (96.25 ± 40.5) and Golden Star (242.85 ± 42.29) fruit varieties tested showed an effective hypoglycemic effect in rats, although a significant difference in the hypoglycemic activity was detected among them.

Determination of plasmid profiles to a collection of *Escherichia coli* strains isolated from urinary tract infection (UPEC)

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Introduction.

Uropathogenic *E. coli*(UPEC) is the main causal agent of urinary trac infection (UTI). Identification of *E. coli* strains is important for both clinical and epidemiological implications. Understanding antibiotic resistance patterns and molecular characterization of plasmids is epidemiologically useful.

Objectives.

Characterization of UPEC strains by plasmid profile and relation between plasmid profile and antimicrobial resistance.

Material and Methods.

101 strains of *E. coli* isolated from patients with urinary tract infection with antibiotic susceptibility profile and of know virulence were used. Were grown on a suitable culture medium with and without antibiotic. Extrachomosomal DNA (plasmids) was extracted by the method of alkaline lysis. An analysis by electrophoresis was carried out and subsequently the molecular weight of the plasmids isolated from each of the strains was determined.

Results

The presence of extrachromosomal genetic material was found at 91.34% of UPEC strains and only 8.65% of the strains presented no plasmids. Totally, isolates harbored plasmid with an average of 3.7 plasmid (range: 1- 13) in each strain.50.0% of the *E. coli* strain has from one to three plasmids, 23.08%% has from four to six plasmids, 13.46% of the strains present of 7 to 9 plasmids and 4.81% has of ten to thirteen plasmids. The molecular weights range was between ≥1 kb to ≥10 kb. The most often plasmids are those <2 kb with a 33.6% while those ranging from 9 to 10 kb found at a frequency of 0.79%. However 31.23% of plasmids have one size >10 kb. It was noted in a experiment with only nine *E. coli* strains that some of these changes its plasmid profile when strain is grown in a medium with ampicillin. We found that six strains change number of plasmids of two to three plasmids and these are of a size of 3 kb to 5 kb.

Conclusions

Plasmid analysis of representative *E. coli* isolates also demonstrates the presence of a wide range of plasmid sizes, with no consistent relationship between plasmid profiles and resistance phenotypes. Presumptively some *E. coli*strains have inducible plasmids by ampicillin.

Association between nonspecific febrile illness and leptospirosis

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Nonspecific febrile syndrome (NFS) is a medical condition widely distributed throughout the world, mainly in warm coastal areas. In Mexico has been reported NFS without a diagnosis of the causal etiological agent. The objective of this study was establish the relationship between nonspecific febrile syndrome and the detection of Leptospira in patients from southern Sonora. A total of 137 blood samples were collected for patients with NFS non etiological detectable in different Health Centers of communities in south of Sonora State. The study was carrying out from January to October of 2013. Blood samples were analyzed by dark-field microscopy as a prediagnosis and subsequently the positive sera samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for detect leptospire-specific immunoglobulin IgM and IgG. The results analysis by dark-field showed forms compatibles for spirochetes, in 130 samples (94% positives), where 26% were positive to IgM and 38% to IgG. In conclusion leptospirosis is present in Sonora, Mexico. The association between NFS and leptospirosis is clinical and epidemiological important and requires more attention to avoid becoming a public health problem.

Keys word: Nonspecific febrile syndrome, leptospirosis, dark-field microscopy

Study of the role of Avin03910 depolymerase in the metabolism of Polyhydroxybutyrate polyester (PHB) in *Azotobacter vinelandii*

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Abstract

Polyhydroxybutyrate (PHB) is polyester that is synthesized and intracellularly accumulated as a carbon and energy reserve in several bacteria. In *A. vinelandii* the genes coding for the PHB biosynthetic enzymes, as well as several regulatory elements involved in the control of its synthesis, have been characterized. However, little is known about the mobilization process allowing the utilization of accumulated PHB when it is needed. The genome sequence of *A. vinelandii* DJ revealed seven genes that are thought to encode intracellular PHB depolymerases (the enzymes responsible for the mobilization of PHB). However, direct evidence for the *in vivo* roles of their gene products is missing. In this study, we selected one candidate gen (*Avin03910*), representing the most probable candidate to be involved in the catabolism of PHB, and investigated the physiological function of their gene product: (i) with recombinant *Escherichia coli* strains expressing AvinPhbZ$_1$ (*Avin03910*), protein; (ii) with *A. vinelandii* null mutant. Evidence for significant PHB depolymerase activity *in vitro* was obtained for the product of *Avin03910* expressed in *E. coli*. The protein was active on amorphous and artificial granules only, but not on crystalline PHB, like other intracellular depolymerases.
On the other hand, we found that the protein Avin03910 is associated with the granules of PHB. The mutant AvinPhbZ$_1^-$ had a phenotype of increased PHB accumulation and its purified native granules showed a diminished PHB mobilization and degraded more slowly than the wild type. This phenotype, together with its inability to degrade intracellular PHB under conditions stimulating mobilization in the wild type strain, suggest that the depolymerase AvinPhbZ$_1$ is the main responsible for intracellular PHB degradation. The presence of other PHB depolymerases in this bacterium cannot be discarded and will also be discussed.

Estudio de la Diguanilato ciclasa E putativa de *Azospirillumbrasilense*, que participa en crecimiento y estrés.

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Azospirillumbrasilense, es una bacteria promotora del crecimiento de plantas. Además, entender los mecanismos de la interacción entre los microorganismos y las plantas, los mecanismos y las estrategias para la colonización representan un aspecto importante para la interacción. La formación de comunidades bacterianas complejas conocidas como biopelículas comienza con la interacción de las células planctónicas con una superficie en respuesta a señales ambientales. El di-GMP cíclico [bis-(3'-5 ') - di-GMP cíclicos, (c - di-GMP)], es una molécula intracelular, segundo mensajero, ampliamente conservada que está implicado en la regulación de la formación de biopelículas en bacterias[2]. Los niveles intracelulares de di-GMPc están reguladas por la actividad antagonista de diguanilatociclasas (DGC), y fosfodiesterasas (PDE), que catalizan la síntesis y la hidrólisis de esta molécula, respectivamente[1]. Las DGCs contienen un dominio conservado GGDEF, mientras que la actividad de PDE se asocia a dominios EAL o HD - GYP.
A partir de la secuencia del genoma de *A. brasilense*Sp245[3], se realizó un análisis "*in silico*", del genoma; la búsqueda fue centrada en los dominios GGDEF, EAL y HD-GYP. Fueron encontrados 35 genes relacionados con la regulación del di-GMPc. 15 proteínas contenían el dominio GGDEF; 5, el dominio EAL, 12 ambos dominios (GGDEF/EAL) y 5 con el dominio HD-GYP. Eneste estudio se clonó y mutó el gen *cdgE* que codifica por una proteína con dominios GGDEF y REC, e involucrada en crecimiento y estrés osmótico.Sugiriendo que como en otras bacterias el di-GMPc modula funciones relacionadas con la capacidad de la bacteria de establecerse en un nicho ecológico.

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Frequency of mutations in the *rpoB, katG* and *inhA* gene that confer drug resistance to rifampin and isoniazid in patients with Tuberculosis in the State of Jalisco: identification molecular basis

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Estimates from the World Health Organization (WHO) report that 2000 million people in the world are infected with tuberculosis, with 8.6 million new cases reported last year. In Mexico, tuberculosis has become a serious public health problem, due to the appearance of multidrug resistance strains (MDR) and associations with diabetes mellitus, HIV / AIDS and malnutrition.

Objective: To identify mutations in the *rpoB, inhA* and *katG* gene in strains of Mycobacterium tuberculosis that demonstrateresistance to rifampicin and isoniazid in patientsin Jalisco, Mexico.

Methods: An observational, retrospective, prospective and clinical study of patients with active tuberculosis, with positive cultures identifiedas being drug resistance was performed. DNA was isolated from strains cultured atthe State Laboratory of Public Health (LESP). Using PCR and sequencing mutations in the genes *rpoB* and *inhA, katG*were identified which confirms resistance to rifampin and isoniazid respectively.

Results: In 2013,1,059 cases of tuberculosis were recorded inthe state of Jalisco, México.Fromthe reported cases, 984 are new cases in all forms: 67% pulmonary, 14.83% nodal, 11.8% in other ways, 3.02% miliary, 1.23% renal and 1.7% urinary bone. Moreover, 18.85% have diabetes mellitus and 9.17% is in coinfection with HIV/AIDS. Tuberculosis is predominantly in men with a 1.6:1 ratio to women Pediatric cases (under 19 years old) are 11.4% of the total cases. Seventeen deaths are associated with complications of pulmonary tuberculosis and 48 deaths from other causes. In 2013, 4 patients were found with a presence of isoniazid and rifampicin resistant strains.So far,in 2014 there have been 462 new cases of which 2 are resistant to isoniazid and rifampinamongst 9 different strains.The most frequently observed mutation was in the geneinhA (27.27%), followed by gene katG (25.0%) and lastly in the gene rpoB (12.5%)The mutations were found by the molecular techniques PCR and sequencing, identifying the substitution of a single nucleotide at codon 516 and 531 for *rpoB*gene, the -15 and -16 position in the*inhA* gene and codon 315 in the *katG* gene.

Conclusion: Molecular methods for identification of rifampicin resistance in Mycobacterium tuberculosis strains identifiesmutation-drug-resistance in patients early on in the state of Jalisco with other regions of the world relationship. Mutations at codons 531, 315 and -15 are frequently found in the study population. Molecular diagnosis of antibiotic resistance tuberculosis will reduce the time to identify the sensitive strain, which contributes to early control and prevents relapse or persistence of strains resistant to conventional treatment and complications.

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Role of theputative domain,of the MucG protein, for the degradation of c-di-GMP, involved in alginate synthesisin *A. vinelandii*

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Azotobactervinelandii is a nitrogen-fixing soil bacterium that produces theexo-polysaccharidealginate; this polymer is produced in copious amount during vegetative growth. Because of its physicochemical properties alginate has a significant industrial applications. In the biomedical science the alginate hydrogels are used for the delivery of a variety of low molecular weight drugs in a controlled way as well as in tissue engineering.Alg44 is an inner membrane protein that, together with Alg8, constitute the alginate-polymerase complex. Alg44 has a domain for binding the second messenger c-di-GMP; upon binding c-di-GMP Alg44 triggers the polymerase activity of Alg8 for the synthesis of alginate. It has been postulated that the final molecular mass of the alginate is determined by the enzyme activity of the Alg8-Alg44 polymerase complex and by the alginate degrading enzymes, called alginatelyases. Additionally, the molecular mass of alginate is strongly influenced by the different aeration conditions or dissolved oxygen tension (DOT). The Tn5 mutant strain GG9 exhibited increased levels of alginate of higher molecular mass. The gene affected in this mutant (Avin07910), named *mucG*, encodes a protein with domains for the synthesis (GGDEF) and degradation (EAL) of the second messenger c-di-GMP; it also contains a PAS domain for the detection of oxygen levels. Based on these results we hypothesized that the MucG protein negatively affects the Alg8-44 polymerase complex activity by reducing the intracellular pool of c-di-GMP through its EAL domain, which degrades this second messenger. This activity, in turn, would be modulated by the intracellular oxygen concentration sensed by the MucG PAS domain. The main objective of this project is to evaluate the role of the MucG EAL domain on alginate synthesis and on its molecular mass. To this end we have generated, by PCR, a MucG derivative protein without the phosphodiesterase activity, called $MucG^{AAA}$. We are trying to replace, in the chromosome of *A. vinelandii*, the wild type*mucG* allele by the $mucG^{AAA}$mutant gene. We are going to present results about the characterization of this mutant regarding the effect on the amount and on the molecular mass of the alginate produced. Furthermore, we will investigate the role of MucG, and particularly that of its EAL domain, on determining the molecular mass of the alginate under different oxygen concentrations.

Isolation, identification and characterizationofcyanobacteriafrom biofilms of the archaeological siteofYohualichan,Puebla.

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Biofilms are communities structured by bacteria, algae, cyanobacteria, fungi and protozoa embedded in a polymeric matrix.This microbial interaction with the materials and their environment can lead to biodeterioration, a common problem in monuments and archaeological sites.
The aim of this work was isolation, characterization and identification of cyanobacteria from biofilms of Yohualichan, an archaeological zone in Puebla, Mexico.
Biofilm samples were collected from archaeological monuments in the area known as “Juego de Pelota” (Ballgame), as well as in the East Building, the WestBuilding and “Las Grecas” (Fretworks). The strains were isolated by using BG-11 medium,solidified with 1.3% bacteriological agar, until obtaining axenic cultures and thepropagation of isolates in liquid BG-11. Six axenic cultures were obtained(three from “Juego de Pelota”, one from "Las Grecas" and two from the West Building). Morphological and molecular (16S rRNA) identification of the cyanobacteria was performed. To detect the presence of exopolysaccharides (EPS) in the six isolated cyanobacteria toluidine blue staining was used and therefore, it wasdemonstrated that some of the strains had exopolysaccharides which give them protection fromenvironmental changes and participate in the biofilms formation in rock materials. Finallythe extraction and quantification of phycobiliproteins was performed in six cyanobacteria, four of which had a higher amount of phycoerythrin, all of which suggests that it can grow in conditions of lower light intensity.
Toconclude it can be said that biofilms present in this archaeological site are primarily madeby microalgae and cyanobacteria that produced EPS in order to protect the microbial community.

Familycharacterization of Cyclindependentkinasesin *Trichomonasvaginalis* Erick Amador Gaytán, Karla López Pacheco, Nataly Morales Galeana y María Imelda López Villaseñor. Instituto de Investigaciones Biomédicas, UNAM.Tercer Circuito Exterior Edificio, Ciudad Universitaria. imelda@biomedicas.unam.mx

*Trichomonasvaginalis*is a protozoan parasite, thecausativeagent of trichomoniasis in humans. Thisdisease has beenclassifiedbytheWorldHealthOrganizationas non-viral sexually transmitted disease with the highest incidence in the world, where the most affected are developing countries. Besides its importance in public health, basic research of *T. vaginalis* is relevant since many biological processes are not defined in this organism, such as cell cycle control.

Fundamentally, the parasite's cell cycle is the same as very other eukaryote's featuring growth, DNA replication, mitosis and cytokinesis. In other eukaryotes, cyclin-dependent kinases (CDKs) act at the boundaries between different cell cycle stages, to prevent premature orin appropriate transition through key checkpoints. Their activity is tightly regulated through a variety of mechanisms including binding of a cyclin partner and phosphorylation.

From an *in silico* analysis by the graduate in IBB Arizmendi Olivia Perez in the laboratory of Dr. Imelda López Villaseñor, were found eight genes present in the annotated genome of *T. vaginalis*, as candidates to encode proteins of type Cdk, denominated in protozoa as Crk's (foritsacronym in English "related Cdc28 kinase"). The objective of this work is the characterization of these putative Crk's genes and their protein products.

So far,we have determined the expression level of messenger RNA of eight Cdk's, suggesting that the protein products of Crk's are present in *T. vaginalis*. We have been obtained eight recombinant proteins, which are expressed by IPTG induction that include a his tidine tag.

To determine the activity of the Tv Crks as kinase-like proteins, phosphorylation as says of Histone H1 were performed with there combinant proteins of TvCrks purified under native conditions, with this test, a basal activity of TvCrk's in vitro was observed in the absence of anactivating cyclin.

Candidate proteins This result does not role of TvCrks within the cell control in *T. vaginalis*, sinceitisknownthatthisprotozoandivergedveryearly in theeukaryoticlineagewhich places phylogeneticallydistanttoS. *cerevisiae*, thisfactcouldgenerate fundamental differences in thewaythe control of cellcycleregulatorymechanisms.

With two-hybridas says with the LexAyeast system we were able to determine the interaction of some *Tv*Crks both cyclins as CksproposedfoT. *vaginalis*.As a nextgoal, phosphorylationas says of histone H1 with complexes candidates of recombinantsTvCrk's and cyclins of *T. vaginalis*will be madewiththoseweredemonstratedinteractionbytwo-hybridassay.

The role of *Avin08930* in biosynthesis of Poly-β-Hydroxybutyrate and alkylresorcinols in *Azotobacter vinelandii*

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Azotobacter vinelandii is a Gram-negative bacterium able to synthesize polyhydroxybutyrate (PHB), a short-chain length polyester of polyhydroxyalkanoate family. Furthermore, A. *vinelandii* can undergoes a differentiation process to produce resistant-desiccation cysts. During the encystment, the bacterium synthesizes phenolic lipids called alkylresorcinols (ARs), which replace the membrane phospholipids.

The *phbBAC* operon encode the enzymes for PHB synthesis. The *phbR* gene encodes the specific transcriptional activator of *phbBAC.* The *arsABCD* operon encodes the enzymes for ARs synthesis and its transcription is activated by ArpR.

The two component regulatory system GacS/GacA is essential for biosynthesis of PHB and ARs, since a *gacA* mutation abrogates their production. GacA activates the transcription of genes encoding sRNAs $rsmZ_{1\text{-}7}$ and *rsmY*, which bind to RsmA, a small protein that blocks the translation of *phbR* and *arpR* mRNAs. Because of inactivation of *rsmA* in a *gacA* mutant did not restore the PHB and AR synthesis, we hypothesize that there is another pathway by which GacS/GacA controls the biosynthesis of PHB and ARs in *A. vinelandii*.

A bioinformatic analysis the *A. vinelandii* genome allowed us to identify a consensus binding site for GacA in the promoter of Avin08930 gene. The Avin08930 gene encodes for hypothetical protein of 52 aa. By using qPCR, we determined that indeed expression of *Avin08930* is reduced in a *gacA* mutant. This was confirmed by using transcriptional fusion of *Avin08930* promoter with *gusA*. The transcription of *Avin08930* was diminished in the *gacA* mutant as compared this in the wild type strain. The *Avin08930* mutant showed reduction in PHB and ARs levels as well as a reduced expression of the *phbB* and *arsA* genes.

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Effect of dichloromethane-methanol extract of *Parthenium hysterophorus* on enteropathogenic *Escherichia coli* (EPEC) adherence.

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Infectious diarrhoeal diseases continue to be major public health problems especially in developing countries. In these countries, enteropathogenic *Escherichia coli* (EPEC) infection is the main cause of morbidity and mortality, predominantly in children under the age of five [1]. Due to evolution of multi- drug resistance in EPEC and the side effects caused to the host by antibiotics, it is necessary to search for alternative therapies.

Medicinal plants are important therapeutic weapons in the fight against many diseases. In many regions, *Parthenium hysterophorus* is a weed used as a remedy against malaria, stomachache, diarrhoea, and many other infections [2].

This study was conducted to investigate the effect of dichloromethane-methanol extract of *P. hysterophorus* on bacterial adherence to epithelial cells and its antimicrobial activity.

The Minimum Inhibitory Concentration was determined using broth dilution method. Adherence of *E. coli* (strain E2348/69 serotype O127:H6) to HeLa cells (human cervix adenocarcinoma cells) was assayed by the method described by Cravioto *et al*. [3]. EPEC were incubated in the absence (control) or in the presence of various dilutions of the extract. HeLa cells having EPEC adhered in characteristic microcolonies [4] were counted under light microscope.

The dichloromethane-methanol extract of *P. hysterophorus* did not have any effect on HeLa viability (measured by trypan blue exclusion test); and did not have a good antimicrobial activity (< than 15% of growth inhibition with 500 µg/mL).

In the case of the adherence assay, the results show that the extract mainly decreases the EPEC localized adherence pattern with all the used concentrations with respect to the negative control.

Considering that localized adherence pattern is essential for EPEC pathogenesis, our findings suggest that *P. hysterophorus* extract could be useful in therapies against EPEC diseases.

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Exploring the molecular mechanisms that maintain the *Rhizopusmicrosporus* – *Burkholderiarhizoxinica* symbiosis

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The β-proteobacterium*Burkholderiarhizoxinica* is an intracellular symbiont of the Mucoralean fungus*Rhizopusmicrosporus*[1].The endosymbiont producesthe toxin Rhizoxin, which its resistant fungal hostuses to infect rice seedlings[2].Furthermore, *R. microsporus* is dependent of *B. rhizoxinica* to achieve sporulation [3]. Unusual for such a close symbiotic relationship, both organisms can be grown independently, potentially modified and brought back together [3].There are also several*Rhizopusmicrosporus* strains that don't harbor bacteria (non-host) and don´t rely on *B. rhizoxinica* to sporulate, representing an interesting comparisonfor the symbiotic system [4]. To date, the genome sequences of *Burkholderiarhizoxinica*[5], *R. microsporus* (host), *R. chinensis* (non-host), and*R. oryzae* (non-host) are available. All of this makes the *R.microsporus*–*B.rhizoxinica*an attractive model system to study symbiosis.

To find proteins that could be related to the symbiosis, we started by comparingthe full protein sets of*Rhizopusmicrosporus* versus thetwo non-host*Rhizopus*spp. and two other Mucoralean genomes.We looked for enrichments in functional categories in sets of proteins exclusive to *R. microsporus* relative tothe other Mucorales,findingproteins related to antibiotic resistance.We also found several genes coding for proteins with kinase activity and functions related to protein localization to organelles in those families that are expanded in *R. microsporus* compared to *R.chinensis* and *R.oryzae*.We then made a prediction of likely horizontal transferred genes to the endosymbiont. *B. rhizoxinica* has many polyketide synthases (PKS) and non-ribosomal peptide synthases (NRPS), some of which may come from *Streptomyces* according to our results. The Rhizoxin toxin, produced by a mixed NRPS-PKS [6], confers *R. microsporus* the capability to cause rice seedling blight disease; other NRPS or PKS may be relevant for the symbiosis. From these results,we have chosen a set of candidate genes with a possible role in symbiosis in both organisms,and wemeasuretheir expression throughout different developmental stages of the fungus.

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The flavonoid (-)-epicatechin affects erythrophagocytosis in *Entamoeba histolytica*

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BACKGROUND: *Entamoeba histolytica* is a protozoan parasite that causes amebiasis in humans. This protozoan invades and destroys the intestine by killing and phagocytosing, erythrocytes, epithelial cells and cells from the immune system. During phagocytosis, actin cytoskeleton and actin-binding proteins like myosin IB are required to regulate extension and retract pseudopods for closing the membrane over the ingested cell. Trophozoites with overexpression of actin and myosin IB showed deficient erythrophagocytosis. Accordingly, the molecules that participate in this process are good targets for developing new drugs for amebiasis. In this search for new drugs some flavonoids had effects in *E. histolytica*. Previously, using a proteomic analysis, we showed that the flavonoid (-)-epicatechin induced an important modification in actin and myosin cytoskeleton proteins expression which could affect cytoskeleton related functions such as erythrophagocytosis. Therefore, we investigated the effect of (-)-epicatechin in this cellular process and actin cytoskeleton rearrangements in *E. histolytica.*

METHODS: Trophozoites grown with 8.27 µM (-)-epicatechin for 48h at 37 °C were incubated with human erythrocytes for 10 min at 37°C and the internalized hemoglobin in trophozoites was quantified by a colorimetric method, trophozoites grown in TYI-S-33 medium and DMSO were used as controls. Using trophozoites interacted with erythrocytes we investigated whether the flavonoid affected the microfilament rearrangements and expression of actin and myosin IB by confocal microscopy. Relative fluorescence intensity was quantified by software Zen lite 2012 de Zeiss.

RESULTS: Erythrophagocytosis assay showed that trophozoites grown in (-)-epicatechin had a significant increase in the amount of internalized hemoglobin (0.64 $\pm$ 0.07) in comparasion with control cells growing in medium (0.34 $\pm$ 0.04) or DMSO (0.33 $\pm$ 0.04). We found increased in the polimerizated actin without change in the microfilaments rearrangement in response to flavonoid treatment. Results evidenced that trophozoites in medium had 95.50 $\pm$ 24.22 fluorescence intensity and this number increased to 117.3 $\pm$ 14.21 in trophozoites treated with the flavonoid. (-)-Epicatechin did not have effect in myosin IB expression (59.38$\pm$ 20.56) and in its cell distribution, in comparison with cells without treatment (66.68 $\pm$ 27.35).

CONCLUSIONS: Our results indicated that (-)-epicatechin modifies phagocytosis capacity of *E. histolytica* trophozoites because of overexpression of polymerized actin without affect the expression and cellular distribution of myosin IB.

Identification of a gene encoding putative flagellin in *Haemophilus influenzae*

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Haemophilus influenzae is a human pathogen. Encapsulated *H. influenzae* type b (Hib) is the most common serotype associated with invasive disease. The non-typed forms of *H. influenzae* (NTHi) are generally associated to moderate diseases of the upper respiratory tract in children and pneumonia in adults. *H. influenzae* has been considered nonmotile and nonflagellate, however, bacteria considered nonmotile like *Actinobacillus* and *Shigella*, have shown the capability of expressing a flagellum. Therefore, in this study we tried to identify flagellar mobility in both strains Hib and NTHi. The strains were analyzed in a semisolid agar plate using Brain Heart Infusion (BHI) medium supplemented with Fildes. Degenerated oligonucleotides based on highly conserved regions of flagellin were designed. Genomic DNA was obtained from the mobility assays for PCR reaction; the product obtained was purified, cloned and sequenced. Indirect immunofluorescence assays (IFA) were performed using anti-Hib and anti-NTHi antibodies and observed through a fluorescence microscope. Transmition Electronic Microscopy (TEM) was used to detect the flagellum. Furthermore, Western blot assays using heterologous antibodies anti-flagellin were performed. The analyses shown motile strains of Hib and NTHi. The sequencing results demonstrated identity with the flagellin genes in the 5 ' and 3' end, in the central region identity with a truncated version of the 3 -hydroxyacyl-CoA dehydrogenase enzyme was observed; however in tertiary structure prediction assays was demonstrate high homology with the flagellin. The presence of the flagellum was observed by IFI and TEM. The western-blot assays showed recognizing of a band of 55 kDa, indicating that a crossover between *E. coli* antibodies and the possible flagellin of *H. influenzae* are occurring. These results shown that exist mobility in *H. influenzae*, but only under certain conditions and this mobility may be mediated for the expression of the flagellum. The findings of this study could be indicating that a chimerical protein can be generated and that may act as structural protein of the flagellum and allow bacteria to reach the different niches in the host to establish themselves and cause an infection.

Modification of the host cell cytoskeleton during *Toxoplasma gondii* encystment

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BACKGROUND: *Toxoplasma gondii* presents 3 infectious stages; sporozoite, located inside the oocyst; tachyzoite, a highly invasive, mobile and replicative form and bradyzoite, a slow replicative form contained in tissue cysts. Tissue cyst is surrounded by a protective wall rich in glicoproteins that provide to the structure a characteristic resistance against cells and molecules of the immune response as well as parasiticidal drugs. Encystment process apparently starts as result of the action of effectors of the immune response against the parasite. Encysment can be induced *in vitro* by exposure of the infected cells to alkaline pH, thermal shock, nutritional stress or presence of cytokines such as IFN-γ. These encystment conditions require long time and their efficiency is low. By exposure of tachyzoites to an inhibitor of inosine monophosphate dehydrogenase (IMPDH), we could induce an efficient and reproducible encystment of *T. gondii*. The aim of the present study is to determine how the host cell cytoskeleton is modified during the tissue cyst formation *in vitro*.
METHODS: HEp-2 cells were infected with tachyzoites previously treated with the IMPDH inhibitor at different concentrations and time exposure. Using confocal microscopy we evaluated distribution of actin cytoskeleton (F actin and G actin) and intermediate filaments during *Toxoplasma* encystment. Formation of tissue cyst was determined by detecting the expression of CST1 glycoprotein.
RESULTS: Our results showed that treatment with the IMPDH inhibitor, did not affect host cell and parasite viability during the course of the experiments. Induction of *Toxoplasma* encystment induced a rearrangement in the distribution of actin filaments and intermediate filaments. These modifications correlated with changes in the host cell shape as a previous phase to the cyst formation.
CONCLUSIONS: The inhibition of the purine synthesis by the IMPDH inhibitor induced differentiation of the tachyzoites to bradyzoites. Parasite´s differentiation modified organization of actin filaments and intermediate filament of vimentin in the host cell. As the parasitophorous vacuole evolved into a pre-cystic structure, host cells exhibited a loss of the filamentous organization of the cytoskeleton. This loss could represent one of the earliest cellular events during cyst formation as a strategy of the parasite to facilitate the host cell transformation into a tissue cyst.

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Potato bacterial endophytesasbiocontrol and biofertilizeragents

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Abstract.The presence of endophytic bacteria in plants provides certain benefits such as: antagonism against pathogens, systemic resistance induction, auxins production, atmospheric nitrogen fixation, phosphatesolubilization and synthesis of siderophores. In this work, we propose the use of these endophyticbacteria showing antagonistic activity against two potato pathogens (*Rhizoctoniasolani and Fusariumoxysporum*) and plant growth promoting activities (PGPA). 74endophytic bacterial strains were isolated from different potato plant tissues; so far 64 strains have been analyzed for pathogen antagonism: 15 of them have 5.5 to 50.83% of inhibition for potato isolated*R. solani* and 8 strains have 5.7 to 38.7% growth inhibition activity against potato isolated *F. oxysporum*, only 4 strains have inhibitory activity for both phytopathogens. All 74 isolated strains where tested for Indole acetic acid production and phosphate solubilization, 34 strains were capable of solubilizing phosphate with an efficiency of 2.2 to 100 according to Nguyen et.al. (1992), and 73 strains were able to synthetize auxins with a production of 1.6 - 27.4 ppm, 24 strains were analyzed for Nitrogen fixation, all of them gave a positive result for growth in selective Norris medium. We have sequenced the 16s rRNA fragment from 44 strains, all identified stains were grouped in 10 genera belonging to Firmicutes and γ Proteobacterias. Among all identified strains, *Pseudomonas*genuswas the most representative with 21 strains, followed by *Enterobacter*with 5 strains, *Bacillus* with 4 isolates, *Pantoea* and *Paenibacillus* with 3 strains each, *Stenotrophomonas*, *Aeromonas* and *Serratia* with 2 isolates each and finally *Delftia* and *Rahnella* with 1 strain. The distribution of the population and diversity of strains varied according to the type of tissue tested, neverthless*Pseudomonas* remains as the most commonly isolated genus.Those strains showing the best antagonistic activity and PGPA will be further tested as biocontrol and biofertilizer agents respectively in greenhouse assays.

Biodiversity and community structure associated to rhizosphere of *Quercus sp*. in Sierra de Lobos, Guanajuato.

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The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1 mm wide, it is an area of intense biological and chemical activity influenced by compounds exuded by the root and organism such as bacteria, actinomycetes, fungi, protozoa, slime moulds, algae, nematodes, enchytraeid worms, earthworms, millipedes, centipedes, insects, mites, snails, small animals and soil viruses compete constantly for water, food and space

.About 2 to 5% of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed plant growth promoting rhizobacteria (PGPR)

.n comparison to the bulk soil, the number of microorganisms in the rhizosphere is always substantially higher because of the plant influence. There are also changes in the biodiversity of microorganisms caused by this "rhizosphere effect" which was defined as any physical, chemical or biological change occurring within the root sphere or even indirectly mediated by its excretions and organic debris

We have a rhizosphere strain collection with around 250 isolates of three species of oak from Sierra de Lobos. The aims of this study was characterize and correlated the cultivable biodiversity, phylogenetics and bacterial community associated to roots by ARDRA analysis and biochemical activities to plant growth promoting.

Functional characterization of the heterodimerization domain of GrlR, a LEE-encoded negative regulator of Enteropathogenic *Escherichia coli*

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EPEC belongs to a group of pathogens that form ‘‘attaching and effacing’’ (A/E) lesions on intestinal epithelia. The A/E lesion is characterized by the localized destruction of the enterocyte apical microvilli and cytoskeleton rearrangements beneath the adherent bacteria, leading to the formation of actin-rich pedestals and intimate bacterium-host cell interactions. The genes required for the formation of the A/E lesion are located within a pathogenicity island known as the locus of enterocyte effacement (LEE), which expression is repressed by H-NS. Ler (LEE encoded regulator) activates the expression of the LEE by disrupting H-NS repression. GrlA is required for the activation of *ler*, whereas GrlR represses the expression of LEE operons. Protein-protein interaction experiments and crystallography showed that GrlR interacts with GrlA, so it was proposed that GrlR inhibits GrlA activity and therefore the expression of LEE genes.

In the absence of GrlR, the transcriptional activity of LEE genes increases under repressing growth conditions, while GrlR overexpression leads to repression of LEE genes in wild type EPEC and even in EPEC Δ*hns* or *E. coli* K12 Δ*hns*. In this work, we generated GrlR mutants in residues forming the crystal structure interphase with GrlA to investigate its role in GrlR-GrlA heterodimerization and repression of LEE gene expression using protein-protein interaction, western blot and secretion assays. GrlR inactive mutants that were still able to interact with wild type GrlA further support the notion that GrlR may act directly as a repressor of LEE gene expression, independently of its interaction with GrlA. Overall, our data illustrates that LEE gene expression is negatively regulated at two levels, which are mediated by both a global regulator (H-NS) and an EPEC specific regulator (GrlR).

Implementingthe BioDAgeneas a transformation markerin *Trichodermaatroviride*.

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*Trichoderma*spp are cosmopolitanfilamentous fungicommonly foundin soil, which arebest known forits ability to carry outbiological controlagainst a great number offungal phytopatogens due to its antagonistic activity. Currently, to protect crops it is prepared as conidia formulations, which are dispersed in the field. For that reason, the mycoparasitic fungus *T. atroviride* has been using as morphogenetic model to study conidiation stimulated by light, injury, stress, and nutrients deprivation.

In filamentousfungi, different methodsforgenetic transformationhave been developed to manipulate their potential in biotechnological processes or for knowledge generation.Worth to mention is the fact that, having access to the genome sequence of five *Trichoderma´s*species more approaches for implementing strategies of functional genomic are required. Just a few selectable markers are currently used in this fungus, which are genes that confer resistance to an antibiotic which are expensive. One alternative is to generate auxotrophic mutants, which could be used as genetic background and by complemention select the mutants on minimal medium.

Biotin isa water-soluble vitamin,it is acofactorof several enzymes known asbiotin-dependent carboxylases. In this work, we identified an ortholog of bioDA from *Aspergillusnidulans* in the genome of *T. atroviride*. Mutants lacking *bio*DAgene were generated by gene replacement using the approaching double-joinPCR. All mutants are unable to growth in minimal medium and their growth is restored adding 120 ng/ml of Biotin in the medium. Delimitation of the minimum length of *bio*DA gene, which is able to complement the auxotrophic phenotype, is in progress.

Dense granule protein 12 (GRA12) is a virulence factor associated to subpelicular cytoskeleton in *Toxoplasma gondii*

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Toxoplasma gondii is the causing agent of toxoplasmosis; dynamic events such as gliding motility, conoid extrusion, host cell invasion and cell egress are involved in pathogenesis and virulence of this infection[1]; these dynamic events are based on the cytoskeleton components that include the pellicle, the subpellicular microtubules and the subpellicular network[2]. Although the protein composition of cytoskeleton have not been fully characterized, in a recent proteomic analysis, we found the presence of the dense granule protein 12 (GRA12) associated to the subpellicular cytoskeleton fraction[3]. We characterized the presence of GRA12 by mass spectrometry analysis (MS) and Western blot (WB); In addition, study of the distribution of GRA12 in the subpellicular cytoskeleton of tachyzoites was achieved by immunoelectron microscopy (IEM) and immunofluorescence (IF). Finally we used a knockout strain (ΔGRA12) to characterize its possible role in the cytoskeleton organization and virulence.

Our MS and WB results indicated that protein GRA12 is associated with the cytoskeleton fraction of *T. gondii;* the IEM and IF analysis, showed that it is distributed throughout the subpellicular network. The ΔGRA12 strain showed a clear reduction in conoid extrusion capability and cellular invasion in comparison with the wild type strain. In addition, ΔGRA12 strain showed an evident reduction in virulence in mice infection model, as well as differences in the arrangement into the parasitophorous vacuole during the intracellular proliferation.

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Microbial Diversity and Community Structure, by ARDRA Analysis to soils of Natural Protected Areas in Guanajuato Mexico.

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Natural protected areas (NPAs), are areas of terrestrial and coastal ecosystems promoting solutions to reconcile the conservation of biodiversity with its sustainable use. NPAs serve in some ways as 'living laboratories' for testing out and demonstrating integrated management of land, water and biodiversity. The state of Guanajuato has 24th NPAs with different categorization, these areas are important for a bioprospection program we started, including the study of microbial diversity and commnunity structure of soils. The structure of bacterial communities, biodiversity and the abundances of individual bacterial taxa have been examined extensively in aquatic ecosystems. However, few studies have focused in growth rates of specific taxa in natural microbial communities. It has been known for some time that the soil hosts a large number of bacteria (often around 10^8 to 10^9 cells per gram of soil) and that the number of culturable bacterial cells in soil is generally only about 1% of the total number of cells present. We have a strain collection with 300 different isolates obtained of Sierra de Penjamo, Sierra Gorda and Sierra de Santa Rosa NPAs. ARDRA is a useful and rapid way of assessing changes of bac-

terial communities. The aim of this study was to characterize the biodiversity and structure of the bacterial community by ARDRA analysis in the three NPAs above mentioned.

Antibacterial activity of extracts and lectins from leaves of Capitaneja (*Verbesina crocata*)

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Medicinal plants have been used from ancient times for the treatment ofvarious diseases caused by bacterial action. Among these is the Capitaneja (*Verbesinacrocata*) aMexican shrub who´s leaves are used to treat urinary tract infections, cutaneous ailments, diarrhea, fever, gynecological problems, between others.Using oral or topical administration. It has been proven larvicidal and hypoglycemic activity. **Objective:**Evaluate the antibacterial activity of extracts and lectins from leaves of Capitaneja (*Verbesina crocata*).**Materials**. Capitaneja (*Verbesina crocata*) leaves. ATCC bacteria. Analytical Grade Reagents. **Methods.**Kirby-Bauer method (Disk diffusion) in brief 3-5 bacterial colonies were inoculated into broth 2-6 hours at 35°C to obtain 0.5 McFarland standard and then was spread evenly on the surface of a petri dish. Next disks were impregnated to serial dilutions samples and controls (positive and negative), were incubated at 35°C for 24 hours and was held reading inhibition halos. **Results:**The crude extract presented antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 with an MIC of 83.25 µg/mL. The protein fraction (FP) and lectins (L1, L2 and L3) purified have activity against *Staphylococcus aureus* ATCC 25923; with an MIC of 75 µg/mL (FP), 95 µg/mL (L1), 68.7 µg/mL (L2) and 22.5 µg/mL (L3) respectively.**Conclusion:**The crude extract, protein fraction and lectins of the Capitaneja(*Verbesina crocata*) have antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853; this can justify the use that is given to this plant in Mexicantraditional medicine.

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Binding of extracellular matrix proteins to *Acanthamoeba castellanii* is modulated by surface molecules

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The acantamoebiosis is a health problem, causes keratitis and encephalitis; with toxic pharmacological therapy and poor selectivity[1]. The A*. castellanii* adhesion to tissues of the host cell, are essential for the development of infection[2]. In this work, the extracellular matrix (ECM) is analyzed as a study model of *A. castellanii* infection. Methodologically, was obtain the ECM of epithelial cells and polyclonal antibodies raised against *A. castellanii*, were used as a tool to elucidate molecules involved in the parasite - ECM host cell interaction. The results shown, majority eight antigenic molecules of Mr ≤ 180, 174, 124, 113, 84, 49, 43 and 40 kDa, localized in the surface of the trophozoite. Some of these molecules have a capacity to bind to fibronectin with a Mr ≤ 188, 179, 114, 97, 86 kDa and Collagen IV with Mr ≤ 115.184, 84, 62, 58 kDa; suggesting that they may be receptors. In the parasite- ECM interaction, the adhesion of trophozoites was of 39% and 80% at 15 and 60min respectively. Whereas, the adhesion to host cells was of 65% and 95% at 15 and 60min respectively. Furtheremore, prior exposure of trophozoites with anti-*A. castellanii* antibody, shown the inhibition of adhesion of trophozoites to the MEC (58%) and host cells (90%) at 60min. Together data indicate that these proteins are involved in the pathogenic process of the parasite and may be therapeutic targets. However, unknown the participation of each of the antigenic molecules in the process and mechanisms of adhesion, dissemination and infection of pathogen.

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"Isolation of beer spoiling bacteria *Lactobacillus* and *Pediococcus* from environmental samples"

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Abstract:

Beer has been recognized as a safe beverage due to its good microbial stability given by the characteristics of beer such as the presence of hops which acts as an antibacterial. Even though, some non-pathogenic bacteria can grow in beer, causing turbidity and sensorial alteration which diminishes the quality of the product. As a result of the restrictive environment of beer, only a handful of microorganisms can cause spoilage. Bacteria from the genera *Lactobacillus* and *Pediococcus* are regarded as the most dangerous due to the frequency of contamination and the negative effects on the sensorial profile of the spoiled beer. This is why in the present work we looked for the presence of beer spoiling *Lactobacillus* and *Pediococcus* in environmental samples, this way we could know if beer spoiling bacteria could find theirway to the brewing process causing a microbial contamination. To do this, air samples were incubated at 25°C in NBB-Aand modified MRS agar in anaerobic conditions. The bacteria isolated were inoculated in beer to confirm the beer spoiling potential and the beer spoiling bacteria were identified by qPCR and the hop resistance gene *horA*was amplified by PCR. We found 4 beer spoiling strains, two of which are *Lactobacillus* and two *Pediococcus.* The hop resistance gene *horA*was found in only one of the isolated strains.

Prevalence of virulence markers cagA and vacA in Mexican patients infected with *Helicobacter pylori*

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Introduction. *Helicobacter pylori* (*H. pylori*) is a microorganism that chronically infects almost half of all people worldwide. *H. pylori* is the main cause of chronic active gastritis, stomach and peptic ulceration, gastric cancer and gastric mucosa associated lymphoid tissue ymphoma. Some genes such as cytotoxin-associated gene A (cagA) and vacuolating cytotoxin (vacA) have been identified as virulence markers and have been associated to gastric diseases.

Materials and Methods. One hundred thirty patients from Hospital Juarez de México were enrolled in this study. All participants were informed about the research and signed a consent format. Endoscopic procedure was used to collect gastric antrum and corpus biopsies. To purification DNA, The High Pure PCR Template Preparation Kit (Roche) was used. The presence of *H. pylori* DNA was done by PCR targeting the 16S rRNA gene as mentioned previously. The H. pylori virulence markers were determined by multiplex PCR using primers targeting cagA gene and vacA s/m regions as previously were described by Chattopadhyay et al. in 2004.

Results. The presence of *H. pylori* was detected in 113 patients (86.9%), whereas 17 (13.1%) patients were negative (21.8%). All *H. pylori*-positive biopsies were further analyzed in their cagA and vacA status. Fifty-five biopsies (59%) were positives for both genes; seven biopsies (7.5%) were positives only to cagA and 31 biopsies (33.3%) were positives only to vacA. In parallel, we characterized the vacA gene allele types; Sixty-nine (80.2%) patients were positives for vacA s1m1 allele, 10 (11.6%) were s2m2, 2 (2.3%) s1m2 and 5 (5.8%) were mixed allelles. **Conclusion**. The presence of *Helicobacter pylori* infection in this population remains high and the s1m1 was the most prevalent genotype from *H. pylori,* and only the half of the *H. pylori* positive population had both genes.

Frequency of *Entamoebagingivalis* in two groups of Mexican patients

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Entamoebagingivalis is a parasitic protozoan of the oral cavity, andit is associated with poor oral hygiene. Periodontitis with its various clinical forms represent one of the most widely distributed types of oral disease. Approximately, 5% to 20% of the world population is affected by severe generalized periodontitis. Periodontal disease is an inflammatory process generally associated withthe bacterial plaque, and it is considered this plaque modifies the medium and allows the growth of other organisms like*Entamoeba*. Previous studies have demonstrated a high incidence of this protozoan in patients with oral disease, including periodontal diseases.
The mean of the present study was to determine the frequency of *Entamoebagingivalis* in both diseased gingival pockets and healthy gingival sites in some Mexican people.Here we show a preliminary report of the frequency of *Entamoebagingivalis* intwo different groups of patients, one with periodontal disease and the other one withthe healthy gingiva, but these wereusing orthodontic brackets.
A writing consent was provided from each patient,in the university ethics, and investigation committeesapproved this study. PCR analysis was used to determine the occurrence of *Entamoeba* in both groups.

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Study of the interaction of SltF and FlgJ, proteins that are involved in the biogenesis of the type 1 flagellar system of*Rhodobacter sphaeroides*

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The flagellar protein FlgJ from *Salmonella enterica* consists of two functional domains: the N-terminus acting as a scaffold for rod assembly and the C-terminus acting as a peptidoglycan hydrolase that facilitates rod penetration. In the photosynthetic α-proteobacterium *R. sphaeroides* FlgJ lacks the hydrolase domain and encodes only for the rod scaffold domain. On the other hand, SltF is the lytic transglycosylase that allows the rod to assembly by creating a hole in the cell wall. SltF is secreted through the general secretion pathway Sec, and an interaction with FlgJ allows SltF to find the spot where the cell wall needs to be degraded.

The details of the interaction are currently being explored by dissecting different regions of SltF and observing the effects in motility by performing swimming assays. We are also studyingon*in vitro*assays the interaction between SltF and FlgJ. Additionally we are performing the enzymatic characterization of the various SltF constructs.

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Detection, mapping and phylogenetic analysis of flavohemoglobins from the genome of rhizobial bacteria.

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Hemoglobins (Hbs) have been detected in living organisms from bacteria to vertebrates. In bacteria six structural types of Hbs have been identified: flavoHbs (fHbs), single domain Hbs, single domain sensor Hbs, globin-coupled sensors, protoglobins and truncated Hbs. With the exception of a *Sinorhizobium meliloti* fHb, nothing is known about the existence of fHbs in the rhizobial bacteria. In this work we report the identification and mapping of *fhb* genes in the genome of α- and β-rhizobia and the phylogeny of rhizobial fHbs using bioinformatic methods. Results showed that in the α-rhizobia analyzed in this work fHbs only exist in *S. meliloti* and *Rhizobium leguminosarum* bv. viciae and that fHbs exist in the β-rhizobia *Burkholderia phymatum* and *Cupriavidus necator*. Mapping analysis showed that most of the rhizobial *fhb* genes are flanked by ORFs coding for either unidentified proteins or identified proteins located >100 bp from the *fhb* genes. However, genes coding for a 2- aminopropane dioxygenase and a nsrR transcriptional regulator were detected
<100 bp up- and down-stream of the *fhb*s from *R. leguminosarum* bv. viciae and *C. necator*, respectively. This observation suggests that a function of the *R. leguminosarum* bv. viciae fHb is oxygenate oxygenases and that a function of the *C. necator* fHb is regulate gene expression. Multiple sequence alignment revealed that proximal His and distal Gln and amino acids located at the FAD- and NAD^+-binding sites are highly conserved in the globin and FAD/NAD^+ reductase domains, respectively. However, the analysis of pairwise sequence alignments showed that identity values among the rhizobial fHbs is rather low (40-50%), thus indicating that the variability of rhizobial fHbs is rather high. Phenetic analysis revealed that the rhizobial fHbs evolved in two lineages which are 39-46% identical. Thus, high evolutionary rates probably occurred after the lineage divergence during the evolution of rhizobial fHbs.

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Characterization of TgArticulin in *Toxoplasma gondii* tachyzoites.

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Toxoplasma gondii, the causative agent of toxoplasmosis in animals and humans, has a subpellicular cytoskeleton that is involved in motility, cell shape and invasion[1]. To date there are several proteins of the cytoskeleton have been described as part of the cytoskeleton, By the successful isolation of the subpellicular cytoskeleton together with the tandem mass spectrometry analysis, we reported the proteome of this subcellular fraction. Ninety-five proteins were identified as part of the subpellicular cytoskeleton[2]. Some of the proteins have been already described but most of the identified proteins are unknown and remain to be studied.

One of the proteins identified in the subpellicular cytoskeleton was TgArticulin. Through bioinformatics analysis we designed a highly immunogenic articulin peptide for its synthesis. The peptide was used to immunize Balb/c mice in order to produce monoclonal antibodies that were purified by affinity chromatography. The purified antibodies were used to characterize the articulin molecule in *T. gondii.*

Presence of TgArticulin was exclusively associated to the cytoskeleton fraction with a cortical distribution apparently associated to the subpellicular microtubules. Immunoprecipitation followed by mass spectrometry analysis, suggested its interaction with other cytoskeletal associated proteins. Distribution by confocal microscopy showed a labeling associated to the pellicle or the parasite, while by transmission electron microscopy and immunogold labeling, we could determine its location in the subpellicular network. Precise function of this molecule remains to be determined.

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Phylogenetic profile of the carotenoid biosynthesis across sequenced bacterial genomes

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The advancement of massive sequencing technologies had increased the number of sequenced genomes and this goes in pace with bioinformatics development, with focus on evolutionary analysis. For example, phylogenomics had studied universally conserved gene families and used them to analyze evolutionary relationships between prokaryotes and eukaryotes. Other practical examples of phylogenomics are involved with the study of the phylogenetic profiles of interest traits (*e.g.* pathogenic genes, etc.).

Carotenoids, are a group of isoprenoids which are synthesized in bacteria, algae and plants. Carotenes are found as accessory pigments in the photosynthesis reaction centers, and perform additional features like preventing radiation damage by means of reactive oxygen species (ROS). Although, up-to-date there are over 700 identified carotenoids compounds, the whole carotene biosynthesis pathway (BC) depends upon a restricted enzyme diversity repertoire (*e.g.* cyclases, synthases, desaturases, isomerases and hydroxylases), making difficult to predict the compounds straight directly from each particular BC gene set. The gene distribution of the BC enzymes is not expected to be evenly distributed across the phylogeny, where additional factors like the organisms' nutritional preferences (*i.e.* autotrophic, heterotrophic) could influence the presence or absence of particular genes of the BC. The BC gene diversity had been studied in plants such as *Arabidopsis thaliana,* as well as in microalgae and some bacteria. However, a global phylogenetic profile with patterns of distribution and abundance of BC genes has not been described in bacteria using hidden Markov models (HMM).

We had chosen a initial dataset comprising 25 bacterial key BC enzymes sequences, with experimental support of its function, as well as sequences retrieved from the KEGG database. Subsequently, a larger dataset of homologous proteins of the original 25 sequences were identified, retrieved, aligned and used to build and expand the HMM. Afterwards, we calibrated the HMM thresholds values for true and false positives. We searched across 150 selected bacteria genomes with each of the 25 HMM. The selection criteria for the species was based upon the universal conserved protein families phylogeny, described by Cicarelli et al. (2006), and publicly available in iTOL (http://itol.embl.de/). Each BC protein homologue presence/abscence/copy number is depicted in the iTOL phylogeny, and thus the phylogenetic profile of the BC is shown as the main result of this work.

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STIM1 and Orai1 Identification of *Entamoeba histolytica*

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Amoebas are parasites of the digestive tract [(2)].*Entamebahistolytica* protozoan commensal of the large intestine, which sometimes invade the intestinal mucosa and may spread via the blood, is the agent responsible for amebiasis[(6)].Worldwide, every year about 500 million people are infected with this parasite are reported, of which 10% show clinical symptoms; intestinal 80% to 98% of cases and extraintestinal from 2 to 20%, causing a mortality rate ranging from 40,000 to 110,000 cases per year.Among Latin American countries, Mexico has been the most endemic with infection rates of up to 75%, followed by Colombia with 45-60% and 18-20% with Chile. *E. histolytica* is registered with higher morbidity and mortality rates in countries like Mexico, India and Africa [(7)]. The number of reported cases in Mexico were 19049.27[(5)]. Amoebas cause this pathogenicity by: adhesion cytolysis, phagocytosis, these mechanisms are regulated by the concentration of calcium in both the parasite and host.Calcium is involved in several biological processes involved in nerve conduction, muscle contractility, secretion and action mechanism of various hormones and cytosolic enzymes, membrane permeability, the coagulation process of the blood and bone mineralization [(1)], the calcium in the body is regulated by ion channels, these ion channels are found in the cellular membranes of animals, plants and bacteria [(4)], the purpose is to study the STIM1 and Orai1 protein *Entamoeba h*, because there is aware of these proteins in this parasite; in this study we use bioinformatics methods.Modeling of these two proteins and was conducted by RT-PCR expression was seen to inmunodetectar technique Western Blot and indirect immunofluorescence in trophozoites of the parasite was used.

In conclusion, we suggest that these two proteins we expressed in *E. histolytica* and also may have a role in virulence mechanisms function.

Understanding copper uptake in Gram-negative bacteria.

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Copper (Cu^{2+}) is an essential micronutrient for all forms of life. Since copper alternates between two oxidation states (Cu^{+}/Cu^{2+}) this metal is ideal cofactor of enzymes catalyzing redox reactions as the cytochrome *c* oxidase a key enzyme of the respiratory electron transport chain. Uptake of Cu^{2+} is the unknowing part of a fine regulated Cu-trafficking network mediated by protein-protein interactions that deliver Cu^{2+} to target proteins and efflux excess of metal to maintain trace concentrations of intracellular Cu^{2+} and avoid toxicity. In Gram negative bacteria Cu^{2+} acquired from the environment must cross the outer membrane before reaching its target proteins. Since Cu^{2+} cannot cross lipid bilayers by simple diffusion it is assumed that Cu^{2+} should cross outer membrane through channels with no or low selectivity. To date, inner and outer membrane proteins (OMP) involved with import of copper has not been characterized in Gram negative bacteria.

In this study we report the identification and partial characterization of a putative OMP involved with Cu^{2+} uptake in *Rhizobium etli* CFN42, a facultative symbiotic diazotroph that must ensure appropriate Cu^{2+} supply for living either free in the soil or as intracellular symbiont of leguminous plants.

The putative Cu^{2+}-uptake OMP described in this study was identified in an isogenic mutant, more tolerant to Cu^{2+} than the parental strain, which spontaneously lost 200 kb of plasmid p42e. This Cu^{2+}-resistance phenotype suggested that a putative Cu^{2+}-uptake protein may be encoded in such region. The maintenance of Cu^{2+}-resistance phenotype in a collection of mutants with smaller deletions of plasmid p42e led us to the identification of the putative OMP encoding gene, *ropAe*, whose disruption increased Cu^{2+} tolerance to *R. etli*. The poor growth of *ropAe* mutant under Cu^{2+} limitation suggests that RopAe is a high affinity Cu^{2+}-uptake transporter.

Proteins with protease activity are secreted by *Leishmania mexicana*, *Entamoeba histolytica* and *Trypanosoma cruzi*

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Introduction: Leishmaniasis, Chagas disease, and Amebiasis are caused by protozoan parasites *Leishmania spp*, *Trypanosoma cruzi,* and *Entamoeba histolytica*, respectively. Unfortunately, there are no vaccines to prevent any of these diseases and parasites have developed resistance to existing drugs. Therefore, it is necessary to identify new therapeutic targets against these parasites. Recent studies have focused on studying proteins secreted by pathogenic parasites, such as *Leishmania spp*, *Toxoplasma gondii,* and *Cryptosporidium parvum*, among others, as probable protection-eliciting proteins in the host. There are few reports of proteins secreted by protozoan parasites and some of them seem to present protease activity. Proteases are classified as serine proteases, aspartic proteases, cysteine proteases, and metalloproteases, depending on the nature of the functional group at the active site. The role that these proteins may have in the parasite biology and/or during interaction with the host is poorly understood. **Objectives:** To analyze and characterize the enzymatic activity of proteins secreted by *Leishmania mexicana*, *Entamoeba histolytica*, and *Trypanosoma cruzi* into the culture medium by using co-polymerized gels and protease inhibitors. **Methods:** Parasites were grown at different temperature and times: *E. histolytica* trophozoites, HM1:IMSS strain, were cultured for three days in TYI-S-33 medium at 37°C; *L. mexicana* amastigotes, MNYC/BZ/H2/M379 strain, were grown in Schneider's culture medium for 7 days at 33°C; and *Trypanosoma cruzi* epimastigotes, TBAR/MX/0000/Querétaro strain, were grown in LIT medium for 7 days at 28°C. To induce protein secretion, parasites were incubated in the different media without fetal bovine serum (modified media) for 0, 6, 12, 18, 24, and 30 h for *E. histolytica* trophozoites; 0, 1, 3, and 5 h for *L. mexicana* amastigotes; 0 and 1 h for *Trypanosoma cruzi* epimastigotes. Modified media were concentrated 10 times of its original volume and used to analyze enzymatic activity in co-polymerized gels, in presence and absence of protease inhibitors. **Results:** We found that *E. histolytica* and *L. mexicana* secreted proteins with protease activity. For *E. histolytica*, we visualized 2 to 5 bands corresponding to proteases with a molecular weight around 37 to 250 kDa. Protease activities were affected in presence of AEBSF (inhibitor of serine proteases) and E-64 (inhibitor of cysteine proteases) inhibitors. For *L. mexicana*, a single band around 75 kDa with protease activity was found. Protease activity was inhibited in presence of AEBSF. However, proteins secreted by *Trypanosoma cruzi* showed no protease activity. **Conclusions:** *E. histolytica* and *L. mexicana* secret proteases with different inhibition patterns.

Functional Analysis of *MNN4*–Like family of *Candida albicans.*

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Candida albicans is an opportunistic fungal pathogen that affects humans, causing either local or systemic infections whose severity largely depends on the host immune response. For the infective process to be efficient, the fungus possesses several virulence factors, but pathogen-host interaction is very strongly influenced by the fungal cell wall, being this structure the immediate point of contact with the host tissues,

C. albicans cell wall is composed of an inner core of chitin, β1,3- and β1,6- glucans and an outer layer of glycosylated proteins rich in mannose-containing oligosaccharides (mannans). Mannans can be modified by mannose residues linked by phosphodiester bonds, called phosphomannans. These structures provide a net negative charge to the cell wall, which has been proved to be relevant for the phagocytosis by macrophages and the action of antimicrobial cationic peptides.

The phosphomannosylation route has been characterized in detail in *Saccharomyces cerevisiae*: Ktr6p is the main phosphomannosyltransferase in this fungus, which is positively regulated by the product of *MNN4* gene. However in *C. albicans* there are two more genes, *MNT3* and *MNT5*, encoding enzymes that contribute 50% of the total phosphomannosyltransferase activity. These data indicate that contrary to *S. cerevisiae*, *C. albicans* has several genes encoding for this biochemical activity.

The *CaMNN4* gene family has seven members, and it is believed that at least one of them has phosphomannosyltransferase activity. We are interested in the study of the members of this family. W previously demonstrated that *MNN41*, *MNN42* and *MNN43* encode phosphomannosyltransferases, while *MNN41* and *MNN42* have an additional role as positive regulators of this process, so far we know that one of the genes does not work as a positive regulator but it works as a phosphomannosyltransferase and another one of them does not work in any function (*MNN43* and *MNN47*). In order to elucidate the phosphomannan biosynthesis in *C. albicans,* we aim to disrupt the last four genes of this family; *MNN44*, *MNN45*, *MNN46* and *MNN47*, and assess the effects on cell growth, morphology, dimorphism and phosphommanosylation levels of the mutants. We will also evaluate the contribution of the products in the phosphomannosylation process to finally propose an integrative model to explain this biosynthetic pathway.

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Identification of nitrogen-fixing bacteria associated with the rhizosphere of different varieties of *Zea mays.*

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In plants, the associationsthat can set up withmicroorganisms areimportantto get the essentialelements for its growth,as the found with atmosphericnitrogen fixers.Nitrogen-fixing bacteriainfluence the growthanddevelopment of plants, sometimes increase yield and production.Bacterial species as *Rhizobium, Azospirillum, Azotobacter, Burkholderia, Paenibacillus* have been reported associated to different crops as bean, peas, grasess, and maize, among other crops. Maize isone of the majorcropsin the world,foritshighfood value and easiness ofcultivation; in Mexico is part of the basic diet of the population. In this study,the nitrogen fixing abilities of diazotrophic bacteriaisolated from therizhosphere of different varieties of maize were evaluated. Plants and rizhosphere soil samples were collected at different localities in Nayarit and Chiapas States. The rhizosphere soil, rhizoplane, roots and seeds were cultured in four different nitrogen-free media, 760 strains with different morphotypes were isolated. DNAfrom some strains was extracted to amplifyand sequencing the 16S rRNAand *nifD* genes. The acetylene reduction test was performed in semisolid media with 24 h of growth of some strains, and in rhizospheric soil supplemented with glucose or glycerol and water. The rhizospheric soil of Chiapas was able to reduce acetylene with a capacity of
9.2nmoles of ethylene/h, proving the presence of a diazotrophic guild associated to maize crop.

Effect of a semi-purified extract of cruzipain on endocytic capacity and cytokine expression in murine macrophage

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Chagas disease is a chronic inflammatory disease caused by infection with *Trypanosomacruzi*.This is anendemic disease in Central and South America and is the first cause of myocarditis in the world. In Mexico is widely distributed and cases are reported all over the country. *T. cruzi* is anhemoflagellate protozoan and produces a large number of enzymes including the cruzipain, a cysteine protease. This enzyme favors the establishment and proliferation of the parasite, promotes its penetration to the cell, favorstrypomastigote output to the extracellular medium, induces metacyclogenesis, modulates the cellular immune response, promotes clonalactivation of B cell, and modifies the balance of iNOS/arginase, among other evasion mechanisms. So, to clarify the mechanisms by which *T. cruzi* evades the body's immune response and to contribute to the knowledge about the parasite-host relationship in this disease, our aim was to analyze the effect of a semi-purified extract of cruzipain (Esczp) in endocytic capacity and in the expression of cytokines by J774 and P388D1 line cells of murine macrophages. The semi-purified extract of cruzipain was obtained from an extract of epimastigotes of *T. cruzi*by affinity chromatography, its enzyme activity was determined by protein electrophoresis in polyacrylamide-gelatin gels and enzimatickinetics by fluorometry. (*T.cruzi* was originally isolated from feces of a *Triatomabarberi* specimen from the state of Queretaro, Mexico, 19 years ago).

Biological assays were performed in the presence of ESczp and ESczp inhibited with E64, the latter in order to determine whether the observed effect was due to the enzymatic activity of the cysteinprotease or itwas an independent effect.Our study of the endocytic activity inJ774 and P388D1 line cells of murine macrophages in the presence of sheep erythrocytes and epimastigotes of *T. cruzi*in the presence of ESczp showed that the endocyticratedecreases in the macrophage line J774 but increases inP388D1macrophages (differences was statistically significant). Finally ESczp effect on the expression of mRNAs for IL-10, IL-12, TNFα and TGF-β by RT-PCR was examined in macrophages of both cell lines and preliminary results showed that cruzipain modifies the expression of the cytokine mRNAs.

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Antibiotic Resistance to First and Second Choice Antibiotics of *E. coli* isolated from Mexican Population with Urinary Trac Infection and its Relationship to Serotype and Phylogenetic Group.

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Introduction: *Escherichia coli* (*E. coli)* is the most frequent urinary pathogen isolated from urinary tract infections (UTI). *E. coli* accounts for as much as 90% of the community- acquired and 50% of nosocomial UTIs.UTI by *Escherichia coli*, although treatable, is now becoming increasingly tough to control because of rampant antimicrobial resistance. Undestanding antibiotic resistance patterns and its relationship with serotypes and phylogenetic groups is epidemiological useful.

Objectives: Determine the antibiotic resistance profile to first and second antibiotic choice for the treatment of urinary tract infection to a collection of *E. coli* strains from a Mexican population with ITU and its relationship with serotype and phylogenetic group.

Materials and Methods: For our study we use 97 *E. coli* strains from hospitalized patients with ITU, donated by Department of Public Health, Faculty of Medicine, UNAM and previously serotyped were used. Resistance profiles to trimethoprim-sulfametosaxol (Tp- Su), nitrofurantoin (NF), ciprofloxacin (CIP), ácidonalidixic acid (NA), ofloxacina (OFX), norfloxacina (NOR) y levofloxacina (LVX) for Kirby Bauer method was determined. Genómic DNA was used to determine the phylogenetic group by multiplex PCR.

Results:71% of *E. coli* strains were resistant to Tp-Su, 21.6% were resistants to NF and 41.2% to CIP. 51.5% of strains were resistant to AN, 44.3% a OFX, 42.3% a NOR y 38.1% a LVX. 11.34% of *E. coli* strains were resistant to all antibiotics tested and 25.77% of *E. coli* strain was resistant to antibiotic second choice and sensible to almost one of first choice antibiotic to ITU terapeutic. The most common serogroups were O4 (9 isolates), O6 (7 strains) and 5 strains of serogroups O2, O25 and O86. Most frequent serotypes were: O4:NM (8 strains), O6:H1 (7 strains) and 4 strains were O1:H6 and O25:H4. The main phylogenetic group was A (39.8%), followed by D group (30.5%), the B2 group was found in 28.8% while the group B1 was present in only 0.85%. The strains of the group A presented more drug resistance than the others, but also presented a greatest serologycal variation. While the strains belonging to phylogenetic group B2 were more sensitive to antibiotics but with a smaller variation in serotype.

Conclusions: It is therefore suggested that appropriate antimicrobial agent be administered to reduce the risk of multi-drug resistance and to avoid the ineffectiveness of antimicrobial agents.

Study of the antimicrobial activity of the venom from honeybee *Apis mellifera*

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The venom from honeybee *A. mellifera* is considered a dangerous toxin, nevertheless its components have different biological properties that could be interesting to apply in the biomedical field. We focused our interest in the antimicrobial activity of the venom from honeybee, *A. mellifera*, by means of an antimicrobial bioassay. It was obtained the Minimal Inhibitory Concentration (MIC), at which the venom was still active versus different Gram positive and Gram-negative bacteria as well as in a microscopic fungus. In parallel, in order to known the toxicity of the venom on mammalian cells, we tested the percentage of vitality of HaCat cells, after 24 hours of incubation. We found not only that the venom has antimicrobial activity on a wide spectrum of microorganisms but also that the MIC values of the Gram positives are in general lower than those from the Gram negatives. This may be attributed to the different components of the cell wall of the bacteria and by the interaction with the elements of the venom. By the way, we found that at the same concentrations at which the venom inhibit bacterial growth, it is not toxic for HaCat cells. The above mentioned is the beginning of a more extended study between the interaction of the different components of the venom with the cell walls, in order to understand how the inhibition is done and it may help to design new antibiotics that do not induce resistance.

The diguanylate cyclase DgcA encoding by *dgcA* has role in biofilm formation in *Azospirillum brasilense.*

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In many well-studied bacteria, proteins containing a GGDEF domain are involved in the production of the second messenger c-di-GMP. However in *Azospirillum brasilense* Sp7 the role of GGDEF domain proteins remains poorly understood.

Here we report that c*gdA* gene encoding for a diguanylate cyclase is involved biofilm formation, exopolysaccharides (EPS) production and fitness colonization to root wheat. It was observed that, quantification of biofilm formation using crystal violet staining revealed that inactivation of *dgcA* affected biofilm formation. In addition, confocal laser scanning microscopy analysis of green-fluorescent-protein-labeled bacteria showed that, during static growth in minimal medium the dynamic imaging showed differently biofilm development, the mutant c*dgA* exhibit biofilm formed with a considerably reduced thickness and loosely attached compared with the wild-type Sp7 strain. Besides, using specific staining for DNA and treatment with DNAse I, epifluorescence studies demonstrated that eDNA is a component of the biofilm matrix in *Azospirillum* strains under the conditions tested. Expression and purification of DgcA were done and the enzymatic activity was determined using GTP as substrate displaying diguanylate cyclase activity. Furthermore, when compared root wheat colonization inoculated with both *dgcA* mutant and wild-type strains, data indicated that *A. brasilense* Sp7 strain was more competitive that *dgcA* mutant in colonization. Thanking together in account all data obtained in this study, we proposed that as other soil bacteria studies, the second messenger cyclic diGMP has a role in association of bacterium with its host plant.

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Motility in *Klebsiella pneumoniae* mediated by flagellum

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Introduction: The genus *Klebsiella* belongs to the family of *Enterobacteriaceae* and is considered as non-motile and non-flagellated. Other members of this family, such as *Escherichia coli* and *Shigella* are considered non-motile, now there are reports that show motility produced by flagella. **Objetive:** Study the motility in *K. pneumonia* isolated, causing nosocomial infection. **Material and methods**: Twenty-five *K. pneumonia* strains were obtained from blood culture of children with nosocomial infections from the Hospital del Niño Poblano, Mexico. These strains were identified by biochemical tests; motility assays were made at different concentrations of Tripticaseine soy soft agar in plate and in tube (with 0.17 to 1.5% agar), at different growth temperatures (25 to 42 °C) and at different incubation times (5 to 72 hours). Control strains were used: *K. pneumoniae* ATCC 7603 (no motile), *E. coli* E2348/69 (motile) and AGT01 strain, (derived from *E. coli* E2348/69, *flic* gene interrupted with a chloramphenicol acetyl transferase cassette). The confirmation of the identification of the *K. pneumoniae* (kpBUAP021) isolated was made with the amplification by PCR and sequencing of the *16S rRNA*, *rpoB* and *gyrA* genes. The phylogenetic group was permormed by Brisse and Verhoef, 2001 method. MLST was performed as described by Diancourt et al., 2005. Detection of *fliC*, *fliA*, *fliD* and *flgH* genes were performed by amplification and sequencing. The flagellum was observed by negative staining for electron microscopy (JEM 1400). **Results:** We show, with a motility assay under different physiological growth conditions, that of a group of 25 *Klebsiella* isolated, one strain (KpBuap021) shows motility due to the presence of a flagellum. This strain was genotype as *Klebsiella pneumoniae* by a sequence analysis of the genes *16S rRNA, rpoB* and *gyrA* and a MLST analysis showed that the strain has a sequence type ST 345 and is part of the fillogenetic group Kpl. We found the presence of the *fliC*, *fliA, flgH* genes (with an identity > 90% with genes of the *E. coli* and *Shigella flexnerii)*, and the absence of the *fliD* gene, this reinforces the findings obtained with electron microscopy of transmission of a polar flagellum. **Conclusion:** This data is important to consider in the evolution of *Klebsiella pneumoniae*.

In silico design of a chimeric protein for a vaccine generated against urinary infections by Uropathogenic *Escherichia coli.*

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Introduction. Urinary tract infections (UTI) are the third cause of morbidity in Mexican pediatric population. Uropathogenic *Escherichia coli* (UPEC) is the cause of 70-90% of community-acquired UTI and 30-50% of nosocomial UTI. UTI by UPEC have been complicated by an increase in multidrug resistance. Therefore, we propose to generate a chimeric protein designed with the FimH, CsgA and PapG adhesins of UPEC in order to implement a viable vaccine that allows us to reduce UTI in the pediatric population of Mexico.
Objective. *In silico* design a chimeric protein based on FimH, PapG and CsgA.
Methods. Bioinformatic tools for the analysis of genomic-expression comparative, secondary structure prediction, physicochemical parameters, three-dimensional molecular modeling and evaluation of the immune response were employed.
Results. Homology was observed in multiple alignment of the *fimH*, *papG* and *csgA* gene of CFT073, UTI89 and ABU83972 strains of *E. coli*. Construction of the chimeric CsgA-FimH-PapG protein (FPC) was generated using *fimH*, *papG* and *csgA* linked with a oligonucleotide encoding for the $(EAAAK)_5$ peptide. Secondary structure (GOR4 server) and three-dimensional molecular structure (*ab initio*) predictions of the FPC protein showed high levels of beta folded structures; while, the peptide showed a rigid alpha helix. The FCP protein was optimized by refinement and minimization cycles (KoBaMIN and NAMD software), showing a ΔG (Gibbs free energy) of -13866.6 kcal/mole, without conformational changes or loss of structural folding (molecular dynamic). Ramachandran plot (PROCHECK program) of the 3D structure FCP showed that 92.5% of amino acids were located in allowed regions. Structure FCP showed Z score of -10.66 (ProSa server) corresponding to data obtained from X-ray structures solved. Our model requires optimal translation conditions that allowing increased expression levels of FCP. Therefore, usage codon optimization (OPTIMIZER server) showed a codon adaptation index of 1.0 as indicative of the FCP expression in BL21 (DE3) *E. coli*. The predicted secondary structure of mRNA (Mfold server) revealed an ideal hairpin at 5' site and ΔG of -844.9 kcal/mole, indicative of stable mRNA in aqueous solution. Moreover, best epitopes were selected of FCP protein showed 22 linear peptides (BCpreds and AAPpreds servers) corresponding to recognized molecules by the B cell receptor and triggers humoral response; also discontinuous epitopes for B cells prediction (Discotope server) showed eight conformational regions: one in the lectin domain of FimH, two in the pilin and lectin domains of PapG and four in the beta turns of CsgA. Additionally, high affinity molecule to major histocompatibility complex class II of the FCP protein were identified (NetMHCII program), showed 25 peptides. Allergen protein prediction and IgE epitope mapping (AlgPred) revealed that FCP was not detected as potential allergen.
Conclusion. FCP protein can be expressed and purified *in vitro* using to *E. coli* as an ideal model. In addition, FCP can be considered a potential target for the generation of a vaccine against urinary tract infections by UPEC.

Characterization of new genes regulated by HilD in *Salmonella enterica* serovar Typhimurium

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Salmonella enterica serovar Typhimurium (*S*. Typhimurium) is a food-borne pathogenic bacteria that causes severe enteritis in humans and different animals, and a systemic disease mainly in mice. Most of the *Salmonella* virulence genes are clustered in chromosomal regions denominated *Salmonella* Pathogenicity Islands (SPIs). SPI-1 encodes a type III secretion system (T3SS), different effector proteins and their respective chaperones, as well as transcriptional regulators that control gene expression within this island. These effector proteins are translocated into the intestinal epithelial cells from the *Salmonella* hosts, through their cognate T3SS, which leads to *Salmonella* invasion of the intestinal epithelium causing enteritis. The SPI-1 genes are expressed *in vivo* when *Salmonella* is in the intestine of its hosts and *in vitro* when growing in nutrient-rich Luria-Bertani (LB).

HilD is a SPI-1-encoded AraC-like transcriptional regulator that induces the expression of all the genes within this island, as well as other genes located outside of SPI-1. HilD directly induces the expression of HilA, an OmpR/ToxR-like regulator that in turns induces the expression of the AraC-like regulator InvF. Therefore, HilD can regulate its target genes directly or through HilA and InvF.

Recently, comparative RNA-seq-based transcriptomic analyses of wild type *S*. Typhimurium and its Δ*hilD* derivative revealed at least 18 HilD-regulated additional genes encoding hypothetical proteins (Colgan *et al*., unpublished). In this work, we started to characterize 3 of these genes, here denominated *grh1-3* (Gene Regulated by HilD). By using transcriptional fusions to the *cat* reporter gene we confirmed that HilD positively regulates *grh1*, *grh2* and *grh3*. Furthermore, our results indicate that HilA and InvF are not required for the expression of these genes, which strongly suggests that HilD directly controls their expression. Currently, in order to address a potential role of these genes in *Salmonella* virulence, we are constructing *S*. Typhimurium *grh1*, *grh2* and *grh3* deletion mutants, as well as analyzing the expression and potential T3SS-dependent secretion of their protein products by tagging the *grh1*, *grh2* and *grh3* chromosomal genes with the 3XFLAG epitope.

Molecular characterizationand Indole-3-acetic acid production of native *Azospirillum* strains from diverse origin.

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The bacteria belong to *Azospirillum* genus are facultative aerobic, nitrogen-fixing species, which are associated with the rhizosphere of various plants. *Azospirillum* strains excrete plant hormones that have beneficial effects on both plant growth and yield, and have been implicated in disease suppression [1]. Members of *Azospirillum* genus exhibit a broad ecological distribution and have been isolated from different geographical regions and plants.

This study focuses on Azospirilla PGPR strains that were isolated from rhizosphere and endophiticregions from rice, wheat, maize, sugar cane and cactus. The majority of the *Azospirillum* isolates affiliated, according to the 16S rRNA gene with *A. brasilense* and *A. lipoferum* and we assessed sequence diversity of internal spacer regions (ISR) in conjunction with 16S rRNA, *ipdC, hisC1* and *hisC2* sequences, to enhanced phylogenetic resolution of these isolates. Then,this approach generates unique fingerprints for each isolates of *Azospirillum*facilitating their discriminationat strain level. Their IAA production was also investigated all *A. brasilense* isolates produced IAA ranged from 10 to 42μg/mg protein, whereas *A. lipoferum* isolates produced lower levels (0.1 to 2.5μg/mg protein), in addition *ipdC* gene was not present, suggesting that IPyA pathway was not present in *A. lipoferum* isolates. Our data demonstrated that all tested strains harbor two types of ISR, ISRL and ISRS, the differences in intensities betweenISRL and ISRS PCR amplicons could reflect the copy number of rRNA operons (*rrns*) containing the respective ITS1. Nine*rrns*operons have been identified within each of sequenced genomes of *A. lipoferum* 4B and *A. brasilense*Sp245, [2]. Our phylogenetic data suggested that strains *A. brasilense*40Ma and 42Mb isolated from the rhizosphere of maize, respectively are closely related. Finally, the present findings, taken together, indicate a combined analysis based on genetics and phenotypes features improve discrimination between strains belonging to *Azospirillum* genus and provide a useful method to identified strains suitable for field studies.

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Role and subcellular localization of EGL-1 and EGL-2, two putative GPI anchored cell wall β (1-3) endoglucanasas, in *Neurospora crassa.*

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Hyphal growthpresumably involvesa balance between synthesis of new cell wall polymers and hydrolysis of pre-existingpolymers. EGL-1 (NCU06381) and EGL-2 (NCU09175) are two putative β-(1,3)-endoglucanases in *Neurospora crassa*, with predicted binding sites for glycosylphosphatidylinositol (GPI anchor). Both GFP-tagged proteins were observed at the plasma membrane (PM),forming a collar in the hyphal apical dome. When co-expressed with CHS-1-mChFP or after staining with FM4-64, both GFP-tagged proteins were observed excluded from the Spitzenkörperand from the foremost PM apical region, where biosynthetic enzymes have been previously found. EGL-1-GFPand EGL-2-GFP were also observed at the leading edge of a new developing septum, which advanced centripetally until reaching the edge of the septal pore.Both endoglucanases were observed at unreleased interconidial septa. During conidial isotropic growth, the fluorescence, initially concentrated at the conidial poles, was found all around the conidial surface. EGL-1-GFP fluorescence at interconidial septa was lost in a *N. crassacsp-2*Δmutant, unable to release conidia from conidiophores. Both tagged proteins were observed at sites of hyphal fusion and emerging branches, confirming arole for these enzymes at sites of cell wall remodeling and at sites of new cell wall synthesis. Under treatment with latrunculin A, an actin inhibitor that induces a loss of hyphal polarity, both proteins no longer accumulated at the apical collar.Single mutant strains *egl-1Δ* and *egl-2Δ*and double mutant *egl-1Δ::egl-2Δ*exhibited a slightlyreduced growth rate.Single mutant *egl-1Δ* presentedmore chains of unreleased conidia and fewer free conidia, while *egl-2Δ* produced both few conidial chains and a significantly lower amount of free conidia, confirming a role for these genes in conidial formation.The double mutant *egl-1Δ::egl-2Δ*produced more conidia than wt, suggesting a compensation or genetic suppression. When exposed to calcofluor white and congo red, two dyes that intercalate betweenchitin fibrils and glucans,and cause a decrease in cell growth in fungal hyphae, the single mutant *egl-2Δ*and the double mutant *egl-1Δ::egl-2Δ*displayed a higher growth rate than the parental strain. This “hyper resistant” response could result from a rougher cell wallpresent in thesemutants, composed of longer β (1-3) glucan chains and therefore fewer residues available to get crosslinkedto chitin. In contrast, the single mutant *egl-1Δ* displayed a reducedgrowth ratewhen exposed to the two inhibitors.We suggest that EGL-1 and EGL-2 mayhave a role in the shortening ofpreformed glucans in the cell wall, whichcould facilitate the construction of new cell wall at the apex, septa and interconidial septa. EGL-2 seems to act primarily at apical sites of growth, while EGL-1 seems to function mainly at sites of conidial formation. These and previous studiessuggest a division of labor during cell wall synthesis at the hyphal dome: at the very tip, long chains of glucans are synthesized by enzymes that accumulate at the Spk prior to their incorporation at the apical plasma membrane, whereas at the subtending zone below the apex glucansare hydrolyzed into oligosaccharideswith reducing ends amenable for further crosslinking with other glucans and chitin.

"Taxonomic diversity of the carnivorous plant microbiome *Utricularia gibba*"

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The study of bacterial and other microorganisms have limitations; the studied microbes from pure cultures represents less than 1% of the diversity from many environmental samples . Genome analysis of microorganisms by metagenomics, allows the analysis of the whole microbial community; metagenomics aims the fine study of biodiversity, both taxonomic and functional levels. Next generation sequencing technologies had allowed to get large amounts of data per sequencing run and consequently a detailed description of the microbial communities, including rare taxa. *Utricularia gibba* is an aqaticous carnivorous plant that usually lives in calm fresh waters which are usually nutrient deprived environments. *U. gibba* complements its autotrophic nutrition by trapping within its small suction traps several preys from crustaceans, mites, nematodes, rotifers and protozoa. Within *U. gibba*'s traps it is suggested that photosynthetically absorbed carbon is secreted, which suggest that the carbon shared by the plant benefits their associated microbial community (microbiome). In the plant-microbiome interaction it is expected that the nitrogen and phosphorous derived from this community may be assimilated by the plant in a manner similar to terrestrial plants' rhizospheres and its microbial interactions. Recently, *U. gibba'*s genome was sequenced and so there is a predicted inventory of the plant gene functions (Ibarra-Laclette et al., 2013) .

We sequenced the the total metagenome (WGS) of *U. gibba'*s traps as well as it surrounding environment (water and sediment), using the Illumina MiSeq (PE-250) platform. The goal of this work was to perform the taxonomic determination of the *U. gibba's* microbiome and its surrounding environment . We compared the the *U. gibba*'s microbiome with seven metagenomes available in databases: three belonging to a plant-microbe microbiome, two of fresh watter, and two of soil. The metagenomic comparisons allowed us to establish a baseline and contextualize the diversity of *U. gibba*'s microbiome.

We calculated parametric and non-parametric diversity indexes, as well as grouping techniques such as correspondence analysis to determine relatedness across the compared metagenomes. Likewise, we described the over-represented bacterial families who highlighted the differences between *U. gibba*'s microbiome and the rest of the analyzed metagenomes. Finally, we made a comparison of different methods for the taxonomic assignment of the metagenomes, using databases as Ribosomal Database Project, the Lowest Common Ancestor method and fragment recruitment for the *U. gibba*'s microbiome against the bacteria sequenced genomes .

Reference.

Ibarra-Laclette, E., Lyons, E., Hernández-Guzmán, G., Pérez-Torres, C. A., Carretero-Paulet, L., Chang, T.-H., … Herrera-Estrella, L. (2013). Architecture and evolution of a minute plant genome. *Nature*, *498*(7452), 94–8. doi:10.1038/nature12132

Mannheimia haemolytica expressesanOmpP2-likeamyloid protein

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Mannheimia haemolytica (Mh) causes significant economic losses to the livestock industry because it causes respiratory disease in cattle. Different virulence factors, such as the leukotoxin, lipopolysaccharides, capsule, adhesins, biofilm formation and several proteases, are expressed during Mhinfection and contribute to disease. In biofilms, amyloid proteins are major protein component, function as scaffolds which aid in the adhesion and confer resistance against a variety of environmental insults. Amyloid proteins are highly resistant to protease digestion and to physical and chemical denaturation. Among its features are its ability to be stained with the dye Congo red (CR) and its property exhibiting green birefringence under polarized light. The purpose of this study was to characterize an amyloid protein of approximately 40 kDa CR binding expressed by Mh, resists digestion with formic acid and is stable to boiling heat. This protein was purified from cultures of Mh grown at 37 ° C for 48 hours; was recognized by polyclonal anti-curli of *E. coli* (a widely characterized amyloid protein), anti-amyloid protein of 40 kDa of *Gallibacterium anatis* and sera from animals infected with Mh, suggesting its expression *in vivo* and its likely involvement in the immune response. By immunolabeling andtransmission electron microscopeobservations, it is appreciated that the antibodies are associated with long, thin fibers attached to the bacterial surface. Mass spectrometry indicates that it is a Mhmembrane protein P2-like which exhibits similarity to outer membrane proteins of other microorganisms members of the *Pasteurellaceae* family. The *in silico* analysis of the amino acid sequence indicates that this protein has auto aggregative properties and have 8 amyloid peptides. Amyloid proteins expressed by Mh may be important in its pathogenicity and biofilm formation. Project supported by PAPIIT IN222313 and PAPCA- FESI (N° 8), UNAM.

Enhancement of insecticidal activity of*Bacillus thuringiensis* Cry1Ab toxin against *Spodopterafrugiperda* (*Lepidoptera:Noctuidae*) through alanine substitution in domain III.

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*Bacillus thuringiensis*Cry1A toxins are environmentally friendly, highly specific insecticides widely used to control important pests of corn and cotton. This pore forming toxins, synthetized as a crystal during sporulation, are solubilized and proteolitically activated in the midgut upon ingestion by susceptible insect larvae. However, certain pests such as *Spodopterafrugiperda,* an important corn pest in Latin America, show low susceptibility to Cry1A toxins.

The proteolitically activated toxin is composed of three functional domains, domain I is involved in pore formation and membrane insertion, whereas domain II and III play a role in the interaction with membrane molecules that function as receptors, previously the β16 (^{509}STLRVN^{514}) and β22 (^{583}VFTLSHV^{590}) sheets located in domain III have been suggested as important regions for the interaction between the toxin and the membrane proteins alkaline phosphatase (ALP) and aminopeptidase (APN)in the lepidopteran*Manducasexta*. We performed alanine substitution on the aminoacids that comprise both sheets in order to asses the effects on toxicity. Interestingly, certain amino acid substitutions in β16 region increased the insecticidal activity of Cry1Ab against*Spodopterafrugiperda*, with none or maginal effect on toxicity to *Manducasexta*, we observed the same phenomenon in mutants from the β22 region. The enhanced insecticidal activity of Cry1Ab mutants correlated with increased binding to *Spodopterafrugiperda* brush border membrane vesicles (BBMV). The domain III mutants characterized in this study could provide useful insecticidal Cry1Ab engineered toxins for *Spodopterafrugiperda*control in the field.

Description of hydrocarbonoclastic activity and putative function of the genes *CYP52* of *Yarrowia lipolytica*

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INTRODUCTION

Yarrowia lipolytica is a yeast of biotechnological interest that has gained importance due to its ability to utilize hydrocarbons as carbon source. It is suspected that in the first step in the degradation of hydrocarbons in this yeast are involved enzymes of the family of cytochrome P450 encoded by *CYP52*genes. The objetives of this study were to describe the spectrum of degradation of several hydrocarbons as carbon source, detect the presence of *CYP52* genes in a collection of strains of *Y. lipolytica,* and predict the possible functions of CYP52 genes by an insilico analysis.

RESULTS AND DISCUTION

From a set of five strains of *Y. lipolytica*isolated from soil contaminated with gasoline and identified by molecular tools, the hydrocarbonclastic ability was evaluated by measuring the growth with linear volatile aliphatic hydrocarbons in the vapor phase as a sole carbon source.In some strains, growth was more efficient when hydrocarbons of 8, 9 and 12 carbon atoms were used.Strains were partially phenotyphicatedby testing assimilation of different carbon and nitrogen sources, and genotyphicated by BOX-PCR. The results demonstrate that *Y. lipolytica* strains are phenotypically and genotypically variable and have anefficient hydrocarbonclasticcapacity.Partial sequences of eight *CYP52* genes of *Y. lipolytica* and the related species were phylogenetically analysed, the presence of 6 paralogous and 2 orthologous groups in this gene familiy indicate that just the 2 orthologous groups present the hydrocarbnoclastic activity. Furthermore, it appeared that the rest of *CYP52* genes found in *Y. lipolytica* strains isolated in our research group may have varied functions, likely involved in lipid and ergosterolbiosynthesis, as well as in the degradation of hydrocarbons under different environmental conditions.

Isolation, characterization, andmolecularidentification ofPGPR fromhalophilic grass*Distichlisspicata*(L.) Poaceae.

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ABSTRACT:

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial bacteria known to rapidly colonize the rhizosphere, suppress soil borne pathogens, and stimulate growth to plants by different mechanisms. Salinity is a major factor reducing crop productivity and major cause of the abandonment of lands for agricultural purposes. An alternative strategy to improve crop salt tolerance may be introduce salt-tolerant microbes that enhance crop growth.The aim of thisstudywas isolate PGPR ofendorhizosphereandectorizósferafrom *Distichlisspicata*an halophilic grass foundsurrounding asalt pond atSobacoValleylocatedin SanPedromunicipality, Coahuila, Mexico. PGPRwere isolatedusingLB, KB,JensenandNFbnitrogenfree culture medias. Theability ofbacteriato promoteplant growth was assayed *in vitro* using *Arabidopsisthaliana*seedssown in MS culture media and inoculated with PGPR. The bacteria identification was made to bacteria with promotion activity in Arabidopsis seedlings, for the molecular identification 16S rRNA amplification and sequencing was used. 75 bacteria was isolated from *Distichlis spicata*rhizosphere and as preliminary results two bacteria promotes growth in Arabidopsis seedlings, these bacteria belong to Bacillus genus according to molecular identification. Until now, the results suggest that in the rhizosphere of *Distichlis spicata* inhabit beneficial bacteria with potential for agriculture use in saline lands.

Identificationof receptor ryanodine (IP3) in*Entamoeba histolytica*

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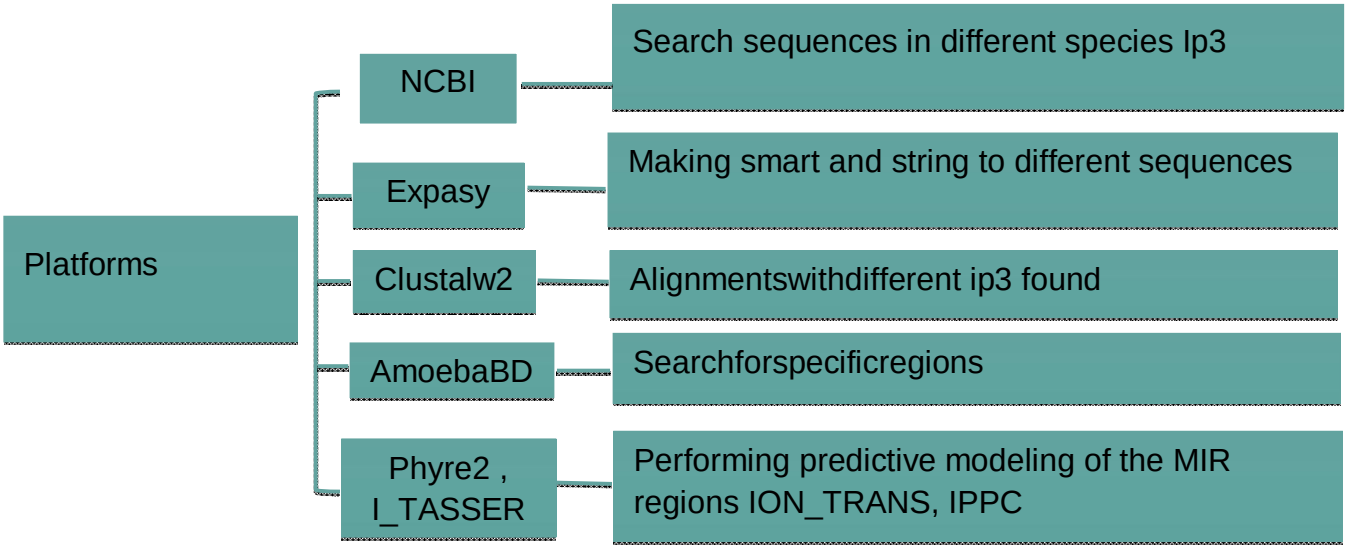
Keywords: *Entamoeba histolytica*, Ryanodine (IP3)

INTRODUCTION. *Entamoeba histolytica*, is responsible for human amoebiasis in protozoan parasite. This disease affects 10% of the world population, are frequent in developing countries with tropical climate and is responsible for about 100,000 deaths per year worldwide. (World Health 1997). Membership, contact dependent cytolysis, and phagocytosis: The pathogenic mechanism of E. histolytica three stages on the target cell are distinguished. (Campos Peralta and Jose Manuel 2010). Inositol-1, 4,5-triphosphate (IP3) a water-soluble product diffuses from the plasma membrane to the endoplasmic reticulum, where specific receptors and via G protein binds produces massive opening Ca2 + channels, allowing the exit of cytoplasmic calcium stored inside the endoplasmic reticulum. Acting as a second messenger responsible for the mobilization of stored Ca2 +. (Jesus Merino and M. José Noriega 2011).

To identify by bioinformatic techniques sequences encoding for ryanodine receptor (IP3) in databases Entamoeba histolytica protozoan parasite.

METHODOLOGY:



RESULTS AND DISCUSSION. In most all living beings IP3 common topology is MIR, RYDR-ITPR, RIH-ASSOC, ION-TRANS however, Entamoeba histolytica only the MIR, ION_TRANS, IPPC localized regions, regions that were not found are: RYDR-ITPR, RIH-ASSOC, these regions probably not in this parasite, are not essential for its virulence mechanisms.

Purification and kinetic analysis of cytosolic and mitochondrial Thioredoxin glutathione reductase extracted from *Taenia solium* cysticerci

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Taenia solium, the agent that produces neurocisticercosis, one of the major central nervous system (CNS) parasitic diseases in humans, lacks both of thioredoxin reductase (TrxR) and glutathione reductase (GR), two major enzymes associated to detoxification mechanisms. Instead of those proteins some platyhelminthes, exhibit a GR and TrxR molecular link with the fusion of glutaredoxin (Grx) and thioredoxin reductase (TrxR) domains into a single protein selenocysteine-containing enzyme thioredoxin glutathione reductase (TGR). In order to establish the presence of TGRs in *T. solium* tissues, protein purification was performed on cytoplasm and mitochondrial from cysticerci. Protein purification chromatography procedures were developed to purify the enzymes and enzyme kinetic techniques were performed to characterize the cytoplasm (cTGR*Ts*) and mitochondrial (mTGR*Ts*) proteins from *T. solium.* Both enzymes are dimers (132,000 kDa) and were more catalytically active towards glutaredoxin, followed by thioredoxin reductase and lastly by glutathione reductase. cTGR*Ts* also showed hydroperoxide reductase activity with a specific activity of 0.21 U mg^{-1} using hydroperoxide as substrate. The $K_{m(DTNB)}$ and $K_{cat(DTNB)}$ values for cTGR*Ts* and mTGR*Ts* (88 µM and 1.9 s^{-1}, 45 µM and 0.04 s^{-1}, respectively) and the $K_{m(GSSG)}$ and $K_{cat(GSSG)}$ values for cTGR*Ts* and mTGR*Ts* (6.3 µM and 0.96 s^{-1}, 4 µM and 0.024 s^{-1}, respectively) were similar or lower than those reported for mammalian TGR. The second to the twelve st amino acids from cTGR*Ts* and mTGR*Ts* N-terminal group were APIGGSAEQVEK indicating that both enzymes are coded by the same gene and this region is identical to *Echinococcus granulosus* TGR. cTGR*Ts* was inhibited by auranofin, a selective inhibitor of thiol-dependent flavoreductases (I_{50} = 3.25 nM, 2.29 nM for DTNB and GSSG substrates respectively). Glutathione reductase activity of cTGR*Ts* and mTGR*Ts* exhibited hysteretic behavior as is observed with TGRs. These data suggest the existence of an effective substitute accounting for the lack of glutathione reductase and thioredoxin reductase in *T. solium.*

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Evaluation of the non-catalytic binding function of Ts26GST a glutathione transferase isoform of *Taenia solium*

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Taenia solium, the agent that produces neurocisticercosis, one of the major central nervous system (CNS) parasitic diseases in humans, presents several isoforms of glutathione transferase (GST). These proteins belong at major phase II detoxification enzymes that catalyze the conjugation of xenobiotics to glutathione. But GSTs have also a ligandin activity, referred to a detoxification mechanism characterized by an absence of catalytic enzyme function. Because there is not information about ligandin activity exhibited by any *T. solium* GST isoforms we decided to evaluate this function in Ts26GST a GST isoform of this cestode. Protein purification chromatography procedures were developed to purify the enzyme and assessed by inhibition kinetics, fluorescence spectroscopy and competitive fluorescence assays with 8-anilino-1-naphthalene sulphonate (ANS). Ts26GST was observed to bind non-catalytically to porphyrins, trans-trans-dienals, bile acids and fatty acids, as assessed by the techniques described above. The quenching of Ts26GST intrinsic fluorescence allowed for the determination of the dissociation constants (K_D) for the twelve ligands employed. Obtained data indicated that Ts26GST binds to all ligands but with different affinity. Porphyrins and lipid peroxide products inhibited Ts26GST catalytic activity up to 100% in contrast with only 20-30% inhibition observed for bile acids and two saturated fatty acids. Non-competitive type inhibition was observed for all enzyme inhibitor ligands except for trans-trans-2,4-decadienal, which exhibited uncompetitive type inhibition. The dissociation constant value K_D= 0.7 µM for the hematin ligand, determined by competitive fluorescence assays with ANS, was in good agreement with its inhibition kinetic value Ki = 0.3 µM and its intrinsic fluorescence quenching K_D = 0.7 µM. The remaining ligands did not displace ANS from the enzyme suggesting the existence of different binding sites. In addition to the catalytic activity of Ts26GST the results obtained suggest that the enzyme exhibits a ligandin function with broad specificity towards nonsubstrate ligands.

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Homologue sequences to Iron Regulatory Protein in *Giardia duodenalis* genome.

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Iron is an important element for the survival of all living cells including protozoan parasites. Recent studies show the great necessity of Giardia by iron and the direct relationship of the presence of this parasite in children with anemia. However, mechanisms to maintain cellular homeostasis in these organisms have been poorly studied. Posttranscriptional regulation by the IRE/IRP system is studied in humans and consists of iron cytoplasmic regulatory proteins that interact with iron responsive elements (IRE). When iron levels are high the IRP binding activity is inhibited, however, when cellular iron levels are low, the IRP binds to IRE. Recently in *T. vaginalis,* were found stem-loop structures which bind to both recombinant human IRP and cytoplasmic extracts of the parasite in low iron conditions and recently, an IRP-like protein. By their evolutionary proximity and the importance of iron in these protozoa, it is hypothesized that *G. duodenalis* may also have some iron regulatory proteins. The aim of this study was to search iron regulatory proteins in *G. duodenalis* to suggest a post-transcriptional IRE/IRP mechanism.

IRP sequences search were performed using as probe: cytoplasmic iron regulatory protein 1 (*Homo sapiens*), iron regulatory protein 1A (*Drosophila melanogaster*), iron regulatory protein 1B (*Drosophila melanogaster*), iron regulatory protein (*Plasmodium falciparum*); which were obtained from GenBank (www.ncbi.nlm.nih.gov), used to search for homologies in *G. duodenalis* genome (www.giardiadb.org).

Our data show that sequences in Giardia genome shared homology with: human IRP GL50803_14790 (32%) and GL50803_7555 (20%), IRP-1A protein of *D. melanogaster* GL50803_11099 (35%), GL50803_103818 (33%), GL50803_14790 (30%), GL50803_16220 (30%) and GL50803_86821 (26%), IRP-1B protein of *D. melanogaster* GL50803_11389 (32%) and GL50803_93551 (27%), IRP-like of *P. falciparum* GL50803_12174 (38%), GL50803_17385 (38%), GL50803_28635 (30%), GL50803_35341 (25%) and GL50803_9327 (23%). All of them encoded for hypothetical proteins.

In conclusion, this is the first evidence of IRP-like proteins in *Giardia* which may suggest the presence of an IRE-IRP mechanism in this protozoon, experimental studies are in process.

Physiological and molecular analysis of *Sclerotiumcepivorum*Berk mutant not forming sclerotia

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*Sclerotiumcepivorum*Berk, a member of*Sclerotiniaceae* family, who can attack crops of onion, garlic, leek, among others, and it can cause losses up to 100% of crops. The main problemin controlling this diseaselies in itsability to formresistance structurescalledsclerotia, which can remain viable insoil in absenceof the hostup to 20 years.

Conidiation process in*S. cepivorum*has been slight studied and whenitformsconidia,there are sterile.There are no scientific reports of strains of *S. cepivorum* unable to form sclerotia; in our group, we obtained a mutant that does not form sclerotia. Thismutantonly formsconidia under conditions that normally would trigger the formation ofsclerotia. The analysis of specific molecular markers, 18Sribosomalgene and ITS sequence, showed that it is a strain of *S. cepivorum*Berk. Southern blotanalysisandRT-PCR have demonstrated thepresence and expression ofsc1gene codingforthe major proteinof thesclerotia(SC1); Howeverinthe mutant, theCS1protein isnotdetectableby SDS-PAGE, suggestinga regulatoryroleinthe formationofSC1sclerotia. Physiological studiesofthemutant straindemonstrated thepresenceoflarge amount of conidiophoreswithconidia,ofthese,3 to 5% have no nucleusandthey areable to germinateinliquidor solid medium.The studies of this mutant allow us toclarifythe role ofproteinSC1and demonstratean alternative pathwayofstructure formationof asexual reproductionfor*S.cepivorum*under normal conditions.

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Identification of a casein kinase 2 (TvCK2) in *Trichomonas vaginalis*

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Three different *pI* isoforms of the initiation factor eIF5A in *Trichomonas vaginalis* (TveIF5A) have been reported. The most acidic isoform (*pI* 5.2) corresponds to the precursor TveIF5A, whereas the mature TveIF5A appears to be the most basic isoform (*pI* 5.5). Besides an intermediary isoform (*pI* 5.3) is found only under polyamine-depleted conditions and restored with exogenous putrescine. Differences in the *pI* of the TveIF5A isoforms are due to posttranslational modifications. The mature TveIF5A contains four phosphorylated residues (S3, T55, T78 and T82). Phosphorylation at S3 and T82 is also identified in the intermediary TveIF5A, while no phosphorylated residues were found in theTveIF5A precursor. The objective of this work was to identify to a kinase that might be involved in the phosphorylations of TveIF5A.

Methodology: The casein kinase 2 (CK2) gene sequences from several species were obtained from the GenBank database. The search for the CK2 gene in the TrichDB genome database was performed by using the consensus sequences of CK2 from *Leishmania major, Homo sapiens, Arabidopsis thaliana and Saccaromyces cerevisiae* as query. The CK2 amino acid sequence of *T. vaginalis* (TvCK2) was aligned by ClustalW program and it was used to determine the percentage of identity and E values with the related proteins by BLAST. Total RNA from *T. vaginalis* isolate CNCD147 was reversely transcribed to obtain cDNA which was used to amplify the *tvck2* mRNA. Finally the *tvck2* was cloned and sequenced.

Results: Since eIF5A proteins from plants and yeast are phosphorylated by CK2, we performed an *in silico* analysis in order to find a homologous protein to CK2 in *T. vaginalis* genome. Our results showed that the locus TVAG_064190 contains a 1023 bp open reading frame (ORF) potentially encoding a protein of the CK2 kinase family (*tvck2*). Protein sequence alignment analysis showed that the TvCK2 residues 50–333 correspond to the protein kinase domain containing the ATP-binding region signature (56- VGTGKYSDVFtAykgdtk...VAIK-77) and the S/T protein kinase active site signature (161-ImHrDVKplNILF-173). In addition, gene expression of *tvck2* was detected in the CNCD147 isolate of *T. vaginalis* by using RT-PCR. Since the consensus substrate sequence for CK2 protein family (S/T-x-x-E/D/pS), was found in the phosphorylated S3 residue (MSSAE...) and in the phosphorylated T82 (..TSHE..) in TveIF5A, these findings suggest that TvCK2 might be involved in the phosphorylation of TveIF5A. However, we cannot rule out the possibility that other kinases are involved in phosphorylation of TveIF5A, since there are approximately 880 putative kinase genes in *T. vaginalis* genome, which encode distinct protein kinases.

Searchingfor molecular targets using the *K1* toxin from *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae is a eukaryotic microorganism,which is amply used in different industrial processes, in laboratory experimentation and food processes,it is being described that are some yeastbelonging to *S.* cerevisiae that arecharacterized for having debris from viral dcRNA. This characteristic let them produce a toxin denominated *K1* (K^+ *strain*) that let the K^+inhibit the cells that don´t have the strain (K^-). The toxin *K1* has a protein origin made by 316 amino acids forming 4 subunits. (1).

Experiments from Ahmen and collaborators in 2001 show that the molecular target of the toxin is the ionic channel Tok1, the interaction between the channel and the toxin increase open probability of single Tok1 chnnels; via reversible destabilization of close states, causing a depletion of potassium y consequently the death of the yeast K^- (2). It is also reported that the K^+ present immunity to the *K1*, this is caused by using the protoxin (*K1 immature*) coupling it in the intracellular side of the channel Tok1. (3)

It is true that the general activity mechanisms form the toxin *K1* are known but the possible effect that it can have on other organisms and the interaction of *K1* with homologous molecular targets has not been wide explore. To determinate this interaction, we proposed to study the inhibitory effect on the next microorganisms: *K. pneumonia; L. monocytogenes; S. typhimurium; S. aureus; A. niger; A. flavus; Penicillum spp.;Fusarium spp.* We propose that these microorganisms can be inhibited, this hypothesis has basis in the *in sillico* analysis made by our laboratory in which we found in this microorganisms proteins that have similarly andidentity to the channel Tok1.

Our principal approximation is based on studies of microbiological competition characterizing different physicochemical interactions between the toxin and the microorganisms. In the second stage, we propose designed electrophysiology experiment to determinate the probable mechanisms of the *K1*interaction with Tok1 channel.

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LEA proteins are involved on desiccation resistance and other abiotic stresses in *Azotobacter vinelandii*.

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Azotobacter vinelandii cells can form cysts. Cysts are metabolically dormant cells which are considerably more resistant than vegetative cells to deleterious physical conditions like desiccation.

Water limitation affects all types of organisms at some stage during their life cycle; therefore, many strategies have been selected through evolution to cope with water deficit. In plants, the expression of Late Embryogenesis Abundant Proteins (LEA) correlates closely with the acquisition of tolerance against drought, freezing and salinity stresses. LEA proteins can act as osmoprotectants or chaperones protecting membranes and enzyme activities to prevent protein aggregation.

In *A. vinelandii* we found two genes with homology sharing identity to putative LEA proteins from *Arabidopsis thaliana* and *Artemia franciscana* the sequence of the genes contains a part of the LEA eukaryotic motif, but differs from the distinctive motive found in other bacteria, so this could be an example for the possibility of horizontal gene transfer between domains of life.

We hypothesized that Avin11010 and Avin11020 genes that encode for LEA proteins in *A. vinelandii* are involved in desiccation tolerance and other abiotic stresses in cyst and vegetative cells of this bacteria.

The aim of this work is to analyze if LEA proteins are involved on the resistance to desiccation, heat, frezzing and osmotic stress in both cysts and vegetative cells of *A. vinelandii*.

We constructed mutants for these genes. Cysts and vegetative cells were treated to desiccation for 3 months; osmotic stress was caused by different concentrations of NaCl and Sorbitol. We tested the tolerance of these mutants to high temperatures (60°C for 15 minutes) and frezzing (-20°C for a month). We constructed transcriptional fusions to determine the gene expression under different conditions.

Results: LEA proteins confer tolerance to desiccation on cysts cells after 3 months of desiccation stress and are necessary to survive high concentrations of osmotic agents like NaCl and sorbitol. LEA proteins also protect cysts and vegetative cells against high and frezzing temperatures. These genes are not necessary for encystment processes in *A. vinelandii* and there are no morphological differences between the mutant and the WT cysts. The gene expression for both genes is twofold under encystment conditions and the Avin11010 gene is expressed threefold over the Avin11020 gene expression. It seems that these genes are

Genomic analysis of cyclic-di-GMP-related genes and their functional in *Azospirillum brasilense* Sp245 strain.

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Azospirillum is one of the best-known plant-growth-promoting rhizobacteria (PGPR) genera.This PGPR is capable of influencing the growth and yield of numerous plants species, many of which have agronomic and ecological significance. Crucial for their complex lifestyle is the ability to sense and respond to diverse environmental stimuli, requiring elaborated signaling pathways. Cyclic di-GMP is a second messenger identified in a growing number of bacterial species. Surprisingly, little is known about the importance of c-diGMPC in *Azospirillum.*

Herein, we analyzed the genome sequence of Sp245 strain (Wisniewski-Dyé *et al* PLoS Genet. 7:e102430),in order to compile an inventory of c-diGMP proteins. The cellular levels of c.diGMP are controlled through the opposing activities of diguanylate cyclases, proteins containing a GGDEF domain, and phosphodiesterases, which contains either en EAL domain or an HD-GYP domain. Additionally, it was carried out a functional analysis of three c-diGMP genes.

The annotated genome of *A. brasilense* Sp245 strain encodes for 15 proteins containing the GGDEF domain, 3 with an EAL domain, 12 carrying both domains (GGDEF/EAL domains), and 5 with HD-GYP domain. It was noticed that many of the enzymes implicated in c-di-GMP metabolism are fused to one or several types of signaling domains or received domains at the N-terminus, such as: PAS-PAC, GAF, BLUF, and REC.

We showed that mutation of locusNC_016596 encoding for a protein displays domain architecture MHYT-MHYT-MHYT-PAS-PAC-GGDEF-EAL and signal peptidewas reduced in quimiotaxis. Interestingly the mutation of locus NC_016595.1 encoding for a protein with REC and GGDEF domains showed slower growth and high sensibility to NaCl stress. Whereas the mutation in locus NC_016617.1 which coding for a protein with REC and GGDEF domains was involved in biofilm production.Taken together the data presented suggested that c-di-GMP is involved in signaling in *Azospirillum.*

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Analysis of *Avibacteriumparagallinarum* operon related to quorum sensing and virulence.

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The gamma-proteobacteria member of the*Pasteurellaceae* family*Avibacteriumparagallinarum*is the etiological agent of the infectious coriza. Aseriousupper respiratory tract diseasethat affects birds and hens. This is well-recognized illness causing important economical losses worldwide because of the poor feed conversion in poultry and diminishing ofegg laying in hens up to 40%.Using the haemagglutination inhibition test of Kumé (1983, J ClinMicrobiol 17:958-964),*A. paragallinarum*strainshave beenclassifiedinto 9 serovars and 3serogroups (A, B, and C), being group Cthe mostprevalentserovar in Mexico. As an intend to control the infectious coriza, vaccines are frequently renewed to maintain its efficacy. New strains expressing a panoply of virulence factors such as haemagglutinin, capsule, hemocins, lipopolysaccharides, secreted metalloproteases, membrane vesicles, RTX-toxins, proteins for iron acquisition areneeded nowadays as antigens,as others in the future will be.DNA sequencesof many genes of the encoding those proposed antigens have been found in the recently releasedgenomic databases of three strains.Currently,*A. paragallinarum*CLstrain, is our model system.The draft sequence of the CL strain have been organized in 109 contigs, containing 2.8 million nucleotides, composed of 41% G + C (Vázquez-Cruz *et al*, unpublished).*In silico*analysis of thecontig65 uncovered two keyoperons: the first contains ten genes with high similitude to the quorum sensing genes*lsr* from *Salmonella thyphimurium,* whereas the second contains three ORFs, one of whichencodes for a putative metalloproteaseand two proteins of the type of divalent-ion regulated transporters.We then sought for the presenceof predicted genes in four*A. paragallinarum*strains (CL, CT, 5 and 6) by PCR assays. From the putative *lsr* operon, allten genes amplified only from two strains, CL and 5, but variability was found in the remaining strains; five from ten genes were absent in strains CT and 6. Amplifications of sequences of metalloprotease and the two transporters were positive from total DNA of the four used strains.As this report is the first evidence for *lsr*-quorum sensing system in *A. paragallinarum* Southern blot analysis will help us to understand better the structure and organization of the *lrs*genes in *A. paragallinarum,* including more strains in the assays. Acknowledgments to CONACYT,project CB-2010-01-157028.

A SPCA1 in the Golgi apparatus of *Entamoeba histolytica*

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The Golgi apparatus (GA) is an organelle located near the core and is formed by groups of spherical vesicles or flattened, called tanks; is primary function is to distribute the proteins to their final destinations: lysosomes, plasma membrane or outside the cell. However, in the protozoan parasite *Entamoeba histolytica*, retains no typical eukaryotic cells such as Golgi, has a vacuolar system that performs the same function: it is a major reservoir of calcium.

ATPases and Ca^{2+} pumps.Ca^{2+} transport against the concentration gradient at the expense of ATP hydrolysis. Have high affinity for Ca^{2+} and are highly expressed in the nervous system and in different cell types. Also belongs to the group of P-type ATPases and is responsible for Ca^{2+} refilling of the Golgi apparatus. This study identified a SPCA1 ATPase in *Entamoeba h*. using bioinformatics techniques, confocal microscopy and RT-PCR.

The SPCA1: is encoded by the gene ATP2C1. By alternative splicing have been identified four variants (SPCA1a-d) in humans. The SPCA1 is the most abundant isoform is expressed in virtually all cell types. It has been shown that this pump is crucial in cell differentiation. However, until now its function is unknown in the protozoan parasite *Entamoeba histolytica*, interestingly this parasite uses calcium for different processes such as transcription regulation, motility and erythrophagocytosis.

Given the importance of this protozoan parasite in public health, it causes a mortality of between 40,000 and 110,000 cases per year in the world and in México is considered as an endemic parasitosis, therefore it is crucial to study calcium homeostasis within mechanisms of pathogenesis of this parasite as adhesion, cytolysis and phagocytosis. This study will help to find new molecular and physiological approaches to the treatment of this parasitosis.

Genotypification of subtypes *Streptococcus mutans* of producing cavity in Querétaro, México.

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The World Health Organization (WHO) says that as oral diseases: cavity, periodontitis, and cancers of the mouth and pharynx, are a serious problem for health, affecting industrialized countries and, increasingly, in developing countries.In the poor communities, tooth cavity is the most common. The young population is the group that urges you to attend because it has been estimated that a high proportion have gingivitis. Without specialized medical care may become a periodontopathic and the result will be the loss of tooth.Studies carried out for decades have identified *Streptococcus mutans* as the main causative agent of decay. However, studies of genotypification subtypes and the relationship with the presence of cavity, have been scarce in the world and in Mexico there is no reported. For this reason we consider it important to create a line of research directed at this studio in Querétaro, and thus obtain the bacteriological information, to set up preventive systems that favor the oral health of our population.

Our results show that 50% women and 50% men represent the population captured in this study. It is notable that more than 50% of the patients belong to the city of Querétaro. There is a predominance of patients of 18 years and that most have a DMFT (decayed, missing or filled tooth) of between 1 and 5. Which shows that the population at risk to generate cavity, is high despite having no tooth cavity. The 84% of captured samples are positive for the growth of *S. mutans*, while 16% even though they do not exhibit growth, have a DMFT rate ranging between 1 and 5 careadas, decayed, missing or filled tooth. We believe that complementary studies could be associated with eating habits with the presence of *S. mutans* and cavity experience. Finally, our studies indicate that we have a reliable method in the identification of *Streptococcus* PCR end point, and our preliminary results indicate that the predominant genotype is the C subtype. This is new since there are no reports for population in Querétaro. Our data compared with other studies carried out in Colombia and Chile, show behave in a similar way. Moreover, our study pointer in Latin America in the genotypification of the subtypes of *S. mutans* PCR multiplex

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Analysis of the effect of benznidazole on the antioxidant metabolism of *Trypanosoma cruzi*

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INTRODUCTION *Trypanosoma cruzi* is the causative agent of Chagas disease. The drugs available for clinical use against the infection are benznidazole (Bnz) and nifurtimox; however, these compounds have severe side effects and are not effective in the chronic phase. Both drugs generate oxidative stress through the formation of free radicals and/or electrophilic metabolites which bind to thiol metabolites, proteins, lipids or DNA[1]. The predominant reducing antioxidant metabolite of *T. cruzi* is trypanothione ($T(SH)_2$), a functional analog of glutathione (GSH) in mammalian cells[2]. It has been previously demonstrated that a unique dose of 100µM of BNZ decreased in average at 20% the $T(SH)_2$ pool of *T. cruzi* epimastigotes, trypomastigotes and amastigotes[3]; nevertheless, there are not studies that monitor the dynamics of the thiol pools (cysteine (Cys), GSH, $T(SH)_2$) at lower and higher BNZ concentrations, and whether compensatory effects are turned on to circumvent the $T(SH)_2$ depletion due to BNZ treatment. The aim of this work is to determine the effect of BNZ on the dynamics of the antioxidant metabolism in the parasite.

METHODOLOGY.*T. cruzi* epimastigotes were grown until the exponential phase. Different BNZ concentrations (15-160µM) were added and the culture continued for 24 h after which the cell viability was determined.

RESULTS. The concentrations that inhibited the cell growth by 50% (IC_{50}) and by 100 % (IC_{100}) were 19 and 39 µM respectively. On the other hand, the concentration that killed 50% of the population (LC_{50}) was 135 µM. At [BNZ]<IC_{100}, the pools of Cys and GSH showed a tendency to decrease, whereas at higher [BNZ] the metabolite concentrations increased. In contrast, the $T(SH)_2$ pool gradually raised as [BNZ] was augmented.

CONCLUSION: This metabolic profiling suggested that below 39 µM BNZ, the Cys and GSH pool may suffice to increase the $T(SH)_2$ concentration to manage the oxidative stress caused by the treatment. At higher and lethal concentrations that affect cell viability, the parasite needed *de novo* syntheses of Cys and GSH or an increase influx from the extracellular milieu. Therefore, at high BNZ concentrations, an increase flux of the Cys, GSH and $T(SH)_2$ synthetic pathways become relevant for a proper management of the oxidative stress caused by BNZ. The enzymatic profile of these pathways is currently studied.

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Comparative study of the secretable proteolytic activity higher than 160kDainthree groups of *Escherichia coli*

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Escherichiacoli(E. coli) is anenterobacteriapartof the normal floraof humans and animals. Thisbacteriumis classified as:(i) commensal,(ii)intestinal pathogenor(iii) extraintestinalpathogenic*E.coli*(ExPEC). ExPEChas 3variants: avianpathogenic*E.coli*(APEC), uropathogenic*E.coli*(UPEC) andneonatalmeningitis *E. coli* (NMEC). Somevirulence factorsof these groups ofpathogenic bacteriaareenterotoxins, hemolysins, colicins, hemagglutinins,fimbriaeand secretedpeptidasesthat degradeimmunoglobulins (Igs). Thelast ones are importantbecause theyhelp the bacteriatoevadehost responsesand initiateinvasive processesofinternal tissues and organs.Currentlysome bacterialproteases degradeIgs(S6 type with >100kDa size). In the case of*E.coli*it is known to havethe ability to interactwithIgsto overcomehost defenses,but still it is notwell understoodhow this interactionoccursand ifthisisassisted bysomeproteolytic accessory function. In our laboratory,we foundhigh frequency ofEXPEC strains producing proteolytic activity (160 kDa) against immunoglobulins and human serum albumin (HSA), surpassing the frequency reported byJohnson(J Infect Dis. 2000, 181(5):1753-4) sowe decided toexplore howthis activityis distributed inother types of*E.coli*. In this investigation,*E. coli* isolateswere obtainedfromraw foods andintestinal samples to search the ability to produce IgG proteases and determine if that were due or not to the presence of*pic, pet* or*tsh* genes. The results of this work showevidence suggesting thatExPECcontainsa non-describedvirulencefactor,a HSA, IgG and IgA degradingprotease (160KDa), different to Pic, Pet or Tshautotransporters (>100kDa).

Homologue sequences to Iron Regulatory Protein in *Entamoeba histolytica* genome.

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In *E. histolytica*, has been reported iron regulation in different virulence genes as *EhCP5* which participates in amoebic liver abscesses formation, *EhFeSOD* involved in resistance to metronidazole and, *Ehrp-L21* in protein synthesis. However, their regulation mechanisms are unknown. Posttranscriptional regulation by the IRE/IRP system in mammalians consists of iron cytoplasmic regulatory proteins that interact with iron responsive elements (IRE). When iron levels are high the IRE binding activity is inhibited, however, when cellular iron levels are low, the IRP binds to IRE. Recently, have been reported IRP-like proteins in *P. falciparum* and a protein with IRP function *in Trichomonas vaginalis*. By their evolutionary proximity and the importance of iron in these protozoa, it is hypothesized that *E. histolytica* may also have an IRP-like protein. The aim of this study was to search IRP-like proteins in *E. histolytica* genome by *in silico* analysis.
Sequences used for *In silico* analysis: IRP 1 (*Homo sapiens*), IRP (*Anopheles gambiae*), IRP (*Plasmodium falciparum*); were obtained from GenBank and used for search homologies in *E. histolytica* genome. Results for *In silico* analysis showed homology to: human IRP 39% identity (EHI_131110), *A. gambiae* IRP 33% (EHI_109920), *P. falciparum* IRP-like protein 24 and 27% (EHI_042140 and EHI_137240 respectively).
In conclusion, the presence of an IRP homologue sequence in *E. histolytica* genome may suggest an IRE/IRP mechanism in this parasite.

Assessment of anticoccidial activity on *Eimeria sp.* oocysts from the yeast *Meyerozyma guilliermondii* isolated from chickens

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Avian coccidiosis is a disease caused worldwide by several species of parasite *Eimeria,* individually or in combination, multiple species of Eimeria Apicomplexan protozoa infecting the intestinal mucosa. It affects chickens development and production, causing economical losses. Control of this disease has been by chemotherapy and by vaccination with oocysts attenuated, but resistance has been developed to alls drugs introduced so far. Although efforts have been made to address this disease, the parasite oocyst remains a problem that must be controlled, as it has a resistant structure that facilitates dispersion. Furthermore, nowadays, consumers request poultry products that are free from residual drugs. The use of others products as an alternative to drugs may be the best solution for this disease.

In this work, we assessed *in vitro* anticoccidial activity of a compound(s) secreted by yeast isolated in oocysts suspension from infected chickens. The yeast was molecularly identified as *Meyerozyma guilliermondii,* and its anticoccidial activity against *Eimeria spp.* oocysts was assessed. Here, we report the damage to oocysts walls *in vitro*, caused by *M. guilliermondii* culture, supernatant and intracellular extract. In all cases, a decreased number of oocysts was observed, showing the potential of this yeast and its secreted products as a feasible method of coccidiosis control.

Spo0M and its role in the formation of the septum; a new role for a regulator of sporulation of *Bacillus subtilis*

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Bacillus subtilis is an aerobic, gram positive bacterium, which has been widely used as a model for studying processes like sporulation and production of proteins. Sporulation in *B. subtilis* may be considered as a mechanism of cell differentiation which allows cells to withstand adverse conditions. There are a number of positive and negative regulators of sporulation. Spo0M is a protein which controls sporulation, yet the mechanism by which performs this function has not been specified.

Although being processes with totally different cellular functions,eukaryotic endocytosis and bacterial sporulation phenomena could converge in remodeling of the plasma membrane. Besides being mechanically similar, it has been demonstrated that several proteins involved in endocytosis are present in *B. subtilis* and have transcendental functions during sporulation, which might suggest that there are evolutionarily conserved protein core functions governing the organization and membrane remodeling.

Arrestins are a family of proteins whose main feature is its ability to function as adapters and as scaffold proteins from a variety of enzymes and their substrates. A primitive functionofarrestins may beassociated to the dynamics of the membrane or the cytoskeleton, and signaling through protein kinases. Recently it has been reported that the sporulation control protein of*B. subtilis*, Spo0M, contains similar structural domains to the N domain of the arrestin family.

Considering the structural similarity between the protein Spo0M andarrestins, and functional conservation associated with the structure of the arrestin family, the question arises: has Spo0M a function that may relate to the family of arrestins and can be involved in septum formation during sporulation?

Transient disulfide reduction is required for EFF-1 mediated cell-cell fusion

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EFF-1 facilitates cell-cell fusions during the embryonic development of *Caenorhabditis elegans*. Despite being structurally homologous to class II viral fusion proteins, most evidence indicates that EFF-1 executes a SNARE-like fusion mechanism that involves the interaction and trimerization of EFF-1 protomers initially located in opposite membranes. The trigger of EFF-1 mediated fusion is still unknown. In some class I viral fusion proteins disulfide reduction and isomerization are required for fusion triggering. EFF-1 and the members of its subfamily conserve a CXXC thioredoxin-like motif that suggests disulfide shuffling. We will show that DTNB, a thiol blocking reagent, inhibited EFF-1 mediated Sf9 cell-cell fusion. EFF-1 was also reacted and precipitated with maleimide-PEG-biotin (MPB), which indicates that DTNB and MPB targets are EFF-1 thiols. CXXC single mutants led to a 70% decrease in EFF-1 activity and to a conspicuous unresolved trimeric adduct. These results, together with previously reported structural data, suggest that a transient disulfide reduction occurs during EFF-1 mediated cell-cell fusion and that CXXC motif may be involved in such process.

In silico analyses of a transcription factor MTF1 in *Trichomonas vaginalis*

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Introduction. The zinc is an element present in high zinc concentration in human prostatic secretions. However, *T. vaginalis* is capable of survive in the environment male urogenital tract. Men are consider as a reservoir of this parasite due that in the majority of cases of men infected are asynthomatics, but little is known about the Zn^{2+} regulation mechanisms in this parasite. *In vitro* studies have showed that high Zn^{2+} concentrations cause a drastic change in the expression of proteins. The response to heavy metal in eukaryotes is given by a mechanism transcriptional through of a transcription factor known as: MTF1 (Metal response element-binding transcription factor-1), which is a six-zinc finger protein that plays an essential role in activating metallothionein expression in response to the heavy metals as Zn^{2+} and Cd^{2+}. *O*ur aim is to search *in silico* in genome of *T. vaginalis* a homologous to the transcription factor MTF1, which possibly is responsible of modulation of genes in response to Zn^{2+} in this parasite.

Methods. The search of a protein homologous to MTF1 in *T. vaginalis* was performed through sequence similitude and identity from database TrichDB (http://trichdb.org/trichdb/). In the database UNIPROT (http://www.uniprot.org/) were obtained the sequences homologous for a Zinc finger putative protein and was realized the phylogenic analyses with MEGA 5 software with Neighbor joining algorithm with 1000 replicates the bootstrap test and was analyzed the conservation of aminoacids from an alignment with Clustal W. The prediction of C2H2 domains was performed in Pfam (http://pfam.xfam.org/) and prediction theoretical structure was modeled with Phyre 2.0 server (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index).

Results The blast showed that the protein localized in the locus DS113312 with the number access TVAG_ 458980 annoted as Zinc finger putative, which we named as TvMTF1, it has an e value $2e^{-}10$, with 30% of identity and 46% of similitude with MTF1 human sequence; TvMTF1 protein is codified by a gene of 831 nucleotides and the protein is conformed of 277 aminoacids. TvMTF1 is phylogenetically related to eukaryotic proteins of protozoan, yeast and human principally. The domains prediction showed that TvMTF1 has seven zinc finger of type C2H2 with prediction value e 0.01 and $3.7e^{-06}$ and bit score 9.8 and 28.6 and TvMTF1 conserved structurally beta-beta-alpha zinc finger DNA-binding domain.

Conclusion. This work suggests that TvMTF1 could be a transcription factor of Zn^{2+}response in *T. vaginalis*.

Ammonia-oligotrophic and/or diazotrophicheavy metal-resistant*Serratia* spp. isolated from pioneer plants and main tailings from Zacatecas.

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In mine tailings, an environment characterized by low concentrations of nutrients and toxic levels of heavy metals, the diazotrophic and nitrogen-oligotrophic bacteria are the main income source of nitrogen to the environment. In this study we evaluated the ammonia-transport and nitrogen-fixing abilities in presence of heavy metals of 29 diazotrophic and/or nitrogen-oligotrophic strains of *Serratia* spp. isolated from the rhizosphere, root and aerial part of pioneer plants and tailings from three mines at Zacatecas State. From 165 potentially diazotrophic and/or oligotrophic strainsisolated from nitrogen-free culture mediaand a screening growth test in nitrogen-free liquid media, the analysis of the 16S rRNA gene indicate that 29of them correspond to *Serratia* spp. The first amplifications of *nifD*and *amtB*genes, and the growth up to 5mM of Zn and 0.1 mM of Cd innitrogen-free MBMM liquid media of*Serratia* sp. strains,show that they are potential members of the community of diazotrophs-oligotrophsand heavy-metal resistant guilds of the tailings. Strains of *S.marcescens*,*S.liquefaciens*,*S.fonticola*,and *S.plymuthica*have been reported*as* tolerants tohigh concentrations of Cd, Ni, Cu,Zn, Pb and Fe; andsolely*Serratiamarcescens*IRBG500 has been reported as a diazotrophic bacteria associated to the root of rice.The strains of *Serratia* spp. isolated from the mine tailings and pioneer plants of Zacatecasappear to introduce nitrogen asdiazotroph or ammonia-oligotrophin presence of heavy metals to this poor and polluted environment.

A Taenia crassiceps metacestode factor enhances ovarian follicle atresia and oocyte degeneration in female mice

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Taenia solium causes taeniasis in humans and cysticercosis in both humans and pigs. Experimental animal models are excellent tools for understanding the host–parasite interactions of human cysticercosis. Experimental murine *Taeniidae* metacestode infections or inoculation with *Taeniidae* secretion products have been used to study structural gonadal tissues and reproductive functions. It has been reported that a substance secreted by *Taenia taeniaeformis* significantly reduced the in vitro testosterone production in rat Leydig cells. Studies of larval *T. taeniaeformis* infection in female and male rats showed that mating, uterine implantation sites and the number of full-term pups were decreased in females 5 months after infection ant that both serum and testicular testosterone levels were significantly decreased in male rats. It has been observed that *Taenia crassiceps* infection disrupted the oestrous cycle and significantly decreased oestradiol levels in female mice. Histological studies of the testis seminiferous epithelium from mice intraperitoneally infected with *T. crassiceps* metacestodes, or subcutaneously inoculated with a low molecular weight *T. crassiceps* metacestode factor (MF), revealed severe disruption and generalized apoptosis of the seminiferous tubule cells. A significant infiltration of macrophages in the tubular lumen was also observed. A low molecular weight (3.5 kDa) *T. solium* MF has previously been isolated and shown to impair the proliferation of human and murine lectin-stimulated lymphocytes and reduce the inflammatory reaction surrounding subcutaneously implanted *T. solium* metacestodes in mice. Subsequently, it was reported that this *T. solium* MF reduced in vitro interleukin (IL)-2, interferon (IFN)-g, IL-4 and tumour necrosis factor (TNF)-a production from lipopolysaccharide (LPS)-stimulated macrophages and also induced DNA damage in human lymphocytes in vitro. Additionally, excretion/secretion products of *T. crassiceps* larvae suppressed the T-cell proliferative responses in vitro as well as IFN-g and IL-4 production early during the infection. The main objective of this study was to determine whether infection of female mice with *T. crassiceps* metacestodes or inoculation with *T. crassiceps* MF leads to structural damage of the ovary.

Sexual hormones and their influence over mitochondria: estrogens

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Cardiovascular disease is the principal cause of morbidity and mortality worldwide. Through observational studies it has found that gender plays an important role, since men are more prone to this type of disease, such situation continues until woman reaches menopause. For this reason it has been endowed to estrogen (Est) the role of protect against cardiovascular events. In this regard, it has been shown that, in cardiovascular system Est increase vasodilation through the regulation of nitric oxide and different antioxidant molecules, as well as a protective effect against atherosclerosis. These effects can occur via specific receptors located in the membrane, cytoplasm, nucleus and mitochondria of target cells. This point is especially relevant in the heart, since mitochondria constitute 30-40% of the cellular protein. A major function in mitochondria is oxidative phosphorylation, to produce the necessary ATP in heart for the excitation-contraction coupling.

Different works appointed the importance of sexual hormones in mitochondria function and structure. This point becomes a primary point of interest, given the rise of transgender people. For women who choose the "sex change" toward men, testosterone in high doses is administered in order to obtain and maintain secondary sexual characteristics and until this moment no one has observed if there is some effect at energetic level.

In order to obtain some data at this respect, in this work, administration of high doses of testosterone in castrated female rats was done.

Ovariectomized(ovx) female rats received testosterone during 4 months. After this time, the heart function was assessed by electrocardiogram. Heart blood was collected and testosterone levels were measured. Mitochondria were obtained by differential centrifugation and Ca^{2+} transport, transmembranal potential and oxygen consumption, were evaluated. Important differences were found.

Isoindoline-1,3-dione derivatives analogous to dopamine as potential agonist to D_2 receptor.

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Introduction

The dopamine D_2 receptor selective compounds of diverse intrinsic activity have the potential to become pharmacotherapeutic agents in the treatment of neurological disorders, neuropsychiatric disorders and psychostimulant drug abuse; as well as imaging agents for the study of expression and regulation of D_2 receptors[1-3]. However, due to the high degree of homology between D_2 and D_3 receptors, it has been difficult to obtain compounds that can selectively bind to either of the two subtypes of D_2 or D_3receptors[4-5]. The aim of the present study was to develop a series of dioxoisoindolinesrelated to dopamine as selective ligands to D_2 receptorusing technological tools to characterize the interaction of these ligands with the receptor and propose the synthesis and characterization of the best ligands.

Results and discussion

Using *in silico* models, it was developed and characterized by physicochemical properties a series of 30 dioxoisoindolinesstructurally related to dopamine. It was carried out the molecular approach of each ligands with the D_2 receptor and the ΔG of the binding energy was evaluated showing that ligands that have more affinity are the molecules D13S, D6S and D18 which interact in average with 10 amino acid residues such as Asp114, Ile183, His393, Phe389, Val115, which have been reported as important in the recognition and selectivity for the D_2 receptor[1-3]. The main interactions of these ligands with the receptor are hydrogen bonds and hydrophobic interactions due to the aromatic rings that possess these ligands. The results also shows that there is more selectivity for those ligands that have an S configuration that those with an R configuration. In order to carry out in vivo experiments in the second phase of the project it was carried out the synthesis of one dioxoisoindoline derived of metoxydopamine in solvent-less condition (green chemistry) and it was characterized by IR, ^{1}H and ^{13}C NMR.

Conclusions

The addition of Dioxo-Isoindoline group to the structure of different amines analogous to dopamine favored their selectivity for the D_2 receptor and also their properties evaluated by Lipinski's rule of 5. These results suggest that these ligands possessing the best characteristics can pass a second test phase to be evaluated as potential agonists of the D_2 receptor, mainly in animal models of Parkinson.

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Aliskiren modifies (Pro)renin receptor associated with canonical Wnt/β-catenin signaling expression on heart and kidney in 5/6 nephrectomy-induced hypertension in rats

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The renin–angiotensin system and Wnt/frizzled receptor signaling pathways are important in the morphogenesis and development of essential organs. In the RAS signaling cascade, prorenin and renin are the initial elements, which allow angiotensin-II synthesis, a majorautocoid, considered a final effector peptidethrough AT1 and AT2 receptor activation.In 2002, Nguyen et al, discovered a new element of this cascade the (pro)renin receptor (P)RR.Cruciat et al, in 2010,characterized the (P)RR as a multifunctionalprotein that associates with vacuolar H+-ATPase (v-H+-ATPase) (independently of renin) and is required for canonical Wnt/β-catenin signaling and functioning.Their aberrant activation results in cardiovascular and renal pathologies.The pharmacologic interruptions of abnormal activation of the RAS at different stages in its signaling cascade have been well known to be effective in treating variouscardiovascular disorders.Recently, Aliskiren, a direct renin inhibitor, has been used to treat these diseases.

The aim of this work was to study Aliskiren effects upon (P)RRandcanonical Wnt/β-catenin signalingexpression in cardiac and renal tissue during 5/6 nephrectomy-induced hypertension. Male Wistar rats underwent 5/6 nephrectomy (5/6 Nx) or sham operation (S). Both groups received Aliskiren, 10 mg/kg/day, po, throughout the experiment. Control groups received vehicle. Seven days later, 24-hour urine was collected and blood pressure was measured. (P)RR β-catenin and dishevelled Dvl-1 expression was determined by immunoblot. Our results showed that, compared to sham animals, 5/6 Nx rats treated with Aliskiren had lowered blood pressure and improved creatinine seric values, proteinuria and creatinine clearance. We found in nephrectomized rats that (P)RR increased seven days after surgery in heart and kidney, and aliskiren reduced (P)RR, β-catenin and dishevelled Dvl-1expression in both tissues. These results confirm the antihypertensive and renoprotective effects of aliskiren and interestingly, showed that aliskiren modifies PRR expression as well as canonical Wnt/β-catenin signalingexpression and function.

Evaluation and synthesis of a series of 2-substituted isoindolines with probably antineoplastic activity and their action with histone deacetylase 8.

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Keywords:isoindoline, docking, histone deacetylase, epigenetic regulation.

Transcriptional regulations in eukaryotic cells are exquisitely influencedby epigenetic regulation, such as methylations, sumoilations, phosphorylations and theacetylations of histones. Both Histone Deacetylase (HDAC) andHistone Acetyltransferase (HAT) are key enzymes in hipo/hyperacetilations; extensively studied mechanisms appear to claim that the inhibition of HDACupregulates genes that lead tocell apoptosis. This gene overexpression, either way, does not eliminate the cell capacity to differentiate or proliferate under certain environmental conditions.[1]One important characteristic of HDAC8 is that it uses Zn^{++} as cofactor, and it is located in the catalytic site of this enzyme. Selective HDAC inhibitors have been tested as anticancerdrugs; however, the adverse effects of these therapeutic agents make necessary to evaluate and developenew molecules.

In response to this urgency, our work group developed a series of isoindolinesderivative of primary amines, and it was performed an*in silico* analysiswith theHDAC8. In addition, it has been evaluated the physiochemical and toxicological properties of the molecules, and three of this compounds weresynthetized. We also performed an*in vitro* analysis of the synthetized molecules on L5178-Y murine leukemia cells, with determination of viability by MTT assay and cell counting.

Docking results on human HDAC8 showed that ligands with higher affinity were molecules MD2a13, MD2b13, and MD2k13, with a ΔG of -11.92, -11.33 and -10.92 kcal / mol. The principal interactions between the ligands and amino acid residues were hydrogen bond, π-π, hydrophobic interactions and π-cation interactions. Among the important aminoacid residues for these interactions are K33, Y306, H180, F152, Y100, D101, K142, W141,C153, F208, H143,I34; all of these interactionsare important for inhibition of HDAC8[2]. The best ligands satisfyLipinski's rule of five, furthermore, have notheorical reproductive, mutagenicity or irritability effects. It was carried outa new green synthesis technique of three isoindolines, these compounds were characterized by IR, 1HNMR, 13CRMN and mass spectrometry.

The*in vitro* experiments showed that at concentration of $1x10^{-3}$M, compounds reduce cell viability up to 70%, whereas at concentrations of $1x10^{-5}$, $1x10^{-6}$and $1x10^{-7}$M, the compounds exhibit interesting biphasic behavior increasing cell viability up to30%.

We suggest that our compounds are likely to interact with HDAC. Also, it is probably thatcertainactivity of the HDAC makes possible not only to reduce but to increase cell viability, since HDAC not only deacetylated histones in the nucleosome,but alsoother proteins. It is necessary to make further experiments, like enzyme kinetics and microarrays to determine with greater precision the mechanisms involved in the biphasic behavior of these compounds.

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Synthesis and characterization of lipophilic bismuthdimercaptopropanolnanoparticles and their effects onoral microorganismsgrowthandbiofilmformation

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Presentación: cartel

Area: Farmacología

The increasingprevalenceofresistanceamongpathogenicmicroorganismstocommonantibioticshasbecomeoneofthemostsignificantconcernsinmodernmedicine.Nanotechnology offers a new alternative to develop materials with interesting applications in many areas of biological sciences and medicine. While some bismuth derivatives havebeenemployedtotreatvomiting,nausea,diarrheaandstomachpain,theantimicrobial propertiesofbismuth in its nanoparticulate form havenotbeen extensively studied.Theobjectiveofthisinvestigationwastoanalyzethebactericidal, fungicidal and antibiofilmactivities of bismuth dimercaptopropanol nanoparticles (BisBAL NPs) against oral microbes.Thenanoparticles are composed of 18.7 nmcrystallitesonaverage and have a rhombohedralstructure, agglomeratingintochains-likeorclusters of smallnanoparticles.Ourresultsshowed that stable colloidal BisBAL NPsinhibited *Streptococcus mutans and Streptococcus gordonii* growth by more than 70% at 0.1 µM, showing a twelve thousand fold higher effectiveness compared with 1.2 mM chlorhexidine, the oral antiseptic most used by dentists.Theminimalinhibitoryconcentration (MIC) ofBisBAL NPs for*S.mutans* and *S. gordonii*was5 µM. MIC of BisBAL NPs for *Candida albicans* was 10 µM. However, 100 µM of BisBALNPs were required to interfere with planktonic growth of and biofilm formation by a multi-species population of bacteria. Our experiments show that bactericidal activity of BisBAL NPs was similar to antibiotics such as vancomycin and rifampicin. Based on MTT cell viability assays we hypothesize that BisBAL NPs potentially act on key enzymes, altering their metabolism, and cause cell lysis. All together, thesefindingsshow the efficacy of BisBAL NPsas a broad spectrum antimicrobialagent which could reduce antibiotic usage.

"Influence of sex hormones on mitochondrial function in male rat heart"

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Cardiovascular disease is the principal cause of morbidity and mortality worldwide. In the range of 45 to 64 years, more men than women die of heart disease (39%) however after 65 years, the range of heart disease in women exceeds that of men by 22%. This difference has been attributed to estrogens low level and testosterone high level.

Testosterone effectstail through via androgenic receptors in the membrane, cytoplasm and nucleus of target cells. It is known that testosterone synthesis occurs in the mitochondria, which constitutes 20-40% of the cell volume in cardiac cells. One of the central functions in mitochondria is the oxidative metabolism, essential in the coupling of cardiac work, which requires continuous energy production, 95% is generated by mitochondrial respiration. This metabolism is altered by sex hormones, a fact that becomes relevant in transsexual people, who uses hormones of the opposite gender for extended periods of time at high doses. Some data have shown that over time,pathologies in the cardiovascular system occur at higher rates than people who do not have this kind of hormonal replacement.

In order to know if testosterone generated some alteration at mitochondrial level, 20 male Wistar rats were used. These animals were randomly assigned to one of two groups:control(Ctrl) and orquiectomized(Oq).In the Oq group testes were removed and estradiol was administered i.m. in a dose 20-fold higher than physiological for 4 months. Functionality of the heart was assessed by electrocardiography; sex hormone and pro-inflammatory cytokines levels were measured. Mitochondrial calcium transport, transmembrane potential and respiration of mitochondria obtained by differential centrifugationwere assessed. The activity of mitochondrial superoxide dismutase (SOD), thiobarbituric acid reactive species (TBARs) and the presence of proteins ANT, 2-KGDH and GA by Western blot was determined. The results indicate significant differences in the parameters evaluated.

Purification and evaluation of the effect of *scammonin I* y *tyrianthin C* isolated from the *Ipomoea tyrianthina* root on gabaergic transmission system.

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Keywords: GABA, Ipomoea, glycolipid.

Introduction. Mexico possesses a great diversity of species from the *Ipomoea* genus, some of them have been used in the treatment of diseases of nervous origin [1, 2]. *Ipomoea tyrianthina* Lindley (Convolvulaceae) is an herb widely distributed in Mexico [1], whose glycosidic resins isolated from their roots have demonstrated activity on central nervous system (CNS). Recently, some chemical compounds have been characterized and isolated from these glycosidic resins [1] and have showed different activities related to the GABAergic system, such as: sedative, antidepressant, anticonvulsant and / or neuroprotective [2, 3]. Since compounds obtained from extracts of *Ipomoea tyrianthina* could eventually be used as potential drugs, the objectives of this project were to isolate *scammonin I* and *tyrianthin C* from methanol extracts of the *Ipomoea tyrianthina* root and to identify the possible mechanism of action by which they could be altering GABAergic systems. Also, their relation with neuroprotective and/or anticonvulsant activities.

Methods. Methanol extracts (EMIT) were obtained from previously dried and ground *Ipomoea tyrianthina* root. The EMIT fractionation and identification of pure compounds were performed according to the protocol of Mirón *et al*. (2007) [4] with some modifications. The chemical characterization was made according to León *et al.* (2014) [5]. Pharmacological evaluation was carried out using cerebral cortex slices from CD-1 mice as experimental model, following the protocol described by Gutiérrez and Delgado (1989) [6] and Castro (2011) [3].

Results. *Scammonin I* and *tyrianthin C* were isolated form EMIT. *Tyrianthin C* was identified as a novel compound to *Ipomoea tyrianthina* (León *et al.*, 2014). *Scammonin I* and *tyrianthin C* have an effect on the *in vitro* release of endogenous GABA, increasing its concentration compared to controls. This effect was calcium- and/or sodium-dependent.

Preliminary conclusions. *Scammonin I* and *tyrianthin C* increase the concentration of endogenous GABA released, possibly by an effect on GABAergic synapses.

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The CB1-receptoragonistPhAR-DBH-Me inhibitstheproliferation of SKOV-3, MCF-7, A549 and HepG2 tumor celllines.

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Recently, wehavesynthesized a new diazabicyclicamidederivative of phenylacetylrincinoleicacid (R,Z)-18-((1S,4S)-5-methyl-2,5-diaza-bicyclo[2.2.1]heptan-2-yl)-18-oxooctadec-9-en- 7-yl phenylacetate (PhAR-DBH-Me), and testeditseffectsonsomephysiological variables, revealingthatitpossessescannabinoidactivity and thatsuchaneffectismediatedbythe CB1R [1]. Numerousreportsmakeclearthe role of thecannabinergicsystem in themodulation of growth and proliferation of cancercells [2]. In thiswork, weperformthecharacterization of theantiproliferativeproperties of PhAR-DBH-Me in several tumor celllines (SKOV-3; ovarian adenocarcinoma, MCF-7; breastcancer, A549; lungcancer and HepG2; human hepatocarcinoma). In addition, theeffectsontheinduction of apoptosis wascarrierby DNA fragmentationassay and theabilitytomodifythepattern of phosphorylationwasperformedby Western Bloth. Theresults shows thatPhAR-DBH-Me inhibitstheproliferation of tumor celllines in a dose-dependentmannerin a nanomolartomicromolarrangeand induce celldeathby apoptosis. Currently, we are studyingthemechanismresponsibleforthisbiologicaleffect and control experimentson non-tumor cells. References: [1]López-Ortíz M., et al. BioorgMedChemLett 2010; 20: 3231-3234. [2] Bifulco M. British J Pharmacol 2006; 148: 123-135. Acknowledgmenttofinancialsupport of project CONACYT 101855.

Design and synthesis of a novel anti-tripanosome drug and description of the binding pocket on TcTIM of fluorine containing benzothiazole

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Background: Triosephosphate isomerase (TIM) has been considered as an important target to combat *Trypanosoma cruzi* (Tc), the etiologic agent of Chagas disease [1]. Recent works have described the structural differences between the TIMs from several species including humans and trypanosomes [2], some of the most important differences are the percent identity in the interface of these proteins that is up to 52% and the amino acid residues present in this region, due to they generate different sized pocket which can be used as a target to selectively inhibit TIM with molecules that block the interaction between important aromatic residues in the stability of this enzyme such as benzothiazole [3]. Because there is still no effective treatment against trypanosomiasis the aim of the present work was to evaluated a set of benzothiazole derivatives in order to explore their affinity and binding mode on TIM compared with hTIM, their physicochemical attributes were determined and an score was generated for each ligand. Finally, the most representative compounds were synthesized.

Methods: We evaluated 24 compounds, included some well-know ligands, all compounds were optimized and docked on TcTIM and hTIM obtained from Protein Data Bank (IDs: 1TCD and 2jk2), the stability of the binding pocket on TcTIM was evaluated by molecular dynamics. Their physicochemical descriptors such as: Log*P*, log*S*, PSA, nON, nOHNH, nrotb and toxicity risk were calculated *online* in Osiris Property Explorer and Molinspiration. Finally, the best compounds were synthesized by modifying the method described elsewere [4] and characterized by ATR/IR, ^{1}H, ^{13}C, ^{19}F NMR and MS.

Results: The docking analysis showed that interaction between TcTIM and the 5-substituted with fluorine moieties benzothiazole have high affinity and suggest that there is a new binding site of nine amino acids in this enzyme that include the following residues: Arg100B, Leu101B, Thr131B, Glu134B, Ala142B, Val143B, Thr146, Gln147B and Val170B. Molecular dynamics showed that this binding pocket on TcTIM is frequent and stable compared to that reported for the interface. The physicochemical attributes suggest that **BZTIOL-J**, **BZTIOL-L**, **BZTIOL-A** and **BZTIOL-C** are the best compounds and were synthesized.

Conclusions: Our theoretical studies have identified a new binding pocket to fluorine containing benzothiazole. We propose the main amino acid residues for selectivity of these ligands to TcTIM as well as the key moieties in these compounds. Finally, based on *in silico* information we suggest that **BZTIOL-J** and **BZTIOL-L** could be effective anti-trypanosome drugs.

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CASPASE 3 ACTIVATION THROUGH 7-HYDROXICOUMARIN IN LUNG ADENOCARCINOMA A-549 CELLS PERFORMING IN VIVO MICROINJECTION.**AZUCENA DÍAZ-MORALES MARIBEL SOTO-NÚÑEZ, PATRICIA CUAUTLE RODRÍGUEZ, JUAN MOLINA-GUARNEROS.**PHARMACOLOGY DEPARTMENT FACULTY OF MEDICINE, UNAM.5556232164, molina_ja2007@yahoo.com.mx

Induced apoptosis through caspase 3 activation via coumarin derivatives in lung adenocarcinoma A-549 cells has been described in literature. After treatment with 300 µg/ml of 7-hidroxicumarina (7-HC), caspase 3's enzymatic activity increases in A-549 cells. Western blot shows the characteristic cleavage of procaspase 3 and PARP following a 24 hour exposure of A-549 cells to 300 µg/ml of 7-HC. This cellular process, however, has never been documented *in vivo*. This work's objective is to photodocument this process's kinetics *in vivo* through single-cell microinjection. This was performed with an IM300 microinjector (Narishige), at an injection pressure of 7psi and an injection lapse of 400 ms, using Texas Red as injection marker (615 nm), and a specific caspase 3 substrate consisting on the DEVD aminoacid sequence coupled to a fluorochrome (Rodamin-110 at 520 nm). We performed time courses and obtained fluorescence pictures of the microinjected cells previously exposed during 3, 6, 12, 18 and 24 hours to 7-HC. Image analysis was performed with NIS-ELEMENTS Ar 3.0 software. With the resulting data we plotted the kinetics of caspase 3 activity. We were able to distinguish the enzyme's activity induced by the studied drug (7HC dissolved in ethanol) from the one produced by the drug's solvent. In the case of the 7HC exposure´s time course, ANOVA revealed statistically significant differences among the group (Kruskal-Wallis).In the 3 hour exposure,initial velocities at times 60 s, 120 s, 180 s were statistically different ($p<0.05$, Tukey test)from time zero. When we compared the ethanol treated group with the 7HC group through Student's T test, we only found statistically significant differences ($p<0.05$) between times 40 s, 60 s, and 120 s. In the 3-hour-exposure's initial velocities, a linear regression with slope different from zero $p=0.033$ was observed in caspase 3 activation trough 7HC. Even though the 7HC solvent's linear regression value is acceptable, the stat test revealed a slope no different from zero $p=0.186$. The rest of the studied times did not present the same initial kinetics and there were no statistically significant differences.

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ON THE MECHANISM OF ACTION OF THE RELAXING EFFECT OF THE 5,4´-DIHIDROXI-6,7,8,3´-TETRAMETOXI-FLAVONE, FLAVONE A, ON VASCULAR SMOOTH MUSCLE OF THE RAT.

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Introduction: Werecentlypurified a vasorelaxant compound: 5,4´-DIHIDROXI-6,7,8,3´-TETRAMETOXI-FLAVONE (FA), fromanextract of *Larrea tridentata*, anendemicplantfromthemexicanhighlands also known as “Gobernadora” .

Aim: To understand the mechanisms which underly vasorelaxation of guinea pig mesenteric artery.

Materials and Methods: For isometric tension experiments, the mesenteric artery was extracted from male guinea pigs, weighing 500-600 gr and endothelium was removed or conserved depending on the experimental protocol. Ionic currents for Ca^{2+} and K^{+} were also measured by the patch clamp technique using mesenteric artery isolated cells from male guinea pigs weighing 300 gr.

Results: FA (10 1M) completely relaxes vascular rings contracted with phenylephrine in a dose-dependent fashion, with and EC_{50} = 3 1Mwith endothelium and EC_{50}=5 1M for endothelium free tissue. In contrast with these results, the effect of Quercetine, a vasorelaxant flavonoid which is commonly used as a standard for these family of compounds, relaxed the tissue with and EC_{50} =100 1M with or without endothelium. The relaxant effect of FA was not affected by maximal doses of nifedipine or verapamil, which means that the voltage dependent Ca^{2+} channels $Ca_v1.2$ are not involved in the process. Moreover, it was also observed that Ca^{2+} currents evoked by depolarization in isolated cells, were not affected by FA. Inhibition of K^{+} currents through BK_{Ca} channels with Iberiotoxin did not affect the relaxation due to FA in artery rings, althoug inhibition of K_V channels with 10mM TEA did cause a partial inhibition of this relaxation. This effect was also observed by measuring K+ currents in isolated cells and adding FV, which increased the ionic current sensitive to TEA.

Conclusions: FA is a more potent vasorelaxant compound than Quercetin in this model. The relaxant effect of FA does not depend on endothelium, inhibition of $Ca_v1.2$ channels of activation of BK_{Ca}channels. The effect seems to rely partly on K_V chanels by increasing their activation and modifying the resting membrane potential

Vasorelaxant effect from chloroform extract of *Justicia spicigera*

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Hypertension is the most common reason for adult office visits and has the highest use of prescription drugs, despite major advances in understanding its pathophysiology and therapeutic approach.High levels on blood pressure are a condition that carries high morbidity and mortality worldwide, so herbal medicine has gained ground in this area of medicine. In Mexico, some plants are used as alternative therapy for their possible vasorelaxant properties. *Justicia spicigera*has the ability to lower blood pressure and is used as antihypertensive. Elsewhere, chloroform extract showed the best antihypertensive effect tested in L-NAME rats. The present study was designed to investigate if fractions from this extract could have a vasorelaxant effect. The chloroform extract was fractionated in a chromatography column and collected in fractions, then were tested (01-1000 µg/mL) in phenylephrine pre-contracted endothelium-intact and denudated aortic rings. The results suggest that the vasorelaxant effect is present in at least three fractions, and the vasorelaxant effect was concentration-dependent.Fraction F3 has the best EC50 in endothelium (1.1456 µg/mL) or -denuded (1.1985 µg/mL) aortic rings.In conclusion,chloroform extract from *J. spicigera* has the ability to therefore exert a vasorelaxant effect. Hence the next step is to elucidate if some (which) component of the extract present in the fractions is the responsible for this effect and it remains to clarify the mechanism by which the effect is shown.

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Natural neuroprotective compounds enhance autophagy to increase cells survival in Parkinson's disease model.

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Summary

Parkinson's disease is an important neurodegenerative disorder that results in the loss of dopaminergic cells in substantianigra. Exposure to the pesticide rotenone has been related to this dopaminergic cell loss through mitochondrial damage and increase of oxidative stress. Recent reports suggest that autophagy is central for the maintenance of neuronal homeostasis and is an essential mechanism for coping with the effects of oxidative stress. We found protective doses of curcumin or β-caryophyllene (BCP) are able to decrease the induced apoptosis by rotenone in SK-N-SH cells using flow citometry methodology; however, their radical scavenger activity is not sufficient to explain their neuroprotective effects. Interestingly, autophagy inhibition with chloroquine overrides protection bycurcumin or BCP. We show that curcumin or BCP increased autophagosomes vesicles in cells under stress conditions by rotenone. These findings suggest that these natural neuroprotective compounds enhance autophagy activity therefore augmenting cell survival.

New isoindoline-1,3dione as possible selective ligands for ß$_2$AR (*in silico*study).

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Selectivity is one of the most important challenges in medicinal chemistry because the actual molecules do not have selectivity enough and also induce side effects. Asthma is an example of the above because the molecules used to promote bronchodilation have affinity for ß$_2$ adrenergic receptor (ß$_2$AR) but also for the ß1 adrenergic receptor (ß$_1$AR). An important discovery of the structure-activity relationship of ß$_2$ agonists (ß$_2$A) is the steric hindrance in the amine group of catecholamines, increasing the affinity for ß$_2$AR and reducing for ß$_1$AR. Another characteristic is electron-donating substituents in the aromatic ring and also is known that agonists have R configuration while antagonists have S configuration[2].One family of structures that have been used in several areas due to their properties is the isoindoline-1,3dione. This structure is related with the isoindoline-1-one this family has been described as selective antagonist for the B$_1$AR with a cardioselectivity comparable to atenolol. For this reason in this contribution we propose the addition of the isoindoline-1,3dionemoietyon the phenylethylamines derivatives to attack the lack of selectivity on this kind of compounds[1].We performed*in silico*experiments: design, molecular modeling and docking studies of 29 isoindoline-1,3dione structures onthe ß$_2$AR(PDB-ID 3PDSm)with Autodock 4.2[3].Additionally we calculated molecular physicochemical properties of the structures with Molinspiration and OSIRIS Property Explorer.Finally we synthesized*de novo* one isoindoline-1,3dione derivate ofphenylethylamine and elucidated the structure by IR, H^{1}NMR, C^{13}NMR and MS.The results of *in silico*experiment with 3PDSm receptorshow that the highest affinity ligands that form the most stable complexare D3S, D10R and D3R thathaveΔG of -8.54kcal/mol, -8.43kcal/mol and -8.3 kcal/molrespectively. D3S interact with TRP109, ASN312, CYS192, ASP113, ASP192, PHE193, VAL117 TYR308, PHE289, PHE290 and SER207. D10R interact with ASP192, PHE193, CYS191, TYR308, PHE290, PHE289, ILE309, ASN312, SER207, VAL117, ASP113, VAL114 and TRP109. D3R interact with CYS191, ASP192, TYR308, TRP109, PHE193, ASN312, PHE289, PHE290, ASP113, VAL114, VAL117 and SER207. The aminoacid residues that are important in the allosteric site and that show interaction with our ligands are SER204, SER207, ASP113 and VAL114. The results of the molecular properties demonstrate that our aforementioned ligands satisfy Lipinski's rules of 5, do not induce mutagenesis or tumorigenesis and do not affect reproduction or cause tissue damage. In other hand we synthesized the isoindoline-1,3dione with α,α'-dibromo-*o*-xylene and phenylethylamine in a new green synthesis technique in solvent-less condition. This molecule will give us the initial steps to synthesize the other ligands.With the actual results we propose to synthesize our best ligands and make *ex vivo* experiment in cobayo trachea in order to visualize the effect either agonist or antagonist.

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CaMKII-dependent mitocondrial calcium uniporter phosphorylation and its potential role in ventricular arrthymias.

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Sudden death is a main cause of death in the western world, the major causes are the arrhythmias, mainly ventricular tachycardia and fibrillation. At cellular level, a wide variety of molecular mechanism contributes to arrhythmias, in particular, abnormal calcium handling can contribute to arrhythmogenesis. Modulating Ca^{2+} release from the sarcoplasmic reticulum via the ryanodine receptor, Ca^{2+} uptake via sarcoplasmic reticulum Ca^{2+} ATPase or Ca^{2+} removal from the cell via the Na^{+}/Ca^{2+} exchanger, are potential approaches to reduce arrhythmias. More recently, the mitochondria seems to be a participant of this dymanic. Mitochondria are thought to play buffering Ca^{2+} signalling in the cardiomyocytes. The recent molecular indentification of mitochondrial Ca^{2+} uniporter (MCU) shows that cytosilic Ca^{2+} peaks are reduced or enhanced by MCU overexpression and siRNA silencing, respectively. The mechanisms of control for MCU are not fully understanded, but recently published data shows that in part, one of the mechanisms that could be involved is the phosphorilation mediated by CaMKII, increasing the influx of Ca^{2+} to the mitochondria. Under cardiovascular pathologic conditions, strategies that prevent the overload of calcium with Ru_{360} (antogonist of MCU), also prevents the apparitions of arrhythmias. This same effect, is also seeing with the decrease in the activity of CaMKII, and in this concern this could be partially dependent on recently described effect of this enzyme over MCU. As CaMKII is an enzyme that could be activated by calcium-calmoduline, an increase in cytosolic calcium can lead to an increment of its activity, increasing the calcium influx to the mitochondria and the phosphorylation state of phospholamban and ryanodine receptor; creating an arrhythmogenic environment. In this scenario, inhibiting the MCU under physiologic conditions could produce an overload of cytosolic calcium and an increase activity of CaMKII, creating an arrhythmogenic environment. This work intent to demonstrate that under physiologic conditions the inhibition of MCU with Ru_{360} increase the cytosolic calcium and the apparition of spontaneous calcium release, dependent of the increased activity of CaMKII because of the cytosolic calcium overload. Using isolated ventricular cardiomycytes, we measure calcium handling with confocal microscopy. Our preliminary results, shown a dose-dependent increase of peak calcium transient amplitude when MCU is blocked (4.23 ± 0.28 and 5.9 ± 0.42, for control and Ru_{360}-treated cells respectively. $P<0.005$). At 1.0 µM Ru_{360} the spontaneous Ca^{2+} release events were more frequent (0.6 increment of events), had a shorter latency and less threshold in isoproterenol stimulated cardiomyocyte. The involvement of CaMKII is still under study with an inhibitor (K-93), that indirectly can tell us if the effects on the increased spontaneous Ca^{2+} release is in part dependent of its activity.

Synergistic effect of α-mangostin on the cytotoxicity of cisplatin in a cell line of cervical cancer.

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Introduction: Cervical cancer is the second leading cause of death in adult female population in Mexico, treatments most commonly used is platinum compounds,such as cis-diamminedichloroplatinum(II) (CDDP) whose mechanism isDNA'sadducts generation, preventing its duplication andproduce cell apoptosis. However, it generates several adverse effects, mainly nephrotoxicity. Mangosteen (*Garciniamangostana Linn*) is a tropical tree from Asia's southeast;mangosteen fruit has been used to treat skin infections, dysentery, arthritis, etc. α-mangostin (α-M) is the most abundant and studiedxanthone isolated from mangosteen fruit;has propertiesantioxidant, antitumor, anti-inflammatory activity. Previous studies have been reported that α-M possessa renoprotective effect on CDDP-induced nephrotoxicity in rats without interfering in the pharmacokinetics.

Objective: Evaluate whether α-M in combination with CDDP produced a synergistic effect in a cell line of cervical cancer.

Method:10,000 HeLacells weregrown in microplates (Tissue culture grade, 96 wells, flat bottom) in a final volume of 100 µL culture medium per well. Cells were exposed for 24 hours with α-M and CDDP independentlyfor construction of dose-response curves, after that the cells wereexposed to a combination of both drugs.Cytotoxicity was expressed as percentage of viability, evaluating with the MTT and crystal violet assay. The inhibitory concentration 50 (IC_{50}) was obtained using the semi-logarithmic mathematical model and the combination index (CI) were calculated to determine synergism or antagonism of combinations tested.

Results: IC_{50} obtained for α-M and CDDP was 19.2µM and 29.4µM respectively. Antiproliferative effect of CDDP was enhanced in combination with α-M dose-dependent. CI below 1 represents synergism, which was achieved with combinations below the individual IC_{50} for each drug.

Conclusion: It was demonstrated that α-M is capable of producing a synergistic effect on cytotoxicity produced by CDDP inHeLa cell line.Those results give us an alternative treatment that help to patient with increased efficacy and decrease nephrotoxicity.

Antimicrobial activity of the venom of scorpion fish *Scorpaena mystes (Scorpaenidae)*

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Studies on biological activity of marine fish venoms are potentially important, due to possible pharmaceutical benefits offered. In this work it was analyzed the chromatographic composition of an extract of scorpion fish venom obtained from a series of spines of a specimen of *S. mystes* collected in November 2013 in Bahía Chamela, Jalisco. The spines were then frozen for transport to the laboratory, and a simple extraction procedure was done, with PBS pH 7.3 and centrifugation at 5,000 rpm for 30 min, the supernatant was analyzed by SDS-PAGE 18%. Also, it was developed the antimicrobial activity of the mentioned venom against Gram negative bacteria (*Escherichia coli*) and Gram positive (*Staphylococcus aureus)* by the bioassay of Dalgard and Peek.

According to the results of the SDS-PAGE 18%, two bands of ~150 kDa, one >75 kDa, another band <75 kDa, one more of ~ 50 kDa, and significantly it was observed a band <10 kDa, this latter may correspond to antimicrobial peptides.

According to the bioassays that were performed in triplicate for each of the bacteria, it was observed that fish venom completely inhibits the growth of both bacteria, and when it was diluted in a dose response fashion the inhibition dissapeared. It is concluded that the venom of scorpion fish *Scorpaena mystes* has antimicrobial activity, potentially applicable in infections caused by pathogenic bacteria. More biological activities of this venom, such as pro and anti inflammatory properties in human keratinocytes will be done.

Rational design of boroarylamines as agonists of beta 3 adrenergic receptor.

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The human adrenergic receptor β3 (β3AR) is mainly expressed principal in the white and brown adipocyte tissue, its activation was associated with lipolysis and thermogenesis; therefore it has become in an attractive target for the treatment of some metabolic diseases. This receptor contain 396 amino acids, the most are organized in 7 transmembrane domains (TM).TM3, TM4, TM5 and TM6 are considered essential for the interaction with the ligand, while TM2 and TM7 are implicated in the activation of protein G and therefore the initiation of an effect.

The aim of this job is design rationale boroarylamines that have affinity and efficacy on the β3AR, through the use of the computational tools. As the techniques based on molecular modeling and bioinformaticwhich have an extensive direct use in chemistry and pharmaceutical areas.

Initiallythe guide compound was identified, and then chemical modifications were performed increase the specificity, besides trying to decrease the adverse effects. In this process are considered functional groups with boron which previously have been used in the generation of compounds more potent and efficient than its precursors.

The proposed ligands were built in 3D and structural optimization through Gaussian 03 at the B3LYP-631G ** level. Receptor was built using the Swiss-Model server, evaluated and validated previous to the simulation of interaction. The simulations were carried out using the technique of protein-ligand docking by Autodock 4.2 program, by bounding with a box cubic of GRID with 60A side, centered in the orthosteric site of β3AR.

Our results suggest that the presence of diarylamines, tricoordinated boron and the exposure of hydroxyl on organoboron compounds are crucial to the ability of interaction of these with the β3AR and its synthesis could generate potent and efficient agonists on the same.

ESTRADIOL ENHANCES GOLD NANOPARTICLES INCORPORATION IN MCF-7 BREAST CANCER CELLS BY MODIFYING MEMBRANE ROUGHNESS

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RESUMEN

Gold nanoparticles (AuNP) have been investigated in various contexts in relation to breast cancer diagnosis and treatment, including as a delivery vehicle for several chemotherapeutic agents. AuNP were recently investigated with regard to cytotoxicity and biocompatibility according to their interaction with cells, since variations in membrane roughness are possibly related for a successful therapy. In different cell models, it has been shown that estradiol (E2) can modulate significantly membrane fluidity; however, this subject has largely remained uninvestigated when incubated with AuNP. We detected changes in the morphology and physical properties of individual human breast cancer MCF-7 cells by atomic force microscopy (AFM) and imaged AuNP (20 nm) intracellular localization by confocal microscopy, in the absence or presence of E2. MCF-7 cells were synchronized to the G0 stage of the cell cycle through serum deprivation techniques. AFM observations clearly showed changes in MCF-7 plasma membrane roughness, measured as RMS_{Rq} values. Maximal effects were observed after 12 hours of incubation with E2 (1 nM), effect that was precluded by the estrogen receptor (ER) blocker ICI 182780. Plasma membrane roughness RMS_{Rq} values increased significantly after 6 hours of incubation with AuNP (20 nm; 80 µg/mL) in combination with E2, compared to controls ($P<0.005$). Surprisingly, AuNP were localized in the cytoplasm and very close to the nucleus at 2 hours of incubation in the presence of E2; meanwhile, in the absence of E2 this process takes up to 12 hours. We confirmed that uptake and transport of the AuNP in breast cancer cells was mediated by lysosomes, since colocalization of AuNP and LysoTracker Red was observed by confocal laser scanning microscopy, both in the absence and presence of E2. AuNP exerted concentration-dependent MCF-7 cell cytotoxicity (at up to 80 µg/mL and 72 hours of incubation), and this effect was enhanced in the presence of E2. This newly revealed correlation between changes in plasma membrane roughness and increased AuNP uptake enhanced by E2 could provide new insight for combined nano-hormonotherapy of human breast carcinoma.

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Evaluation of acute and sub-acute oral toxicity of *Callistemon citrinus* extract in male Wistar rats

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Callistemon citrinus has been used as ornamental plant in Mexico; however, in other countries is used in the treatment of hemorrhoids, anti-inflammatory and antifungal. In our group the antimicrobial activities of the ethanolic extract of leaves and flowers from C. *citrinus* plants were evaluated against six human pathogens strains. Both extracts showed strong antimicrobial activities (1). This study aims to evaluate the toxic effects of the leaves and flowers extracts after a single dose toxicity study, as well as 28 days sub-acute toxicity study in male rats.

Twenty four rats were divided into 2 groups for the acute and sub-acute toxicity evaluations. One group was the control which receives dimethylsulfoxide and water in a proportion 1:1. For the acute toxicity study, the 3 treatment groups received a single oral dose of the leaves and flower extracts at 100, 316 and 1000 mg/kg body weight. The extract from leaves and flowers at a single dose did not produced treatment related signs of toxicity or mortality in any of the animals tested during the 12, 24 and 72 hours observation period.

For the sub-acute toxicity study each groups received a daily oral dose of the leaves and flowers extracts at 1000 mg/Kg body weight for 28 days. The toxicity was evaluated by the incidence of lethality, body weight measurements, hematological and biochemical parameters, liver weights, and histopathology examinations of liver did not reveal morphological alterations. Analysis of these results with the information of signs, behaviors and health monitoring could lead to conclusion that the long term oral administration of leaves and flowers extract from *C. citrinus* does not cause sub-chronic toxicity.

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Role ofRhoandRacproteinsin renalvascularreactivitytoangiotensinIIinhypertension

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Introduction: Hypertension induces an increase of vasoconstrictive substances such as Angiotensin II (Ang II), a hormonethat mainly affects the kidneys physiology. RhoandRacaremonomeric Gproteinsthat function asactivatorsofa wide variety ofsignaling pathways,some of whichlead tovascular contraction, so it has been suggested toplay an important roleinhypertension. The aim of thisstudy is to determinethe role ofRho and Racproteinsinrenalvascular reactivityto angiotensinIIin hypertension inducedbynitric oxidedeficiency.**Methods:**Vascular reactivity assays have been developed byconcentration-response curvesto AngIIinthe presence and absenceof the inhibitors ofRho(C3) and Racprotein(NSC23766) inisolated kidneys of normotensive andL-NAME-hypertensiverats.**Results:** The Ang II caused renal vasoconstriction in a concentration-dependent manner in both groups, although the contractile effect was slightly lower in the group of hypertensive rats compared to that observed in control group. Ang II induced a significant increase of the perfusion pressure in presence of Rho inhibitor (C3) ($p<0.05$) in hypertensive group. On the other hand, Ang II produced a significant decrease of perfusion pressure in the presence of Rac selective inhibitor (NSC23766) in L-NAME-hypertensive rats ($p<0.05$). However, no significant differences in perfusion pressure in presence of Rho and Rac inhibitors in control group was observed.**Conclusions:** Inthehypertensioninduced bynitric oxide deficiency, the inhibition of RhoandRacproteins,increasedor decreasedrenalvasoconstrictiontoAngII,respectively. However, the Rho and Rac proteins inhibition have not effect on kidney perfusion pressure of normotensive rats to Ang II.**Acknowledgments:** The authors appreciate the partial economic support from the grants of: CIC-UMSNH (2.16,ASM; 2.37,SMA).

Ambroxolhydrohclorideantimicrobialand antibiofilmactivityand thestudy of itsmechanism of action.

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Introduction:Multiresistance to commonantibioticsbypathogenicmicroorganisms, has become a majorproblemformodern medicine worldwide, includingMexico. Microorganismsgrowing in a biofilmdisplaygreaterresistance to antimicrobialagents, being up to 1000 times more resistantcompared to cellsgrown in planktonicstate. Theambroxol (AMB) is a mucolyticagentused in thetreatment of chronicbronchitis, howeveritsantimicrobialpropertieshavenotbeenstudied. **Theaim** of thispaperis to analyzethepropertiesbactericidal, fungicidal and antibiofilmof AMB and studyitspossiblemechanism of action. **Methodology:**Thebactericidal and fungicidalactivity of the AMB wasdeterminedby MTT cellviabilityassayusing*Candidaalbicans* and *Streptococcusgordonii*respectively. MinimumInhibitoryConcentration (MIC) required to inhibitthegrowth of bothmicroorganismswasestablished. The anti-biofilmactivityagainst S. gordoniiambroxol and C. albicanswasevaluatedbyfluorescencemicroscopy. Theeffect of AMB ongenomic DNA of S. gordoniiwasanalyzedby 1) cometassay and fluorescencemicroscopy. 2) Analysis of thegenomic DNA bycolumnextraction, detectionby gel electrophoresis and stainingwithethidiumbromide.3) PCR amplification of SAGP-Like*S. gordonii* gene usinggenomic DNA as templateexposed to AMB overnight. Thepossiblealteration of *S. gordonii*proteinsynthesisbyAMB wasanalyzedbyelectrophoresisunderdenaturingconditions (SDS-PAGE). Finally, theeffect of AMBonmembranepermeabilitywasmeasuredbyCalcein AM assayusingfluorescencemicroscopy. **Results:**Ambroxolinhibitedgrowthby 90% of *S. gordonii* and *C. albicans* to 2mg/ml, whichfarexceedstheeffect of AMB as compared to theeffectobtainedbychlorhexidine and terbinafine. The MIC wasestablishedbythe AMB of 0.5mg/ml and a decreasedbiofilmwasobserved at a concentration of 2 mg / ml fordifferent times of exposure toAMB. Byanalyzingthepossiblemechanism of action, bacterial DNA doesnotseemalteredbythepresence of AMB duringbacterialgrowth. Inhibition of proteinsynthesisby AMB wasdiscardedby SDS-PAGE. However, alteration of cellularpermeabilitywasdetectedbyassayscalcein AM. **Conclusion:**Theseresultsindicatethatambroxolpossesses a highbactericidal, fungicidal and antibiofilmactivity. Itspossiblemechanism ofactioncould be destroyingthebacterialmembraneintegrity, alteringthepermeability and possiblyleading to celllysis. Therefore, ambroxolisexcellenttherapeuticoption to combatinfectionscausedbypathogensthatgrow as biofilms.

Pharmaceutical Thesaurus: unity, structure and the relationship of biomedical terminology. Vanessa Sánchez Delgadillo, Eniak Hernández Alarcón, Daniel Ramírez Martínez, Diana Ramírez Álvarez, LaylaMichán Aguirre*.

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Introduction

Currently, biomedical information is one of the utmost interests for those inquiring scientific topics, since it provides a wide variety of data starting from detailed clinical information about patients to essays on a specific medicine reaction. Hence, though the use of technology and information systems, just as a thesaurus works efficiently for the Pharmaceutics area, biological information can be organized in such a way that offers to those interested a better and personalized way to consult it.

Pharmaceutical Standards

Pharmacology uses a particular language that allows people to recognize, describe and ascribe different areas related to each other such as anatomy, biochemistry, biology, and even other administrative branches that refer to drugs, expenses, inventory, among others. This specialized language is enriched with the theory and practice of health sciences, generating a complex vocabulary. The terms that integrate this language are subject to certain rules depending on the environment of the area of application and approval (standardization) of the area, and these can be stored in databases and related to other terms via a thesaurus.

The term thesaurus etymologically comes from the Latin term *thesaurus* that means "treasure"; just focusing on its documentary aspect, UNESCO, in 1975, defined thesauri as a tools to control the terminology that can be commonly used as a communication bridge between the natural language used for human society and the "artificial" language used to describe computational processes. The latter can integrate information in virtual places, databases, which can be as diverse in themes and constitution as the language itself can be, and the network of relationships around the syntactic and semantic relationships that are created between them.

Justification

Due to the large amount of information available in the pharmacological field and the constant incorporation of terms in this area, it is requisite the construction of a thesaurus that allows unifying concepts (by equivalence relations), make sense of the content of the documents contains, allow recovery of information (useful), and make them easier available.

Objective

The purpose of this work was to create a thesaurus that would respond to the pharmacological information needs and serve as a relational database that contains the terms, allow indexing new information to databases, and been a relational framework for efficient retrieval of information in bibliographic management systems.

Results

The pharmacological thesaurus created in this work, contains over 10,000 pharmacological terms organized in 15 different catalogs with different subject topics and related between them by a structural scheme.

Conclusions

Thesauri facilitate the analysis and retrieval of the information from specific areas through a structure that relates the terms, which allows to obtain new knowledge and eliminates redundancy in the results. In addition, via processing it can provide a more complete response and enables to sustain hypotheses of new knowledge of pharmacological area that might be implemented in future studies, noting more direct questions or topics more targeted as relations between common adverse reactions in medicines and active substances that cause, for example.

Antiproliferativeand apoptoticeffects of Bcr-Ablsecondgenerationinhibitors in SKOV-3 and MCF-7 tumor celllines.

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SKOV-3 ovarian carcinoma and MCF-7 breast adenocarcinomacelllineshavebeenused as modelforscreeningand biochemicalstudy of severalmolecules in thediscovery of new anticancerdrugs. SKOV-3 cells are tumorigenic and resistantto Tumor Necrosis Factor (TNF) and toseveralcytotoxicdrugsincludingcis-platinum. MCF-7 are tumorigeniccells, theirproliferationisinhibitedby TNF-alpha and are resistanttoseveraldrugsincludingdoxorubicin. Thesecondgeneration of Bcr-Abltyrosine-kinaseinhibitorsthatNilotinib, Bosutinib and Ponatinib are relatively new anticanceragentsdevelopedforthetreatment of ChronicMyeloidLeukemia BCR-ABL positive. However, itsantiproliferative and apoptoticproperties in several tumor cellisunknown. In thisstudywasevaluatedthe potencial antiprolifetiveactivity of thisdrugs in SKOV-3 and MCF-7 tumor celllines. In additionthe pro-apoptoticeffectswasstudiedby DNA fragmentationassay. Theresults show thatthreecompoundsinhibitthecellproliferation in a dose-dependentmannerbothcellines in a nanomolartomicromolarrangeand ourthe preliminar observationssuggestthatthesecompoundshaveanapoptoticeffect.
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Evaluation of the glutathione system associated to enzymes in brain ischaemia

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Cerebral ischemia is a public health problem, and is considered as the second cause of death worldwide. The damage produced by the cerebral ischemia has been related with oxidative stress conditions. Previously we studied the relationships between nitric oxide and levels of glutathione, and we showed that the increase of NO can produce the lost of glutathione, generating oxidative stress and cell death. Therefore, the aim of this study was analyse the behavior of enzymes associated with glutathione metabolism, such as glutathione peroxidase, glutathione reductase and glutathione transferase as well as oxidative damage. Male wistar rats were divided in 2 experimental groups: Ischaemia and L-NAME + Ischaemia. Ischaemia was produced by OLCCA for 10 min. Glutathione peroxidase, glutathione reductase and glutathione transferase activity were analyzed after the surgery in the temporoparietal cortex, and oxidative damage was analyzed by the determination of malondialdehyde and 4-hydroxyalkenals. Results obtained showed that in the early stages of cerebral ischemia, oxidized glutathione is reduced by glutathione reductase, and removed from the cell by glutathione transferase, however, an increase in lipid peroxidation is observed. In the late stage of cerebral ischemia, the activity of glutathione peroxidase, glutathione reductase and glutathione transferase increase, while lipoperoxidation products increased with respect to time. In the presence of the inhibitor of nitric oxide production, is observed a decrease of both the enzyme activity and the concentration of the products of lipid peroxidation compared to the ischemia group without inhibitor. We conclude that the enzymes associated with glutathione system modify their activity in response to cellular detoxification needs in the post-reperfusion stage of cerebral ischemia.

Assessment of major depressive disorder model in rat

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Introduction: Major depressive disorder (MDD) is a disease that is based on sadness, characterized by the presence of two or more consecutive episodes of major depression. It is considered one of the highest risk factors for suicide that has increased alarmingly; in Mexico, has increased mainly in the range of 15 to 29 years old. There are various theories that are handled on this pathology, one of that implicates the serotonergic system because their biological roles include mood control. The most commons antidepressant therapies are based on maintaining the serotonin (5-HT) in the synaptic cleft and hold; however it has been reported that in the MDD choice therapies are not always effective, for this reason it is suggested that there are any disturbances in serotonin receptors. It has been reported that the 5-HT1A receptor in the raphe nuclei may be increased in patients with major depression, but have not delved into the involvement of this receptor in other brain areas that have relevance in the control of mood as hippocampus. To study depression there are several animal models that help in the progress of this disease, one of the most accepted model is the bilateral olfactory bulbectomy (OBX) in rat; this is due to the removal of the olfactory bulbs results in a number of physiological and behavioral changes that are comparable to human depression.**Methodology:** Male Wistar rats were divided into three initial groups: Control, Surgery and OBX. After three weeks behavioral tests were performed, that was consisted of: olfactory discrimination test, avoidance to light test and analysis of motor behavior in the open field model. Upon completion of this phase of behavioral tests, the OBX group was subdivided to get: OBX, OBX+vehicle and OBX+SSRI groups; 21 days after treatment with antidepressants all groups were assessed behaviorally in the same tests. At the end of behavioral testing craniotomy is performed to include in the analysis only animals with bilateral olfactory bulbectomy successfully without injury in another area of the brain.
Results and conclusions: So far there has the bilateral olfactory bulbectomy model in rat successfully; bulbectomized rats have decreased weight compared to the control and surgery without extraction groups,the presence of hyperactivity is reduced after 21 days of treatment with antidepressants (SSRI), altered response to adverse stimuli and a decrease of grooming can be equated a symptom of depression in humans.

The mitochondria: an integrator of damage signals that result in neurodegeneration in an Alzheimer's disease model.

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Alzheimer's disease (AD) is a progressive neurodegenerative disease with elderly as its main risk factor. During aging several physiological processes (e.g. autophagy, oxidative phosphorylation, and mitochondrial biogenesis) decline, becoming less efficient and thus affecting cellular homeostasis. Aged and AD-brains are also characterized by increased levels of reactive oxygen species, an augmented DNA mitochondrial concentration, dysfunctional mitochondria and lysosomes, and exacerbated inflammation. Recently, it has been hypothesized that the major hallmarks of AD progression such as senile plaques of β-amyloid peptides coupled with τ-hyperphosphorylation result from the deregulation of these processes, which contribute to neurodegeneration. Particularly, emerging evidences have established a link between autophagy, inflammation and mitochondria. Suppression of mitochondrial autophagy strongly potentiates NLRP3 inflammasome-dependent caspase-1 activation and thus, the proinflammatory response. However, the molecular mechanism that triggers inflammation in response to deficient mitochondrial autophagy in the central nervous system under pathological conditions is essentially unknown. Therefore, this study was oriented to analyze the relationship between the axis composed of these three major factors: mitochondria-inflammation-autophagy focusing on mitochondria as the organelle that integrates and transduce damage signals evoked mainly by β-amyloid peptides. We used the 5XFAD transgenic mouse as Alzheimer's disease model, since it reproduces amyloidosis, memory deficits and neuronal death. To analyze the inflammasome mediated proinflammatory response induced by β-amyloid peptides we crossed the 5XFAD transgenic mouse with the caspase-1 knock out mouse (5XFAD X Csp1 KO) and the morphological changes in mitochondria ultrastructure where determined by electron microscopy. We have observed that β-amyloid peptides induce morphological mitochondrial abnormalities, since the mitochondrion in the brain cortex of 5XFAD transgenic mouse were irregular and elongated; we also observed disrupted mitochondrial membranes and cristae breakage compared to the mitochondria from wild type animals. Interestingly, these mitochondrial abnormalities were reduced in the cortex of the 5XFAD X Csp1 KO mice. Accordingly, the increased brain mitochondrial DNA content observed in the 5XFAD transgenic mouse was reduced in the
5XFAD X Csp1 KO to levels compared to those observed in the brains of wild-type mice. Current experiments are aimed to determine the inflammasome activation and autophagy in different brain regions of the 5XFAD, 5XFAD X Csp1 KO and wild-type mice. This work was partially supported by grants from CONACYT (155290 and 154542) and DAGPA/UNAM (IN209212 and IN227510).

Short-term high-fat-and-fructose feeding produces hippocampal insulin resistance,dendrite and spine reduction, tau and MAP-2 alterations,synaptic mitochondrial dysfunction and neuroinflammation

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Abstract:

Chronic consumption of high-fat and high-fructose diets (HFFD)cause metabolic disorders including: obesity and insulin resistance, which in turn promote a deficient execution in memory and learning tasks, associated with neuronal changes such as reduced number and complexity of dendritic spines, altered establishment of long-term potentiation(LTP) and long-term depression, and diminished dentate gyrus neurogenesis.It has not been investigated whether HFFD feeding also produces hippocampal insulin resistance that could be associated with learning and memory impairment. Therefore, we investigated if short-term HFFD feeding produces hippocampal insulin resistance and if this is associated with hippocampal alterations. Male Sprague-Dawley rats were fed for seven days, ad libitum as follows: control group was fed a standard rodent chow and the HFD group was fed a high-fat high-fructose diet consisting of standard rodent chow supplemented with 10% lard, and 20% high-fructose corn syrup in the water. We assessed insulin signaling and morphological alterations in the hippocampus. Our results show that HFFD feeding for seven days induces: 1) a decreased insulin signaling in the hippocampus 2) a reduction on hippocampal weight associated with a reduction in dendritic arborization in CA1; 3) reduced spine number in CA1 neurons and expression of synaptophysin; 4) an increase in tau phosphorylation, along a decreased MAP2 staining in the hippocampus;5) a decreased insulin-induced mitochondrial activity in synaptic terminals, and 6) glial and microglial activation.To our knowledge, this is the first report showing that short-term HFFD feeding causes insulin resistance in the hippocampus along biochemical and structural alterations.

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Effect of Ketone bodies on glucose deprivation-induced autophagy in rat cultured cortical neurons

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Glucose is the main energy substrate in brain. Whenever its physiological levels (70-100 mg/dl) fall below 40 mg/dl there is risk of hypoglycemic coma and even neuronal death. Alternative substrates to glucose like the ketone bodies (KB), acetoacetate and β-hydroxybutyrate (BHB), can be used as metabolic fuel in certain conditions such as prolonged fasting, the ketogenic diet, severe hypoglycemia and breastfeeding. KB are formed as an end product of lipid catabolism in the liver and reach peripheral tissues, enter the cell through monocarboxilate transporters and are oxidized in the mitochondria. Increasing evidence supports the neuroprotective action of KB in models of neurodegenerative disorders, epilepsy and ischemia. However, the mechanisms of protection of KB are not completely understood. Previous studies of our laboratory show that KB prevent neuronal death by a mechanism involving a decrease in reactive oxygen species (ROS) and the preservation of ATP levels (Exp.Neurol. 2008, 211, 85; Julio-Amilpas et al. non published). The mechanisms of neuronal death induced by hypoglycemia or GD have not been completely elucidated and the role of autophagy is poorly understood. Autophagy is a degradation process activated during energy failure, which recycles damaged molecules and organelles sequestered in double membrane vesicles (autophagosomas), which later fuse with lysosomes (autophagolysosomes) to degrade the cellular components providing ATP. However, its role in cell death and survival is still controversial. Results from our group show that autophagy is early activated during GD in cultured cortical neurons and contributes to neuronal death (unpublished data). In the present study we aimed to investigate whether protection by BHB of GD-induced neuronal death involves the regulation of autophagy. Cortical cells obtained from rat brain embryos of 17-18 days of gestation were plated in Neurobasal medium. At day 8 cultures were exposed to GD (DMEM media without glucose) in the presence or absence of BHB for different times, and glucose was replenished (GR) afterwards either with or without BHB. The levels of different markers of autophagy were determined by western blot at different times of GD and GR, cell survival was estimated by MTT reduction (an index of mitochondrial viability) and the release of lactic acid dehydrogenase (LDH) and ATP levels determined by the luciferin-luciferase assay. Results show that BHB reduces the GD-induced increase in the levels of Beclin1 and LC3-II, two proteins involved in the formation of autophagosomes, and notably abates the content of p62, a protein degraded within the autophagolysosome. BHB prevents neuronal death and restores ATP production. Results suggest that BHB negatively modulates autophagy preventing the excessive sequestration and degradation of cytoplasmic content, while promoting the “healthy” degradation of damaged cell components. In addition, BHB can be used as metabolic fuel through the Kreb´s cycle.

Conjugation of Sema3C with a biodegradable hydrogel causes enhanced axonal growth of dopamine neurons

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Transplants of dopamine neurons in animal models of Parkinson´s disease (PD), as well as in patients, restore dopamine release and improve motor performance. In these studies the somata of dopaminergic (DA) neurons are grafted in the striatum rather than in the substantia nigra (SN), where they normally reside. Transplantation of DA neurons in the SN caused inconsistent recovery in patients and experimental animals, due to the fact that the adult brain is an adverse environment for axonal growth, so that axons cannot reach the striatum. A possibility for the guidance of DA axons is the use of chemotropic agents. Semaphorin 3C (Sema 3C) has been proved to enhance and attract axonal growth of embryonic stem (ES) cell-derived dopaminergic neurons *in vitro*. Our group has previously demonstrated that this strategy is also effective in establishing a new nigrostriatal dopaminergic pathway, grafting ES cell-derived dopaminergic neurons in SN in rats with unilateral depletion of DA neurons, using a transfected HEK cells as a system to deliver Sema3C. Although this strategy showed good results, it is not recommended for pre-clinical studies. In this study we started studying if a hydrogel polymer can substitute transfected HEK cells, because it has the ability to retain proteins in its structure, and release them later. It has been reported that this polymer has not toxicity when is implanted in brain. Using an ELISA assay, we found that Sema3C can be released by the hydrogel, and this delivery is sustained at least for 28 days. We demonstrated *in vitro,* using axonal growth chambers, that Sema3C (200 µg/ml) conjugated with hydrogel can promote dopaminergic neuron axonal growth similar to Sema3C soluble in medium (20 µg/ml). As controls, we use only medium and fluorescent gelatin, which has similar characteristics to Sema3C. Control medium induce an axonal growth of 406 µm, in contrast to 777 µm induced by soluble Sema 3C in medium. On the other hand, hydrogel conjugated with the control fluorescent protein induced an axonal growth of 509 µm, and hydrogel conjugated with Sema 3C induced a growth of 865 µm. We showed that the hydrogel can release Sema3C and that dopaminergic axons grow more after Sema3C application. These results encourage the use of this hydrogel in *in vivo* studies.

Standardizing a Method Based on qPCR to Determine the Loss of Dopaminergic Neurons in *Drosophila melanogaster.*

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Parkinson's disease is the second most frequent neurodegenerative disease worldwide, only behind Alzheimer's disease. Parkinson's disease is a progressive disease characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta*, and by the formation of protein inclusions called Lewy bodies.

The presence of α-Synuclein, in the Lewy bodies is characteristic of Parkinson disease. α-Synuclein is a protein capable of interacting with itself forming oligomers and amyloid aggregates. In this sense, this protein is one of the main factors involved in the pathogenesis of Parkinson's disease. Synphilin is another protein involved in the pathogenesis of Parkinson's disease, it is capable of interacting directly with α-Synuclein. Moreover, the expression of either α-Synuclein or Synphilin has been demonstrated to promote dopaminergic neuron death. However, when both proteins are expressed together, aggregation of α-Synuclein is promoted by Synphilin preventing the formation of toxic oligomers.

The fruit fly, *Drosophila melanogaster*, has been used as a model for the study of Parkinson's disease. The specific expression of the human genes, SNCA and SNCAIP that code for the α-Synuclein and Synphilin proteins respectively, has been achieved through the use of the GAL4/UAS system.

To determine the rate of dopaminergic neuron loss caused by the expression of these genes in the flies, real time polymerase chain reactions were performed in different age groups. After that, the correlation between the expression of both SNCAIP and SNCA and the death of dopaminergic neurons was assessed by counting the number of surviving dopaminergic cells marked with green fluorescent protein these data was also correlated with the levels of expression of the TH gene which is a specific dopaminergic neuron marker. Simultaneously, a method of cuticle transparentation was standardized to observe dopaminergic cells thus eliminating the need of dissection and using microscopy techniques. We were able to make the flies fully transparent but unfortunately the GFP fluorescence was lost.

Expression profile of cytokine mRNAs in the retina of Fyn-/- mice: Possible involvement of Fyn during the process of reactive gliosis.

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Fyn kinase phosphorylates tyrosine residues to regulate various functions including the immune response. Deletion of Fyn increases the damage caused by pathogens in induced inflammatory ophthalmic disease models. Müller cells are characterized by spanning all layers of the retina and maintaining retinal homeostasis, are able to respond to injury through molecular, structural and secretory changes, called reactive gliosis. During reactive gliosis, Müller cells produce proinflammatory cytokines and proteins such as glial fibrillary acidic protein (GFAP) that is subject to regulation by phosphorylation. Little is known about the mechanisms and kinases involved in defending the retina. In this work, we characterized the role of Fyn kinase on basal expression of cytokine mRNAs and GFAP expression in retinal and Müller cells derived from WT and Fyn knock out (Fyn -/-) mice.

Eyes from adult mice of C57BL/6 wild type (WT) and Fyn -/- strains (Jackson Laboratories) were enucleated in order to obtain retinal sections (10 µm). In addition, Müller cells were obtained from retinas of 5 days old WT and Fyn -/- mice. Both sections of retina and Müller cells were treated with blocking solution (3% BSA, 5% goat serum and 0.1% Tween 20 in PBS 1X) for 1 hour at room temperature and incubated with specific antibodies against Fyn, GFAP and glutamine synthetase (GS) overnight at 4°C. The next day, samples were washed and incubated with secondary antibodies coupled to fluorophores and counterstained with DAPI (5 mg / mL). Finally, the sections were observed in the fluorescence microscope. Total RNA was extracted from cultures of Müller by Trizol method and cDNA was synthesized. Using endpoint PCR we analyzed interleukin-2 (IL-2), IL-4, IL-6, IL-10, CCL2, VEGF, IL-1B and vimentin expression in Müller cells. CCL-2, VEGF and IL-1β mRNAs changed their expression in Fyn -/- Müller cells. Real time PCR (RT-PCR) with specific probes (TaqMan) of these cytokines was used for quantification of these effects.

Fyn deletion caused important defects on the cellular organization of mouse retinal layers. In Müller cells, the absence of Fyn caused decreased basal levels of CCL-2 and VEGF, increasing GFAP expression in retinal sections. These results contribute to explain the increased susceptibility to infections ophthalmic of Fyn -/- mice in comparison to WT.

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Análisis de los mecanismos de daño del 27-hidroxicolesterol: Implicaciones para la Enfermedad de Alzheimer.

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La enfermedad de Alzheimer (EA) es la causa más frecuente de trastornos neurodegenerativos. Histopatológicamente se caracteriza por una degeneración neuronal y sináptica, acumulación de péptido β-amiloide (Aβ) formando las llamadas placas seniles y por la deposición de la proteína Tau hiperfosforilada intracelular. La causa de la EA es aún desconocida, sin embargo se cree que comprende diversos factores tanto genéticos como ambientales. Dentro de estos últimos, estudios epidemiológicos indican una mayor prevalencia de EA en sujetos con hipercolesterolemia y estudios experimentales sugieren que el colesterol juega un papel central en el procesamiento de la proteína precursora del amiloide (APP), así como en la sobreproducción y toxicidad de Aβ. Sin embargo, los mecanismos por los cuales el colesterol periférico incrementa los niveles de Aβ siguen siendo desconocidos debido a la impermeabilidad de la barrera hematoencefálica a las lipoproteínas plasmáticas. Contrariamente al colesterol, los oxiesteroles provenientes del metabolismo del colesterol, 27-hidroxicolesterol (27-OHC) y 24-hidroxicolesterol (24-OHC), atraviesan la barrera hematoencefálica. El 27-OHC proviene principalmente de los tejidos periféricos y fluye en su totalidad de la circulación sanguínea hacía el cerebro. De modo que, un aumento en los niveles de colesterol plasmático resultaría en un incremento de 27-OHC y por lo tanto en un mayor flujo intracerebral. Este metabolito podría entonces ser un vínculo entre la hipercolesterolemia y la EA. Sin embargo, a la fecha aún falta por esclarecer esta relación. En este trabajo nos proponemos evaluar en modelos in vitro e in vivo el efecto del 27-OHC en la viabilidad celular y en la expresión de marcadores de la EA. Para ello, células diferenciadas de neuroblastoma humano MSN fueron tratadas por 48 hrs con diferentes concentraciones de 27-OHC (5-150 µM). Se observó una disminución de la viabilidad celular a altas concentraciones por un método colorimétrico de reducción mitocondrial (MTT) y por ensayo de muerte/vida. Resultados preliminares indican una disminución en la expresión de APP y un aumento en la expresión de BACE-1 por análisis de western blot. Estos resultados pueden apoyar el efecto de los metobolitos del colesterol sobre la expresión de APP y el aumento de la vulnerabilidad neuronal.

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KChIP3 mediates cholinergic SN56 neuronal cell death induced by oxidative stress.

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KChIP3, an EF-hand protein, is a negative transcriptional regulator of different genes including those involved in neuronal viability like *c-fos* and *bdnf;* it has been suggested that its nuclear translocation can be modulated by intracellular calcium levels. During neuronal activity, calcium influx induces the activation of intracellular signaling pathways that regulate the transcription of activity-regulated genes (*c-fos* and *bdnf*) and these events involve the dissociation of KChIP3 from regulatory elements of the *c-fos* and *bdnf* genes. Different studies have shown that oxidative stress increases KChIP3 levels leading to neuronal cell death. In this study we show that hyperglycemic conditions and peroxide hydrogen modulates *kchip3* expression in cholinergic neurons and compromise the cell viability. Interestingly, the activation of cAMP-dependent pathways protects neuronal cells from the oxidative stress toxicity by inducing the expression of *c-fos* and *bdnf*. The results from current experiments aimed to determine whether cAMP relieves KChIP3-mediated repression of *c-fos* and *bdnf* expression to promote cell survival will be discussed.

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Keywords: Oxidative stress, KChIP3, cholinergic cells, neurodegeneration.

Sensibilidad diferencial de células de neuroblastoma humano a los anti-inflamatorios no esteroideos y al péptido β-amiloide: efectos sobre la sobrevivencia y expresión de ciclina D.

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Los anti-inflamatorios no-esteroideos (NSAD) son compuestos muy utilizados como analgésicos y antipiréticos. Sin embargo recientemente se ha valorado también su efecto como drogas que evitan la proliferación celular a través de su capacidad para inhibir a las enzimas cicloxigenasa (COX-1 y 2) y la lipoxigenasa (LOX). Por otro lado se ha descrito que el péptidoβ-amiloide (Aβ), que se ha relacionado con la Enfermedad de Alzheimer, tiene efectos neurotóxicos y es capaz de reactivar ciclo celular en neuronas maduras. En este sentido decidimos explorar la sensibilidad de las células de neuroblastoma, en estado indiferenciado y diferenciado a neuronas, al péptido Aβ solo y en combinación con indometacina, ibuprofeno y NDGA. Se utilizaron células de neuroblastoma humano (MSN) y en algunos casos de diferenciaron con ácido retinoico y factor de crecimiento neuronal (NGF). Se incubaron con diferentes dosis (10 y 50 µM) de los anti-inflamatorios mencionados y 20 µM de Aβ. Se valoró la supervivencia neuronal utilizando el método de reducción de MTT, inmunofluorescencia para activación de caspasas y se realizaron Wester-blots para ciclina D. Nuestros resultados hasta el momento demuestran que la viabilidad de células MSN indiferenciadas disminuye significativamente en presencia de NDGA (inhibidor de LOX) y en menor grado en presencia deAβ. Aunque las neuronas diferenciadas también mostraron reducción de la viabilidad con los mismos compuestos, ésta fue menor. La exposición a ibuprofeno a dosis altas no modificó la viabilidad y la indometacina, solo a dosis de 100 µM fue neurotóxica. Se observaron cambios en la expresión de ciclina D en presencia de Aβ en las células MSN indiferenciadas. Estos resultados hasta el momento demuestran el potencial anti-proliferativo del NDGA y la vulnerabilidad neuronal al Aβ.
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Expression of connexins 32, 36 and 43 in rat hippocampus during seizures induced by 4-aminopyridine.

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Epilepsy is a neurological disorder that affects 2-5% of worldwide. This disease is characterized by sustained hypersynchronic electric discharge of a group of neurons. Connexins (Cx) are structural proteins that conforms de gap junctions (GJs) in the nervous system (electrical synapses). Experimental evidence support the antiepileptic effects of GJs blockers as well as the epileptiform activity occur in the absence of chemical synapses. These facts support the important role of GJs in epilepsy. In the present study, the principal goal was to evaluate the transcripts expression of Cx32, 36 and 43 in the hippocampus after seizures induced by 4-aminopyridine (4-AP) administration in the right entorhinal cortex (rEC).

For this purpose, Wistar rats (250g) were implanted with a guide cannula (0.5 mm, internal diameter) in the rEC (AP = -8 mm, L= 4.6 mm and V= 4 mm to Bregma), this cannula was used to insert a needle for injections of saline solution (control group, 0.9%) or 4-AP (10mM) trough a infusion pump with a flow of 0.5 µl/min, during 2 min. Animals with 4-AP were observed to analyze the convulsive behavior according a modified Racine scale, and 60 min after 4-AP administration when epileptiform pattern was established , the rats were sacrificed to obtain the brains and dissected right and left hippocampus. These brain structures were weighed and stored in TRIzol® (-70°C). To evaluate the mRNA expression of connexins, the RT-PCR semi-quantitative was used. Data obtained from RT-PCR were normalized with respect a constitutive gen β-actine. These data were analyzed with a statistical test (t-student).

The results showed that after 4-AP administration no significant changes in connexins expression were found when compared with control data. However, an increase tendency in the expression of Cx32 in right hippocampus was observed and in minor extent in Cx43, in the same region while Cx36 expression was minor compared with Cx32 and 43, in control as well as in experimental group. In conclusion, the expression of transcripts Cx32, 36 and 43 were not changed after an epileptiform pattern established and it is necessary more experimental evidence to determine the role of connexins in seizures. Support contributed by the CONACYT grant from LMC 106179.

The role of glutathione and the reactive oxygen species in postnatal development of rat cerebellar cortex

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Reactive oxygen species (ROS) are produced by several sources, including NADPH oxidases enzymes. Recently, it has been shown that the activity of this enzymes changes during the development of the cerebellar cortex, supporting the idea that ROS produced by NOX are signaling molecules involved in proliferation, migration and apoptosis of granule cells. On the other hand, cells have antioxidant defenses to keep the redox balance that permits counteracting the cytotoxicity produced by ROS. Among these, glutathione (GSH) is one major antioxidant system in the nervous system. Therefore, our hypothesis is that a decrease in GSH levels should induce an alteration in some processes such as proliferation, migration and apoptosis that occur during the postnatal development of rat cerebellar cortex. This condition will induce morphological modifications and will affect motor behavior controlled by the cerebellum. To evaluate this possibility we treated newborn rats with an inhibitor of GSH synthesis (BSO, 3mmol /K, i.p). Rats were treated from P2 to P22. Under these conditions, we observed a significant decrease of GSH. At P16, animals were perfused and immunohistochemistry was performed against caspase 3. We found no significant difference in the number of apoptotic cells between controls and treated rats; however, it was found a tendency to increase the number of caspase 3 positive cells in the layer of Purkinje cells in folia VI. The morphology of the cerebellum was also analyzed without significant changes. Another group of rats were treated from P2 to P22 and motor behavior was evaluated by measuring the beam walking and rotarod performance. However, no significant differences were observed between treated and control animals. These results suggest that GSH inhibition synthesis could not be critical for the redox balance during particular periods of cerebellar development.

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Searching new compounds with better selectivity intoβA in α-helix than β-sheet conformation under *In silico*studies for targeting the Alzheimer disease.

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Introduction: The AD physiopathology has been attributed mainly to the increased production and aggregation of the β-amyloid (βA) peptide of 42 amino acids residues (βA_{1-42}), which is produced following the amyloidogenic pathway in which the secretase enzymes are involved. Once βA_{1-42} is released to water environments,its conformation changes from α-helix to β-sheet folded during the oligomerization process forming highly neurotoxic oligomers and fibrils. It has been shown that the limiting step during the conformational change is the formation of an electrostatic interaction between Lys28 and Asp23. Studies made by our work group have shown that compounds or metals with a positive charge are capable to make an electrostatic interaction with the residues Asp23 and Glu22 of βA_{1-42} avoiding the oligomerization process. In addition, the presence of aromatic rings and aliphatic moieties in the molecule confers π-π andhydrophobic interactions with Phe19 and Phe20 to stabilize theβA_{1-42} in α-helix conformation avoiding additionalthe peptide oligomerization.Therefore, in this study we were focused to design new molecules taking into account the chemical characteristics aforementioned and their affinity on both conformations of$\beta A_{1-42}$to be evaluated by *in silico* studies.

Development: Four families of compounds were designed having within their chemical structure an amine and aromatic groups withdifferent chemical substituents including other additional amine. The geometry of the compounds was performed using ChemBioDraw Ultra 12.0,for later be optimized using HYPERCHEM at the MM+ level. The state of protonation at physiological pH was adjusted using Avogadro program. Once obtained the 3D structure of the ligands, molecular docking studies were performed with the βA1-42in α-helix conformation (PDB ID 1Z0Q) and β-folded (obtained through molecular dynamics studies) using Autodock 3.4. Binding affinity (ΔG) and mode were evaluated and compare between two βA1-42 conformations.
Results:According to the results,4 compounds (F1S4, F2S4, F3S4 and F4S4) from the 28 compounds designed showed better affinity to βA_{1-42} in α-helix than in β-sheet conformation. These compounds have in the aromatic ring substitution other tertiary amine able to be protonated at pH physiological.

Discussion: The distance between the tertiary amines could be important because the **F2S4** and **F3S4** compounds showed better ΔG values (-8.06 and -8.32 respectively), to βA_{1-42} α-helix than **F1S4** and **F4S4** (-7.83 and -7.57 respectively).

Conclusion: F2S4 and **F3S4** compounds could be evaluated *in vitro* as possible inhibitors of βA_{1-42} aggregation.

Early activation of autophagy during glucose deprivation contributes to cortical neuronal death

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A decrease of blood glucose levels or insufficient supply to the brain results in impairment of neuronal function. Whenever blood glucose decreases below 20 mg/dl, the hypoglycemic coma and brain injury take place. Hypoglycemia can occur as a complication of insulin treatment in diabetic patients, leading to brain glucose deprivation (GD). Neuronal damage induced by hypoglycemia is initiated by an excitotoxic mechanism triggered by the release of glutamate and aspartate. However, several molecular and cellular mechanisms are activated that ultimately lead to cellular death. A variety of stress stimuli are able to induce autophagy including nutrient and energy deprivation. Autophagy is a lysosome-mediated intracellular catabolic mechanism characterized by the appearance of double- or multiple-membrane cytoplasmic vesicles and the lipidation and redistribution of the cytoplasmic protein LC3-I, towards vesicles. The main role of autophagy is to maintain the cellular homeostasis and supply of building blocks for protein synthesis and energy in response to starvation. However, excessive autophagic degradation can lead to cell death, known as autophagic cell death. The aim of the present study is to analyze the role of autophagy in GD-induced neuronal death.

Cortical cultures of 7-8 DIV were exposed for different times to GD followed by glucose reintroduction (GR) to evaluate morphological (the presence of double membrane vesicles) and biochemical (LC3-I/LC3-II, beclin1 and p62 protein levels) features of the autophagic pathway. To evaluate the role of autophagy in neuronal death by the MTT reduction and LDH release assays, cultures were exposed for 2 h to GD and 22 h to GR. We found a rapid activation of autophagy and accumulation of autophagosomes during the GD period. However, the autophagic flux was completed until the first stages of GR. Autophagy inhibition by 3-MA incubation during or after GD prevented neuronal death. Conversely, inhibition of autophagic degradation with chloroquine (CQ) during the GD period resulted in aggravation of neuronal death. Nonetheless, CQ treatment during GR improved cell viability, suggesting that regulated activation of autophagy during GD is beneficial for cortical cell survival.

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Role of the NADPH oxidases during the excitotoxic damage in the striatum in mice.

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The excitotoxic damage is a common phenomenon in various pathologies of the central nervous system (CNS). The underlying mechanisms of excitotoxicity depend on several factors, including an increase in the intracellular concentration of calcium (Ca^{++}) and in the production of nitrogen species and reactive oxygen species (ROS), whichcontribute to neuronal death and subsequent loss of function. Recently,we found evidence pointing out to the NADPH oxidases (NOX), particularly NOX-2 isoform, as the main source of ROS responsible for oxidative stress during this process both, in vitro and in vivo models. The only known function for NOX enzymes isROS (H2O2 and O2•) production that seem to be involved in redox regulation processes during physiological and pathological conditions. For instance, it has been observed that a rise in its activity is related to the inflammatory phenomena, as well as necrosis and apoptosis. These features postulate NOX as an excellent therapeutic target in the control and prevention of excitotoxic damage. In an in vivo model of excitotoxicity, C57-BL6 mice were injected intracerebrallywith glutamate and we evaluated the time course of NOX activation in the striata of wild type and NOX2 $KO^{-/-}$mice. Under these conditions, we found a significant increase in NOXactivity with a biphasic fashion (1-12 h) in wild type animals, which was not observed in animals deficient in NOX2. The observed increased in NOX activity correlated with the results obtained from the cylinder test since,in contrast to wild type animals, NOX-2 deficient animals showed a satisfactory performance after glutamate administration. Protein levels, determined by Western blot analysis, indicated an increase inNOX-2 expressionthat could be related to the second peak of NOX activity detected;however, the increase detected in the first hour after glutamate administration appears to be independent of such regulation.It is possible that NOX-2 deficient animals could have an increased expression of NOX-4, as a compensatory mechanism, which has been observed in other models.

The administration of S-allyl cysteine reduces the neurological deficit and mortality in the middle cerebral artery occlusion model in the rat.

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Stroke is the second leading cause of death worldwide and the leading cause of adult neurological disability. Since the brain is the organ with the largest demand of oxygen and glucose, a sudden interruption of the blood flow produce a fast depletion of both substrates, accumulation of toxic metabolites, energetic failure and generation of reactive oxygen species (ROS) leading to functional and structural damage of the cells. Actually, the recombinant tissue plasminogen activator (tPA) is the only drug approved by the FDA, unfortunately the tPA is inefficient in patients with a severe stroke, and its therapeutic window is of 3-4 honce the stroke started.The actual research to improve the outcome of these patients is based in the reduction of damage induced by the ROS once the reperfusion started. More than 800 antioxidants compounds have been proved with promising results in animal model but failed in the clinical proves, the main reason for these failures is that the antioxidants are administrated before the stroke. Our aim is to evaluate the therapeutic effect of S-allyl cysteine (SAC), the main compound from the aged garlic extract, on neurological deficit and the histological damage induced by stroke.

Male Wistar rats (280-320 g) were separated in 4 groups (n = 7-10): 1) Sham, animals submitted to the surgical procedure without occlusion and saline solution i.g.; 2) IR, animals submitted to 1 h of occlusion and 7 days of reperfusionand saline solution i.g.; 3: SAC, animals submitted to the surgical procedure without occlusion and SAC i.g.; and 4) IR+SAC, animals submitted to 1 h of occlusion and 7 days of reperfusionand SAC i.g. Stroke was induced by middle cerebral artery occlusion using a nylon filament. Saline solution and SAC were administrated the 25 h after cerebral occlusion and daily during 7 days. Twenty four hours and 7 days after cerebral occlusion the neurological deficit was evaluated using 5 tests. SAC treatment improved the neurological deficit in a dose dependent manner and reduced the mortality, being the dose of SAC 50 mg/Kg the most effective. This is the first study that showed the therapeutic effect of SAC in a experimental model of stroke.

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STIM1 and ORAI1 importance in Alzheimer's

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Introduction: Alzheimer's disease (AE) is a neurodegenerative disease of the central nervous system (CNS) characterized by the progressive and irreversible loss of neurons. It was described in 1906 by German neurologist Alois Alzheimer, who histologically characterized by two structural abnormalities, neuritic plaques and tangles neurofibrales (Guimerá et al., 2002, Carrillo et al., 2013).Its high rate of prevalence is the fourth leading cause of death (WHO. 2012). Currently there are 35.6 million sufferers in the world, Mexico 2030 350,000 cases of which were reported killed annually. Despite extensive research is still unclear the etiopathogenic process, limiting the development of efficient drugs and timely diagnosis. This work is based on the "calcium hypothesis" which states that brain atrophy is due to altered calcium homeostasis and this, a key process in the pathogenesis of the disease.The calcium ion channels that were studied in this work are STIM1 and Orai1, in order to discuss their participation in the AE. Molecular models were used to see their relationship with the Tau and Beta-amyloid protein prediction analysis on topology and molecular modeling suggest that STIM1 and Orai1 ion channels may play an important role in the onset and during the disease.

In conclusion, the in silico analyzes suggest that calcium homeostasis, ion channels regulated by STIM1 and Orai1, could be crucial in the initiation and during illness.

ALPHA $GABA_A$ RECEPTOR SUBUNIT IS EXPRESSED IN WHITE MATTER ASTROCYTES FROM THE CEREBELLUM

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ABSTRACT

Recent studies on white matter of the cerebellum showed that a fraction of astrocytes have neurogenic activity within a restricted window of postnatal development (from postnatal day 5 to postnatal day 12). On the other hand, gamma-aminobutyric acid (GABA) is released by 4 of the 5 neuronal types of the cerebellum and astrocytes are known to express functional $GABA_A$ receptors. These ionotropic receptors are pentameric proteins where 2 alpha subunits are required for proper function, at present 6 different subunits are known (alpha1-6). Thus, the aim of this study was to investigate if alpha subunits of the $GABA_A$ receptor are differentially expressed by neurogenic astrocytes and astrocytes without neurogenic activity in the white matter of the mouse cerebellum. Our electrophysiological recordings showed that GABA induced inward currents in astrocytes recorded from the white matter of the cerebellum, suggesting functional expression of $GABA_A$ receptors. Immunohistofluorescence studies for the six alpha subunits confirmed this hypothesis and showed inmmunoreactivity for white matter astrocytes. Specific studies on alpha-1 subunit revealed that it is expressed only in astrocytes of the deep cerebellar nuclei, while astrocytes at the lobes showed no immunoreactivity, at postnatal day 8. Interestingly, no expression of alpha-1 subunit was detected at postnatal day 18 neither at the deep cerebellar nuclei nor at the lobes. Our conclusion is that white matter astrocytes from the cerebellum express functional $GABA_A$ receptors with a differential expression of the alfa subunit during postnatal development.

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Searching for negative regulators of microglia activation: an alternative for Alzheimer's disease treatment?

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Microglia are immune cells of mesodermal origin that reach the central nervous system during development. Microglia belong to the macrophage lineage and therefore, they play a key role in responding to inflammation and to different immune challenges within the brain. Recently, microglia has been described to participate not only in regulating immune surveillance and response but also, in controlling processes such as synaptic pruning during the development, maintenance of the neural environment as well as embryonic and adult neurogenesis. In addition, recent evidencesindicate a role for activated microglia in Alzheimer's disease as in this state; microglial cells release pro-inflammatory cytokines that induce neuroinflammation triggering the amplification of the pathology. Thus, the blockage of microglia activation has been proposed as a potential therapeutic strategy in this class of disorders. The present study was focused on the anti-inflammatory effect of a hydroalcoholic extract of *Malva parviflora*(*M. parviflora*) using primary cultures of mouse microglia. Primary microglial cells were isolated from wild-type CD1 mice and from 5xFAD used as model system for Alzheimer´s disease.We demonstrated that the hydroalcoholic extract of *M. parviflora*possesses immunomodulatory properties as it significantly decrease the activation of NF-kB and AP-1 in cells stimulated with LPS. We also observed an anti-inflammatory effect of *M. parviflora*in neonatal mice microgliaas itreversed the amoeboid phenotype (associated with activated microglia) of these cells when treated with LPS. Likewise,microglia cells treated with the hydroalcoholic extract of *M. parviflora* exhibited an enhanced phagocytic capacity and a multipolar morphology. The characterization of the microglia isolated from 5xFAD adult mice at different pathological stages will be discussed.This work was partially supported by grants from CONACYT (155290 and 154542) and DAGPA/UNAM (IN209212 and IN227510).

Keywords: Microglial cells, microglia activation, phagocytic and anti-inflammatory responses, Alzheimer's disease, *Malva parviflora.*

Effect on acetylcholine by ionic and oxidizing environments involved in Alzheimer's disease.

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Acetylcholine (ACh) is a very important neurotransmitter involved in memory and learning. However, there are factors which modify them, such factors as the accumulation of metals in the brain and hydrogen peroxide (H_2O_2) generated by the β-amyloid (βA). Therefore, in this work, it was studied *in vitro* how ACh levels are affected by the metals iron, copper and zinc; taking into account the metal concentrations involved in Alzheimer's disease.Bonting and Featherstone method was used to calculate the concentration of ACh hydrolyzed. This neurotransmitter is affected by metals, but more by the oxidizing environment which is generated by H_2O_2. Therefore, it is important to generate drugs in order to diminish the ionic and oxidizing environments to reduce the damage observed on acetylcholine.

Role of *Nurr1* gene reduction and prenatal stress on depression-like behavior

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Introduction: The exposure to prenatal stress alters brain development and the expression of the transcription factor Nurr1. The *Nurr1* gene encodes an orphan nuclear receptor and acts as a transcription factor essential for the development and maintenance of dopaminergic neurons in the brain. We have reported that in C-57 black/6J mice exposed to stress rapidly increases the expression of *Nurr1* in the hippocampus. The aim of this study was to analyze the behavior of animals with reduction of *Nurr1* gene in hippocampus and exposed to prenatal stress, on depression-like behavior

Methods: Pregnant Wistar rats were exposed to restraint stress. Thus, neonates with prenatal stress and unstressed controls were obtained. Ninety days later, these animals were implanted with guide cannulae to the hippocampus through stereotactic procedures. After seven days, antisense oligonucleotide (or its scrambled control) for the *Nurr1* gene was administered to reduce its expression. An hour later, the forced swiming test was performed to analyze their behavior. This behavioral test, besides being used to analyze depression-like behavior, involves the exposure of animals to acute stress.

Results and Conclusion: Animals with reduced *Nurr1* gene and exposed to prenatal stress had significantly lower immobility time (indicator of lower depressive-like behavior) in the forced swimming test, in comparison with control animals. The results demonstrate that *Nurr1* gene and / or prenatal stress may be related to adaptations of animals, allowing them to cope with future stressful situations. **Partially supported by CONACYT-CB No. 106619.**

The ketone body β-hydroxybutyrate (BHB) reduces the production of reactive oxygen species and prevents neuronal death induced by glucose deprivation *in vivo* and *in vitro* models.

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Glucose is the most important energy source in brain and whenever its concentration in blood decreases to less than 20 mg/dl, neuronal death can take place. The mechanisms leading to neuronal death during glucose deprivation have not been completely elucidated but the role of reactive oxygen species (ROS) has been suggested. Under certain conditions the brain can consume alternative substrates to glucose, such as the ketone bodies (KB), beta-hydroxybutyrate (BHB) and acetoacetate (AcAc). KB blood levels substantially increase (from 0.1 mM to 1-8 mM) during the ketogenicdiet, prolonged fasting and sustained hypoglycemia. It has been shown that the ketogenic dietreduces the number of seizures in epileptic patients and BHB prevents neuronal death induced in several models of brain ischemia, trauma and neurodegenerative diseases. In previous studies we have shown that BHB prevents neuronal death inducedbyglycolysis inhibition in hippocampal cultured neurons by a mechanism involving the reduction of ROS production (Exp. Neurol. 2008, 211, 85).We have now investigated whether BHB can prevent neuronal death and (ROS) production induced by glucose deprivation (GD) in cultured cortical neurons and in the cerebral cortex of rats subjected to severe hypoglycemiainduced by insulin administration and recovered with glucose infusion. Cortical cultureswere exposed during 1-2 h to GDfollowed by glucose reintroduction (GR). ROS production was assessed by the oxidation of the fluorescent dye, dihydroethidium (DHE), ATP levels measured by the luciferin-luciferase determinationkit and cellviability by the MTT reduction and the LDH release assays. The presence of ROS-producing cells in vivo was analyzed in brain sections,after DHE administration to rats and neuronal death by Fluoro Jade-B staining. Results showthat D-BHB reduces60% ROS generated during GD and GR, prevents the decline in ATP and reduces neuronal death between 50 and 70%. In vivo results show that the systemic administration of D-BHB reduces ROS levels between 80 and 40% in distinct cortical areas and notably prevents neuronal death in the cortex of hypoglycemic animals.
Results suggest that protection by BHB, results from its metabolic action combined with its capability to reduce ROS levels, probably due to its contribution to the maintenance of mitochondrial activity. They also suggest that BHB may be a good candidate for the treatment of ischemia and traumatic injury.
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Cytochrome P450 2J3 regulation in a LPS-induced model of neuroinflammation in astrocytes

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Introduction:

Cytochromes P450 (CYP) constitutes a family of enzymes characterized by carrying out the oxidation of organic compounds. In addition, CYP also metabolize many endogenous substrates to biologically active intermediates. CYP 2C and 2J isoforms, which display epoxygenase activity, are involucrated in arachidonic acid oxidative metabolism yielding epoxyeicosatrienoic acids (EETs) metabolites. Among their diverse biological properties, EETs exhibit anti-inflammatory activity. CYP epoxygenases as well as the resulted metabolites have been proposed as important therapeutic targets for the treatment of both systemic and organ specific inflammatory processes, including the Central Nervous System (CNS). However, little has been described about the regulation of these enzymes during inflammation. It has been reported that the expression of some CYP can be modified by pro-inflammatory cytokines such as IL-6, IL-1b and TNF-a. Cytokine-mediated down regulation of some CYP has been related to NF-kB binding to the promoter region of the regulated genes. Our goal is to elucidate whether an inflammatory process in cells of the CNS is able to modify CYP 2J3 expression and the mechanism by which this process is carried out.

Materials and methods: Rat brain primary astroglial cultures were obtained from the cortex of newborn rats. Immunocytochemical identification of astrocytes was performed to confirm its purity. Cultures were treated with 100 ng/mL LPS. As inflammation marker, TNF-α level was determined by ELISA. Additionally, cultures were treated with 5 ng/ml TNF-α to determine its role in CYP2J3 regulation after LPS treatment. Since NF-kB is an important transcriptional factor dependent of pro-inflammatory cytokines like TNF-α, cultures were also treated with 5 ng/ml TNF-α + 1 ng/ml IMD-0354 (selective NF-kB inhibitor) to determine NF-kB role in the response to TNF-α treatment. CYP2J3 mRNA expression was determined by qRT-PCR. CYP2J3 protein expression was determined by Western blot.

Results: The addition of LPS and TNF-α to astrocyte cultures caused a decrease in CYP2J3mRNA and protein expression. Concurrent addition of IMD-0354 to TNF-α treated cultures caused an inhibition of observed TNF-α effect on CYP2J3 mRNA expression.

Conclusions: The inflammatory process triggered by the addition of LPS to astrocytes cultures is able to down-regulate CYP2J3 mRNA and protein expression. LPS mediated down-regulation of CYP2J3 expression may be due in part to the production of pro-inflammatory cytokines like TNF-α, since this cytokine is also able to down-regulate CYP2J3 mRNA expression independently of LPS addition. Transcription factor NF-kB may play an important role in TNF-α mediated down-regulation of CYP2J3 since its inhibition by IMD-0354 reversed CYP down regulation.

NEUROLOGICAL RECOVERY IN RATS TREATED WITH A COMBINATION THERAPY AFTER CHRONIC SPINAL CORD INJURY

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Spinal cord injury (SCI) frequently occurs in young people and leads to permanent damage in motor, sensorial and autonomous nervous system functions. After SCI autoreactive mechanisms enhance neural tissue damage. Research in this field has shown that immunization with neural-derived peptides (INDPs) could provide the necessary conditions to achieve the beneficial and avoid the detrimental effect of immune cells. Recent studies have suggested that modulation, rather than suppression, of immune response could be the best way to attain neuroprotection and neuroregeneration after SC injury. The protective autoreactivity (PA) is supposed to modulate autoreactive mechanisms in order to promote neuroprotection activating microglia under a particular phenotype which low free radicals production. A91 is a modified neuropeptide that is able to diminish secondary neurons degeneration and promote motor recuperation in animals after SCI. Therefore A91 used in combination with other therapies might enhance its protective effect. Hence, the aim of this work is to explore the effect of combination therapy during SCI chronic stage. Neurological recovery was evaluated in 25 female Sprague Dawley rats after 60 days post-injury. Animals were divided in five groups: 1) Rats subjected to chronic SCI without further treatment; 2) Rats subjected to chronic SCI + scar removal; 3) Rats subjected to chronic SCI + scar removal + A91 immunization; 4) Rats subjected to chronic SCI + scar removal + A91 immunization + tissucol with mesenchimal cells; 5) Rats subjected to chronic + A91 immunization + tissucol with mesenchimal cells. Neurological recovery was determined using BBB test weekly during 60 days. Our results showed a significant recovery in motor capacity in rats from group 3, chronic SCI + scar removal + A91 immunization. Suggesting that this combined therapy was the most effective in preserving motor skills.
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Differential expression of Dystrophins during murine brain development Griselda Rodríguez-Martínez[1], José Romo-Yañez[1,2], Anayansi Molina-Hernández[2] and Cecilia Montañez[1]

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Duchenne Muscular Dystrophy (DMD) is a muscle degenerative disease caused by mutations in the dystrophin gene expression. In addition to the progressive muscle degeneration, cognitive impairments in one third of the patients with DMD have been observed. Dystrophin gene is composed of several internal promoters leading to the expression of different protein products. In the last years, a correlation between alterations in the Dp140 and Dp71 dystrophin isoforms expression and the presence of cognitive deficits has been found; however, their functions in brain still remain elusive. Since brain is one of the most complex organs in mammals, its formation requires a tight regulation for giving rise the proper number and pattern of the different neural cells. As it is known that most of key regulators in brain formation are found since early stages of development, we analyzed the expression pattern of dystrophins during embryonic brain development and early stages of postnatal brain development in order to gain new knowledge into the dystrophins field. Dp427, Dp140 and Dp71 showed a low transcript expression in embryonic stages, but its expression increased before birth and postnatal ages. In addition, we also analyzed the expression patterns for the alternative-spliced isoforms. Thus, in early stages of development most of the mRNA lacked of alternative splicing, but it increased as development progressed, and in postnatal stages, most of the mRNA showed alternative splicing for 71-74 and 78 exons. Regarding protein expression, Dp427 protein was only detected in postnatal and adult stages, while Dp140 protein was found since embryonic stages, reaching its maximum level at 18 embryonic days (E18), Dp71 protein, on the other hand, was barely detected at E16, but it was highly enriched as development progresses, being Dp71 the predominant isoform in adult brain. Interestingly, at E14-16 dystrophins were detected in both basal and apical neuroepithelium in neural stem/progenitor cells, neurons and astrocytes, being higher in the basal side where undifferentiated cells were predominant. But, since the antibody used recognizes most of dystrophin isoforms, we were unable to determine whether any isoform is specific for any neural cell. In summary, our results show that dystrophins could have a role in the early processes of brain development, likely having isoforma-dependent functions; thus, as Dp140 pattern expression coincides with neurogenic stages, it could be involved in neuronal differentiation, whereas Dp427 and/or Dp71 could be involved in neural maturation since they are expressed at later stages. This work was supported by CONACyT CB-2009-127600 and SEP-CONACyT-ECOS-ANUIES M11-S02 B000/064/12.

Early life stress increases cytokine expression in the hippocampus and stimulates the release of cytokines in the circulation of rat pups

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Early life stress impactsthe development of the central nervous system (CNS) and the immune systemand increases the vulnerability to suffer a wide spectrum of diseases in adulthood.Glial cells (microglia and astrocytes) are the CNS immune and secrete pro- and anti- inflammatory cytokines. During development these cytokines are trophic factors that contribute tothe maturation of neurons. Thus, early life stress might cause an imbalance in cytokine function within the CNS contributing to psychopathology in adult life.The aim of this workwas toanalyze theimmediate effectsof early life stress on the activation and cellular densityof astrocytes and microglia, as well as to examine the expression of cytokinesin thehippocampus andtheir presence inthe circulation of male rat pups at postnatal day(PD) 15.A group of rat pups were separated from the dam (Maternal separation group, MS)for 3 hours/day from PD1 to PD14; other group waskept in the mother's nest without manipulations (control group). Pups weresacrificed at PD15 and thebrains wereperfused and sectioned for immunohistochemistry, or were immediately dissected to isolate the hippocampus to analyze the expression of cytokinesby q-PCR. Trunk blood was collectedto analyze cytokine concentrations. Half of the groups were sacrificed under non-stressed (basal) conditions and the other half was subjected to a single event of 3h maternal separation (stress) before sacrifice.Our results showed an increased expression of IL-1β(~2 fold) in the hippocampus of the MS group compared to control under basal conditions. Stress-induced expression of IL-1β was significantly higher in MS than in control basal group and SM control (~18 and 9 fold, respectively). The expression of IL-6 was unchanged in both groups under basal and stress-induced conditions and the expression of TNF-αwas significantly increased in basal conditions but not in response to stressin MS pups. Stress-induced TNF-α expression was significantly increased in control pups (~2.5 fold).Circulating IL-6 increased after stress in theMS group (~2.5 fold) but not in basal conditionscompared to controls.No changes were foundin the concentrations of TNF-α in basal or stress-induced conditions in both groups. Analyses in the hippocampalhilusshowed a similar number of microglial cells in both groups but we detected a significant increase in the number ofactivated microglia in the MS pups (~1.7 fold). The number of astrocytic cells was decreased in the MS group compared to control group. In conclusion we found a differential expression of cytokines in the hippocampus and a selective releaseof circulating cytokines under basal or stress-induced conditions in MS pups. Glial cells were affected in a different way bypostnatal stress. This suggests that early life stress induces a preactivated state of the brain immune system anda selective peripheral cytokine response.

IncreasedmitochondrialComplex IV activitybycopper pre-treatment reducesMPP+-inducedmitochondrialstriataldamage

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Parkinson'sdisease (PD) is a neurodegenerativedisordercharacterizedbybothreducedactivity in mitochondrialcomplex I activity and electron transfer ratediminished in substantianigra of thesepatients. Laboratoryanimalstreatedwith 1-methyl-4- phenylpyridinium (MPP+) reproduces themainbiochemicalfeatures of PD through of complex I inhibition and free radicalsoverproduction. Copper (Cu) playsanimportant role as prostheticgroup of severalproteinsinvolved in themetabolismsuch as mitochondrialcomplex IV, a multi-proteincomplexinvolved in cellularrespiration. In previousstudies, wehavefoundthat Cu pretreatmentprovidesprotectionagainstneurotoxicity MPP+-induced in thestriatum of rats. In thiswork, weexploredtheeffect of Cu pre-treatment in themitochondrialelectron transportchain in thestriatum of MPP+-treatedrats. MaleWistarrats (270-300 g) weretreatedwith $CuSO_4$or Na_2SO_4 (10 µmol/kg), and sixteenhourslateranimalswereinjected in therightstriatumwith 10 µg/8 µL MPP+ solutionorsaline as controlgroup. Sixhoursafterstriatalinjury, animalswerekilled and striataltissuewasdissected and enrichedmitochondrialfractionwasobtainedbycentrifugation. MitochondrialComplex IV activitywasmeasured as therate of cytochrome c oxidation (recorded to 550 nm and 30°C). Thefirst-orderrateconstant (k) wascalculated and theactivityisexpressed in k/min/mg protein. Weobservedthatcopper pre-treatmentgroupshown a slighttendency to increasecomplex IV activity (0.148±0.035) above control group (0.130±0.03). MPP+ produced a significantreduction ($P < 0.05$) in IV Complexactivity (0.042±0.010), whereasthechallenge Cu/MPP+ shown a slightincreasing (0.144±0.023) respect to control group. Also, MPP+ produced a significantreduction in electronchain transfer Complex I and II activities in mitochondria, thiseffectwascoincidentwithreduced ATP production. Copper pre-treatmentpreservedComplex I and II activities, thus preservingmitochondrialfunction. Copperalsopreserved ATP synthesiswithComplex I substrates, and partiallypreserved ATP levelswithComplex II substrates in mitochondriafrom MPP+ treatedrats. Theeffectsobserved can be related in firstterm to copperinductionuponmitochondrialComplex IV activity.

Epicatechin administration reduces circling behavior and dopamine depletion MPP+-inducted in rat.

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Parkinson's disease is a neurodegenerative disease characterized for movement disorders caused for diminished dopaminergic neurotransmission in the nigrostriatal pathway apparently produced by oxidative stress (OS). Administration of 1-methyl-4-phenylpyrinium (MPP+) in laboratory rats reproduce the main biochemical characteristics of PD. MPP+ inhibits the complex I of the chain transport electron in the mitocondrial which overproduces free radicals (FR). Increasing FR formation produce OS, decline of DA and behavioral disorders. Catechins (phenolic compounds of green tea), have shown ability as radical scavengers, inductors of antioxidant enzymes redox state modulators and transition metal chelators. In the present study we evaluated the effect of the oral chronic administration increasing doses of epicatechin (EC) to estimate behavioral disfunctions induced by intrastriatal microinjection of MPP+ (10 µg/8µL). Results showed a dose-dependent effect in the study, 100 mg/kg of EC administration is able to significantly decrease ($P < 0.05$) ipsilateral turns behavior induced by apomorphine administration in injured rats with MPP+. Additionally MPP+ administration induced significant ($P < 0.05$) decreases in DA content, however the oral acute administration of 100 mg/kg of EC pre-treatment was sufficient to preserve dopamine levels. The present study also demonstrated that acute administration of 100 mg/kg of EC pre-treatment significantly ($P < 0.05$) decreased both the formation of fluorescent lipid products and reactive oxigen species induced by the infusion of MPP+. The results of this study indicate that pre-treatment with 100 mg/kg of EC has no effect on classical markers of liver damage and is effective against the damage induced by MPP+; nevertheless, future studies are needed for better understanding of mechanism action of EC.

"Alterated levels of histamine and H_1 receptor expression during central nervous system development in embryos from diabetic rats."

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Histamine is an important neurotransmitter and neuromodulator monoamine, in the adult and neonate central nervous system. During brain embryo development is one of the first neurotransmitters to appear, reaching it maximum level at embryo day 14 in the rat. Moreover, high histamine concentrations lead to an increase in FoxP2 neuron differentiation of neuroepithelium stem cells *in vitro* and *in vivo* by H_1 receptor subtype, a transcription factor related to language. Furthermore during neural stem cell proliferation *in vitro*, histamine promotes an increase in apoptosis in a concentration dependent manner. It has been reported that maternal diabetes cause changes during fetal neurodevelopment in animal models promoting early maturation and proliferation, while in human children born from gestational diabetic women present memory, intelligence, verbal and motor impairment. In this study in the rat model the effects of diabetes during pregnancy induced by steptozotocine in the levels of histamine and the expression of H_1 receptor in the developing forebrain at embryo days 12, 14, 16, 18 and 20 of embryos. The histamine level was assessed by ELISA, while the H_1 receptor expression was evaluated by RT-qPCR and western blot in dissected brain tissue of the corresponding area that will give rise to the neocortex. Our results showed an increase of 3.4 fold in histamine concentration in embryo from diabetic rats at day 14 and a decrease in the same group of 1.25, 2.6 and 3.7 times at embryo days 12, 18 and 20 respectively. Regarding H_1 receptor expression our result showed a significant increase in embryos from diabetic rats at days 12, 14 and 20 of 2.1, 1.8 and 3 fold respectively, while decreasing of 5.5 and 1.25 times were observed at developmental days 16 and 18. Although, H_1 receptor mRNA were affected in embryos from diabetic rats, the western bot analysis did not detect any changes in its protein levels. Our results suggest that diabetes during pregnancy can affect embryo histamine levels and H_1 receptor expression, without affecting the protein levels of the receptor. It is necessary to further study the relationship among the decrease of the histamine level at embryo day 12 and H_1 receptors activation with FoxP2 neuron differentiation, since we have previously reported that H_1 receptor inhibition at embryo day 12 *in vivo* decreased FoxP2 positive neurons in dorsal cerebral cortex epithelium, as well as the relationship between the rise of histamine at embryo day 14 with early maturation and/or the apoptotic cell death in early maturated neurons, in other to know if the altered developing histaminergic system in the diabetic rat embryos may be one important participant in the alteration reported in the diabetic animal models during embryo development and in children from diabetic mothers during pregnancy.

Analysis of cellular and viral RNA-expression by RNAseq in normalhuman cellsinfectedwithadenovirus 5.

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Adenovirus type 5 (Ad5) are non-enveloped, icosahedral viruses, containing a linear double stranded DNA genome. Both strands are transcribed, and their expression is divided in temporal transcription units. The products of the early transcription units (E1A, E1B, E2, E3 and E4) establish the cellular conditions in the infected cell for the efficient replication of the virus, while the late transcription unit, known as Major Late, encodes about 20 products among which are the structural proteins that form the viral capsid. All these transcripts are produced by RNA polymerase II; however, during the infection, Ad5 produce other types of non-coding RNAs that are synthesized by RNA polymerase III.

During adenovirus infection, the pattern of production of different types of RNA changes drastically in the infected-host cell, suggesting that cellular or viral RNAs could participate in the modulation of cellular defense mechanisms, such as cell cycle regulation, apoptosis and the innate immune response.

Performing an analysis of RNA isolated from Ad5-infected normal human cells by high throughput sequencing we have found that by the late phase of infection different subsets of cellular RNA are produced compared with the non-infected cells. Our data showed differential expression of cellular miRNAs during the infection, suggesting Ad5 may induce regulatory mechanism that impact cellular miRNAs expression. Interestingly, prediction of possible targets for these miRNAs revealed they could regulate cancer related mRNAs.

Antiviral activity of *Walteriaamericana* L. extracts against human rotavirus strains

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Background. Rotavirus is the main causative agent of viral gastroenteritis. Each year, rotavirus is responsible of 440,000 children deaths. Efforts to prevent and control outbreaks annually have derived in two vaccines: Rotarix^{TM}(GSK) and RotaTeq^{TM} (Merck). However, during co-infections genetic exchanges and genetic recombination can occur between different strains of rotavirus. In addition, during virus replication, mutationcan accumulate due to low fidelity RNA viral polymerase. Therefore, new genetic and antigenic varieties of rotavirus could emerge. For that reason, an approach to use plant extracts with therapeutic activity may represents a simple and natural alternative method to complement the treatment of viral diarrhea. **Metodology**. Hydro-alcoholic and aqueous extracts of *Waltheriaamericana* were used to evaluate the reduction of infectious foci in MA104 cell infected with rotavirus.Each extract effect wasalso analyzed on the reactivity of antibodies anti rotavirus as well as the production of genomic RNA and viral proteins. **Results**. Both aqueous and hydro-alcoholic extracts showed a reduction from 60% to 100% of infectious foci. In addition, 0.1 mg/ml added before rotavirus infection was enough to reduce 99% of infectious foci of both P[4] and P[8] rotavirus genotype. ELISA analysis showed that polyclonal Antibodies were able to recognize these rotaviruses pretreated with aqueous or hydro-ethanolic extracts. Moreover, analysis of electrophoresis showed production genomic RNA and Western blot analysis exhibited the presence of structural VP4 and non-structural NSP2 proteins.**Conclusions**. These results suggest that the plant extracts could accomplish its antiviral activity after virus infection.

Viral incidence and diversity in strawberry fields oflrapuato, Mexico

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Viruses are one or the simplest life forms on earth,howeverits size, compact and highly organized genome has allowed them to be the most adapted organism to theirecological niches. Unfortunately the total viral diversity is unknown. Several studies havededicated effortsto know the viruses that have an impact in the human life, orfood production.

In the case ofstrawberry, a small fruit of great importance throughout the world, near to 30 viruses have been found affecting its production.Mexico is an important strawberry producer worldwide, beingZamora, Ensenadaand Irapuato in the States of Michoacán, Baja California and Guanajuato respectively,the major producers. In the case of Irapuato,the introduction of new and more profitable horticultural crops, as well of phytopathological problems(some of them caused by viral infections), affectedstrawberry production,displacing it from themain producer, to the third position atthe national level.

Just a couple of works havestudied viruses present in strawberry fields inIrapuato,where single and mixed infections of *Strawberry mottle virus* (SMoV) and *Strawberry crinkle virus* (SCV) were found in 1989, andanother one reporting *Strawberry latent ringspot virus* (SLRSV), in2004.

This survey has been focused to investigate the viral diversity prevailing in the strawberry producingregion of Irapuato in Guanajuato State.

Strawberry plants showing symptoms associated to viral diseases were collected in commercial plots, within the Irapuatocounty during April and December 2007, July and December 2008, July 2013 and April-May 2014.RT-PCR and sequencing of viral fragments, revealed the presence of seven viral speciesin single, and/or mixed infections: SCV, SMoV, *Fragariachiloensis cryptic virus* (FClCV), *Fragariachiloensis latent virus* (FClLV), *Strawberry pallidosis associated virus* (SPaV), *Strawberry necrotic shock virus* (SNSV), and *Strawberry mild yellow edge virus* (SMYEV), the last five were reported for first time in strawberry fields in Mexico.Viral sequences obtained werecompared with other viral isolates, and we found some changes such as deletions, silent and non-silent mutations and agreat similarity with viral isolates from the USA.

The presence of some viruses in Irapuato,may be due to the flux of vegetativegermplasm between USA and Mexico, where the virusesinfectnew hosts, that became source of inoculum for the next growing season. These findings encourage us to continue analyzing these viral isolates in more detail, to have a better understanding of their genetic variability and the impact on strawberry production, in conjunction with other pathogens.

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Missense mutationsidentified intoT-cell and B-cell epitopes in the Surface Antigen (HBsAg) of Hepatitis B Virus (HBV) Genotype H.

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BACKGROUND

The surface antigen of hepatitis B virus (HBsAg) is the major diagnosis serological marker of HBV infection; consists of 226 amino acids (aa). This antigen contains the main target for neutralizing antibodies named the “a”-determinant (aa 122-148) and along the entire protein are localized different T-cell and B-cell epitopes. Mutations in these epitopes can alter the HBsAgstructure with consequences like failure to serological diagnosis, vaccine escape,and possiblyimmune escape favoring the perpetuation of the disease.

AIM

To determine the presence of missense mutations into T-cell and B-cell epitopes in theHBsAgfromMexican patients with HBV infection genotype H.

MATERIAL AND METHODS

Twenty-two positive HBV blood samples were analyzed from patients with chronic hepatitis B from the Center and Western of Mexico. The HBV-S gene was amplified by PCR. Cycle sequencing was done by the chain termination method using BigDye® Terminator v3.1 with the sequencing primers.The aminoacids sequence corresponding to HBsAg were deduced from the nucleotide sequence.The amino acid differences were identified according to the corresponding genotype using a hepatitis virus database, which include approximately 6,800 HBV strains.

RESULTS

The genotypes identified in the 22 samples analyzed were, H (82%), F (9%) and G (9%).Amino acid changes were identified in 11/22 samples.We found two of the main escape mutations (sI195M and sQ129H) in two of the samples. Eightmore missense mutations were localized within T-cell epitopes (aa 11 to 33 and 28 to 51) and three in B-cell epitopes (aa 160 to 207). Three additional missense mutations were identified out of B-cell and T-cell epitopes.

CONCLUSIONS AND DISCUSSION

We identified two of the most important and well-documented immune escape mutations (Q129H and I195M), and 11missense mutations within T-cell and B-cell epitopes. These amino acid changes could modify the conformation of these immunogenic determinants with possible failure to HBV vaccine and serologic diagnosis. Also, mutations identified into B-cell and T-cell epitopes could affect theimmune response evoked to eradicate the HBV, with the consequent development ofchronic HBV infection.

Bilirubin defines cytokine profiles by modulating STATs function during hepatitis A virus infection in children

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Summary

There are approximately 1.5 million cases of Hepatitis A virus (HAV) infection reported worldwide annually. In children, HAV is recognized as the most frequent cause of hepatitis, acute liver failure and liver transplantation. The clinical spectrum of infection is variable, and liver injury is determined by altered hepatic enzyme function and bilirubin concentration. We recently reported differences in cytokine profiles between distinct HAV-induced clinical courses, and bilirubin has been recognized as a potential immune-modulator. However, how bilirubin may affect cytokine profiles underlying the variability in the course of infection has not been defined. Herein, we used a transcription factor (TF) binding site identification approach to predict the entire set of TFs associated with the expression of specific cytokine profiles. The results suggested that modulation of the activity of signal transducers and activators of transcription proteins (STATs) may play a central role during HAV infection. We then compared the degree of STAT phosphorylation in peripheral blood lymphoid cells (PBLCs) from pediatric patients with distinct levels of conjugated bilirubin (CB). Low CB levels in sera were associated with increased STAT-1 and STAT-5 phosphorylation. A positive correlation was observed between the serum IL-6 content and CB values, whereas higher levels of CB resulted in reduced serum IL-8 values and in a reduction in the proportion of PBLCs positive for STAT-5 phosphorylation. When CB was used to stimulate patients' PBLCs *in vitro*, the levels of IL-6 and TNF-α were increased. The data showed that bilirubin plays a role in modulating STAT function and defining cytokine profiles during HAV infection. Funding from CONACYT and COECYTJAL-UdeG.

"HPV Infection Modulates the Phosphoproteome Of Cervical Cells Of Women With A High Grade Squamous Intraepithelial Lesion (HSIL)"

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Introduction. The main etiologic factor to develop cervical cancer is the infection with human papillomavirus (HPV). Advances in proteomics knowledge had brought new insights into the molecular pathways of HPV infection and the interactions of HPV oncoproteins with host proteins. The proteome is modified by post-translational modifications (PTMs) such as phosphorylations, which is fundamental for protein-function regulations. It had been reported how the HPV affects the abundance of host proteins using a proteomic approach, however the PTMs that they could suffer during HPV infection had not been studied until now. Therefore, the aim of this study was to evaluate the phosphoproteome of HSILs in Mexican patients and compare it with healthy controls.

Methodology. Herein, we analyzed cervical swabs from Mexican patients with HSIL and healthy women attending a routine colposcopy at Juarez Hospital of Mexico. The samples were stored in buffer solution containing proteases and phosphatases inhibitors. HPV presence was evaluated by PCR using the MY09 and MY11 primers. The protein extracts were obtained by sonication and TCA precipitation. Samples were resuspended in Laemmli buffer and quantified by the Bradford method. To compare equivalent amounts of protein extracts of patients with HSIL and healthy women were analyzed by SDS-PAGE and stained with silver and ProQDiamond stains methods. As controls, samples were dephosphorylated using shrimp alkaline phosphatase (SAP). Extracts were analyzed by Western blot using anti-phospho-threonine, and anti-β-actin antibodies as a control.

Results. A fragment of ~450 bp of the L1 region of the HPV genome was detected in samples from patients with HSIL but not from healthy women. Interestingly, we observed differences between phosphoproteins of the cervical samples from the patients with HSIL and healthy women. We identified the threonine-phosphorylated proteins present in the samples by immunodetection with anti-phosphothreonine antibodies. The specificity of this system was evaluated dephosphorylating the protein samples with SAP. No phosphoproteins bands were observed after the SAP treatment and detection by ProQDiamond stain and Western blot assay.

Conclusion. The phosphoproteome of patients with HSIL is different from normal cervical tissue. Further studies will be needed to find a possible relationship between PTMs and HPV infection.

Coinfection study of HPV in biopsies from Mexican patients with histopathological diagnosis of intraepithelial lesions and cervical cancer

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Human Papilloma Viruses (HPV)are classified in two different types according to their oncogenic properties, in low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV).mRNA expression profile of the HPV oncoproteins (E6 and E7) is one of the main differences in those viruses. It had been reported that the LR-HPVpoorly expressed mRNA of E6 and E7, whereaslarge amounts of these mRNA are found in the HR-HPV (Evans *et al*, 2014).The majority of HPV infections are naturally controlled through the immune system. However, when the HR-HPV infection persist, resulting in cervical cancer (Brendle*et al*, 2014). According to previous reports, the coinfection with several HPV types occurs randomly, although it leads to cervical disease independently of the virus genotypes (Chaturvedi*et al*, 2011).The aim of this research was identify the HPV genotypes that coinfectthe intraepithelial lesions of Mexican patients with neoplasic lesions.

METHODOLOGY: This study includes 38 patientswith cervical damage diagnosed by colposcopy. From each patient two biopsies were obtained: one from the cervical lesion and other from an adjacent uninjured area. The HVP present was analyzed by PCR using oligonucleotides MY09/MY11 that amplify a fragment of the L1 region (Hiroyuki *et al,* 1999). The PCR product was visualized by agarose gel electrophoresis. Genotyping was performed using low-density microarrays (CLART HPV 2) to detect 35 different HPV types both LR-HPV and HR-HPV.

RESULTS: Oneamplicon of 450 bp, corresponding to a fragment of the L1 region was observed in all samples, suggesting that even in the injured area HPV is present. We observed 88% of patients showed coinfection with more than one HPV type. Interestingly, in samples from the same patient we identify HR-HPV genotypes (HPV16, HPV18 and HPV31) in the lesion area, whereas in the uninjured area, several genotypes from both HR- and LR-HPV were identify.

CONCLUSION:We found 88%coinfection in the patients. There is a huge distribution of several HPV genotypes in uninjured areas.

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Effect of adenovirus infection on the activity of the Interferon Regulatory Factor 3 (IRF3).

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During adenovirus (Ad) infection, the virus enters the cell through receptor-mediated endocytosis. The partially disassembled viral capsid escapes from the endosome and travels to the nuclear pore complex, where the viral genome is translocated into the nucleus. The E1A gene is then expressed and transactivates the remaining Ad early genes (E1B, E2, E3, and E4), that in turn optimize the cellular milieu for viral replication, counteract a variety of antiviral defenses, and promote viral replication. In infected cell nuclei, the E1B 55 kDa and E4 Orf6 proteins colocalize to the peripheral zones of specific nuclear microenvironments called Replication Centers (RCs) where viral DNA replication, transcription of viral late genes and initial postranscriptional processing of viral late mRNAs take place. Several proteins that participate in the anti-viral response to infection are recruited to RCs where their activities are regulated or inhibited. The recognition of adenovirus DNA by Pattern Recognition Receptors (PRRs) induces a type I interferon (IFN) response through activation of Interferon Response Factor 3. IRF3 activation is driven by phosphorylation of its C-terminal serine-rich region (p-IRF3), resulting in the protein's dimerization and translocation to the nucleus, where, in association with CBP/p300 cofactors, it activates transcription of Interferon Stimulated Response Element (ISRE)- and Gamma Interferon Activation Site (GAS)-controlled genes. We have recently found that Ad-infection leads to relocalization of p-IRF3 to RCs within infected cell nuclei, suggesting that although activated p-IRF3 may be sequestered and possibly regulated within RCs. Therefore, we decided to evaluate whether the recruitment of p-IRF3 to RCsprecludes activation of IRF3 target promoters.Our data provide information that should help understand how Ad controls the host cell innate immune response by regulating the activity of IRF3 through a mechanism that involves its recruitment to RCs.

EFFECT OF RECRUITMENT p53 TO ADENOVIRAL REPLICATION CENTERS ON DNA VIRAL SYNTHESIS.

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Infection with human species C adenoviruses, such as adenovirus type 5 (Ad5), leads to the accumulation of viral gene products that modulate the activity of the master regulator of the cellular response to genotoxic stress, p53. Theim mediate early E1A proteins induce activation and stabilization of p53, resulting in a transientincrease in p53 concentration in numerouscelltypes (Debbas and Lowe 1993. Grand RJ 1994. Nakajima T 1998). Potential antiviral effects of p53 activity, such as induction of growth, arrestor apoptosis, are precludedbyseveralearly viral gene products: theE1B 55-kDa proteinbindsto p53 and inactivatesitstranscriptionaltransactivationfunction, alsoE1B and E4Orf6 proteins and severalcellularproteinsforman E3 ubiquitin ligase that targets p53 forproteasomaldegradation(Querido E, Marcellus 1997 and Querido E, Morrison MR 2001), the E1B 19-kDa protein blocks apoptosis downstream of p53 (White E. 1992).and the E4 Orf3 protein can preventactivation of transcription of p53-dependent genes (KC, O'Shea CC. 2010).

Paradoxically, despitethesevariousmechanismstoprecludeorcircumventdeleteriousconsequenc es of p53 activation, therehavebeenreportsthatfunctional p53 facilitates adenovirus replication (González et al 2006,Cardoso et al 2008, Jordan Wright et al 2013).somestudieshaveindicatedthat p53 mayplay a positive role in thelifecycle of adenovirus byenhancinglate gene expression and increasingthecytopathiceffect.(Hall AR, Dix BR, O'Carroll SJ, Braithwaite AW. 1998).

Duringthereplicationcycle of adenovirusthereorganizationofnucleicomponentsisinduced, this leads totherecruitment of cellularmachinerythatis responsable of viral gene expresión tospecific nuclear sitesknown as viral replication centers (RC),where viral DNA islocated, and viral and celular proteins are recruited, these are responsable forreplication, transcription and thestart of pos-traduccionalprocessing of viral genes; futhermorethelocalization of p53 in RC correlateswiththepresence of E1B-55kDa and E4 Orf6 in thesesites, so it has beensuggestedthat E1B could be involved in recruitment of moleculesthatfacilitatetheformation of RC.

The E1B protein has threephosphorylationsites in thecarboxyl terminal región, S490, S491 and T495; whenthethreephosphorylationsites are remplacedbyalanines, theproteinnotislocalizedefficiently at replication centers, p53 isnotrelocated at thesesites, in addition DNA synthesis and production of viral progeny are deficient; Additionallygroupobservationssuggestthatphosphorylation of thethreesitesisimportantforthelocalization of p53 in the CR; whenphosphorylationisinhibited S490/491, p53 islocated in CRbut DNA synthesis and of viral progeny are deficient; whenthethreephosphorylationsites are replacedbyasparticacidsmimickingthephosphorylatedstate, the E1B protein is localized efficiently and p53 isrecruitedto RC also DNA viral synthesis and production of viral progeny are efficiently. Theseobservationssuggestthatphosphorylation of E1B

Detection of Equine Influenza in the State of Nuevo León

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Introduction: Equine influenza is a highly contagious disease of horses caused by the subtypes H3N2 y H7N7 of Influenza virus (IA). This virus have a highly capacity of dissemination between animal species mainly pigs as well as wild and domestic birds. Serologic tests for antibodies from equine influenza virus can provide useful information about the circulation of the virus, and the retrospective diagnosis of this disease. However, for the presence of virus, it is necessary to use molecular techniques to corroborate the presence of viral RNA. The matrix gene is widely used for the detection of IA in different species because of its conservation in different strains.**Methodology**: The matrix gene of influenza virus was used for cloning and expression of the M1 matrix protein. The later protein was used to produce polyclonal antibodies anti-M1, subsequently we used an ELISA- HADAS system HADAS. In this study 21 serum were analyzed from 3 regions of Nuevo León and 7 nasal swabs. The colorimetric reaction was analyzed in an ELISA reader at an absorbance of 405 nm. From the nasal swab samples, we performed an RNA extraction by TRIZOL reagent method, subsequently a RT-PCR was performed to obtain cDNA and finally was the conserved region of the matrix gene (244 bp) were amplified with oligonucleotides recommended by WHO for the classification of the Influenza A virus.**Results:** Samples were collected in January – May season in three regions of the state of Nuevo León. In 9 (42.85%) of the 21 serums analyzed from pigs birds and horses, the presence of anti-M1 antibodies was detected. From this positive samples, 8 (50%) are from equine samples and one of them was from pigs. Of the samples analyzed by PCR, 5 (71.42 %) were positive for amplification of the conserved region of M1. **Conclusion:** These results show the presence of influenza A virus in horses in the state of Nuevo León and reaffirm the importance constant surveillance of the virus in various animal species.

Molecular analysis of VP4 and VP7 of human rotavirus strains isolated in Sonora, México.

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Rotavirus is the most common cause of severe gastroenteritis in children up to five years old. Although the incidence of rotavirus gastroenteritis is similar in developed and developing countries, most of child deaths occur in the poorest countries (Desselberg*et al,* 2009; Tate *et al*, 2012). Rotavirus gastroenteritis symptoms are vomit, fever, abdominal pain, diarrhea and dehydration (Hernández, 2011; Ramig, 2004). Rotavirus vaccines have been shown to be safe and effective against rotavirus severe gastroenteritis (WHO, 2007). The aims of this study were to analyze the rotavirus incidence and gastroenteritis severity together with the molecular characterization of the genotypes VP4 and VP7 of the human rotavirus strains isolated. The rotavirus positive samples from children with gastroenteritis were identified with SD BIOLINE Rota/Adenorapid test and then the VP4 and VP7 genotypes were characterized by transcriptase reverse-PCR and seminested PCR. A total of 100 fecal samples were collected from children less than five years old with gastroenteritis in the Hospital General de Navojoa and Hospital Infantil de Cd. Obregon from August 2012 to August 2013. 14 (14%) of the samples were positive to rotavirus most of the cases were associated to mild gastroenteritis. On the other hand, the rotavirus genotypes were 3(21%) genotypes of G1P[8], 2(14%)G2P[8], 1 (7%) G3-non typedP genotype. Two (14%)coinfections were detected: G1/G2P[8] and G2/G4P[8]. We fail to genotype the rest of the rotavirus positive samples. This work showed a decreased incidence of rotavirus in comparison with other studies in Mexico during pre-vaccine period. At the same time, in the same comparison,most of the children with rotavirus gastroenteritis did not require hospitalization. It is important to consider the study of rotavirus incidence and genotype variants in specific periods of time for monitoring the impact and effectiveness of rotavirus vaccines.

Effect of the HPV-16 E5 gene expression on the level and location of β-catenin protein in the α6-integrin^{bri}CD71^{dim} subpopulation ofHaCaT cells.

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Cervical Cancer (CC) is the cervix neoplasia caused by the persistent infection of human papillomavirus (HPV) been the viral type 16 the most frequently detected in human samples. The transformant activity of this virus is performed mostly by its oncogenes E6 and E7. However in recent years the participation of E5 protein in the transformation process has been reported, mainly by enhancingthe tumorigenic activity of E6 and E7 proteins. Besides, E5 alters the gene expression of many genes involved in cell adhesion, motility, proliferation and differentiation process in human keratinocytes, within them, β-catenin has shown a slightly upraise. The β-catenin protein is tightly associated with cell-cell adhesion (adherens junctions) and when it is not as this function, it works as transcriptional factor at the canonical pathway of Wnt. This signaling pathway manages cell proliferation as well as differentiation throughout embryogenesis and homeostasis of adult somatic cell. Furthermore, the non-regularized function of β-catenin enhances and promotes the cervical cancer development in transgenic mice.

HPV infects keratinocytes on the basal membrane of the stratified squamous epithelium. In this specific epithelial layer resides a very small amount of cells having characteristics of somatic stem cells. Currently, this population of human keratinocytes is well phenotyped with specific surface molecules: α6-integrin^{bri}CD71^{dim}.

Thus, the main objective in the present work is to analyze the variation in abundance of β-catenin protein as well as its location in the α6-integrin^{bri}CD71^{dim} cell subpopulation of immortalized human keratinocytes (HaCaT cells), induced by HPV-16 E5 expression.The HaCaT cell line was transduced by infection with a lentivirus vector in order to express the E5 gene of HPV-16 (HaCaT-E5). In the total population of infected cells, the β-catenin protein level was evaluated by Western Blot. Additionally,α6-integrina^{bri}CD71^{dim} HaCaT-E5 cells were sorted by fluorescence-activated cell sorting (FACS), and theβ-catenin protein level and location were detected by immunofluorescence.

The β-catenin protein is involved in several cellular processes such as differentiation and proliferation,and its level and location could be modified by HPV-16 E5 expression in epithelial stem cells, having important implications in determining its fate and possibly on the tumorigenic development.

Effect of the HPV-16 E5 expression on the abundance of α6-integrin^{bri}CD71^{dim} stem-like cells of human keratinocytes HaCaT

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In the high risk human papillomaviruses (HPV), the oncoproteins E5, E6 and E7 are the main viral factors responsible for the beginning and progression of cervical cancer. The HPV-16 E5 protein is able to transform immortalized keratinocytes and fibroblast, and contributes to the skin and cervical carcinogenesis. It has been suggested that this protein mainly participates in the early stages of the malignant progression. In addition, E5 induces alterations on the proliferation and differentiation programs of the stratified squamous epithelium *in vitro* and *in vivo.*

The HPV infect cells from the basal layer of the stratified squamous epithelium. In this epithelial layer there is a small subpopulation of stem cells and it has been suggested that they could be infected by HPV. Infection of stem cells could guarantee the maintenance of the viral genome in tissue for long time. In addition, stem cells in the epithelium are probably able to accumulate the initial genetic alterations for the malignant transformation, due to their highest life expectancy. In the basal layer of the epidermis, it is possible to distinguish a subpopulation with stem cell characteristics, based on the presence of the superficial phenotype α6-Integrina^{bri}CD71^{dim} (α6^{bri}CD71^{dim}). Therefore it is interesting to determine the biological effects of the HPV-16 E5 expression on this cell subpopulation.

In the present work, the HPV-16 E5 gene was transduced by lentiviral infection and expressed in human keratinocytes HaCaT. The expression of the E5 protein was confirmed by immunofluorescence and the relative level of the α6^{bri}CD71^{dim} subpopulation was analyzed by flow cytometry at 7, 9, 12 and 15 days after infection. At 12 days post-infection an important increase on the relative level of α6^{bri}CD71^{dim} subpopulation was observed.

These results suggest that HPV-16 E5 induces the proliferation of cells with stem cell characteristics α6^{bri}CD71^{dim}. Considering the important role that stem cells could have in tumor development, it is important to determine the effect of the HPV-16 E5 expression on the processes of self-renewal and differentiation of these cells.

Comparative 2D-DIGE analysis of depleted serum samples of patients with 2009 influenza A (H1N1).

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Introduction and objectives

The Influenza virus is a member of the Orthomyxoviridae family. There are three types of influenza viruses: A, B and C. In the spring of 2009, a new influenza A (H1N1) virus emerged to cause illness in people. This virus was very different from regular human influenza A (H1N1) viruses and the new virus caused the first influenza pandemic in more than 40 years.

Using a proteomic approach the principal objective was to provide an experimental evidences of a differential expression pattern using depleted samples of patients with a confirmatory probe for pandemic influenza A (H1N1) and control samples.

Methods

Serum samples were quantified, the integrity determined by SDS-PAGE and depleted using a column Agilent Human 6 Multiple Affinity Removal System. The depleted serum samples were separated using 2D-DIGE gels, the first dimension using 24- cm IPG strips (pH 4-7or 4.5-5.5), and the second dimension using 12% polyacrylamide gels, the spot detection and relative quantification of proteins was done using the DeCyder software.

Results and Discussion

In the narrow range of pH (4-7), 94 % of the proteins had the same expression level, however 32 proteins were over or under-expressed. On the other hand, for using a micro-range pH 4.4-5.5, 34 had a differential expression; all the analysis was using a threshold of ± 2 fold change.

Conclusions

Using the IPG strips with a micro-range of 4.5 to 5.5 a better resolution is obtained.

Several proteins were analyzed using MALDI-MS and the antiviral protein CCCH-type zinc finger (ZC3HAV1)validated using WB.

Genetic and antigenic relationship of rotavirus strains in stools of children in northern Mexico.

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Severe acute illness in infants under 5 years are mainly caused by Rotavirus. Surface antigens of the VP4 protein is posing greater genetic variability. The P [8] predominant genotype strains worldwide followed by genotype P [4]. Both are found in about 80% of the circulating strains. During the infection process, the VP4 protein undergoes proteolytic cleavage generating two subunits, VP8 * (amino terminus) and VP5 * (carboxyl terminus). Different studies showing VP8 * as the most immunogenic region of the VP4 protein. For this reason the following work is focused on determining whether genetic changes in VP8 * subunit of rotavirus could affect the antigenic characteristics of the VP4 protein that prevent recognition by antibodies raised against this subunit. **Methodology**. The viral RNA was extracted from feces by Trizol method. The purified RNA was subjected to RT-PCR with primers specific for the coding region of VP8 *. The amplified products were cloned into pGEM-T plasmid for subsequent sequencing. The analysis of the nucleotide sequence was performed using the Clustal W / BioEdit software V7.0. Subsequently subcloned into the expression plasmid pET-28a (+) for the production of recombinant proteins in E. coli strains BL21 (DE3). These were used for the production of polyclonal antibodies in goats and rabbits. Antigenic variability was assessed by Elisa. **Results**. For this study, 122 stool samples obtained from children younger than 3 years were analyzed by Elisa. VP8 subunit antibodies * were used for detection of the complete protein VP4 directly in fecal samples. ELISA analysis showed that these antibodies had reactivity recognizing 110 (90.1%) of the samples tested. Of the 73 positive samples (59.8%) were P [8] genotype, 16 (13.1%) to P [4] and 19 (15.5%) to a mixture between both genotypes. Negative samples are previously identified by RT-PCR than 6 (4.9%) are belonged to genotype P [8] and 3 (2.4%) to P [4]. **Conclusions**. These results show that polyclonal antibodies against VP8 * subunit of rotavirus are unable to recognize the VP4 protein of rotavirus strains with identical genetic characteristics in the VP8 * subunit. Therefore, the studies of antigenic variability of the VP4 protein can be expanded to assess antibodies against VP5 * subunit and identifying the effect of the genetic changes on the antigenic subunit changes the VP4 protein.

Genotyping and Analysis of Mutations in the Core Protein of Hepatitis C Virus (HCV) isolated from Mexican Patients with Chronic Hepatitis C

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BACKGROUND

HCV infection is one of the main causes of cirrhosis and carcinoma hepatocellular (HCC). The sustained viral response (SVR) due to combined pegylated interferon (PEG-IFN) and ribavirin (RBV) depends on the viral genotype. The core protein is an important protein for viral replication and for the pathogenesis of the HCV-related liver disease. Viral mutations in amino acids (aa) R70Q and L91M in this protein in HCV genotype 1b, have been associated in the result of the SVR and HCC development.

AIM

To identify the HCV genotype and viral mutations in thecore protein at residues 70 and 91 in Mexican patients with chronic hepatitis C.

MATERIAL AND METHODS

A total of 27 serum samples from patients with chronic hepatitis C were included. Samples were divided in two groups: Group I: No responders to treatment with PEG-IFN and RBV, N = 13 and Group 2:from treatment-naïve patients, N = 14. A PCR was performed to amplify a fragment from nucleotide 67 of the NC 5' region to nucleotide 1234 of the structural region of HCV. Cycle sequencing was done by the chain termination method. Viral genotyping was performed with the nucleotide sequences using agenotyping tool online. Amino acid sequences from the core protein were deduced from the nucleotide sequence.

RESULTS

The HCV genotype identified in Group I were: 7 (54%), 1a; 5 (38%), 1b; and 1 (8%), 2a. And form Group 2: genotype 1a, 10 (71%) and 1b, 4 (29%). Samples from genotype 1b were selected to analysis of mutations R70Q and L91M. Four (80%) of samples from Group 1, had a mutated aa at position 70 (3 had R70Q, and 1 R70H), whereas all samples from Group 2 had the wild type aa (R) at position 70. Most of the samples from patients no responders to treatment and naïve-treatment had the mutation L91M (80% and 75%, respectively).

CONCLUSION AND DISCUSSION

Genotype 1 is frequent in this population analyzed: 26/27 (96.7%); 1a, 17/26 (65%) and 1b, 9/26 (35%). Mutation at residue 70 in the HCV core protein was most frequent in the group I compared to group 2 (80% vs 0%). No differences were identified in the mutated residue 91 in both groups of patients. Analysis of mutation at aa 70 in the core protein must be analyzed in samples from patients with genotype 1b before and after the treatment, in order to analyze if this mutation is an important predictor, for the treatment response in Mexican patients infected with HCV genotype 1b.

Effect of mutations in the hydrophobic core of E1B 55kDa on the adenoviral replication cycle.

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The E1B 55 kDa (E1B) protein from type C adenoviruses is a multifunctional protein required for the selective export of viral late mRNA and for the inhibition of key cellular antiviral defenses. The tridimensional structure of the polypeptide has not been resolved, but certain structural features that are important for the protein's activities have been described. The E1B can be postranslationally modified by SUMOylation and by phosphorylation. These modifications regulate many activities of the protein and are situated near the N- and C-terminus, respectively. The N- and C-termini also include NES and NLS sequences that are responsible for the protein's nucleocytoplasmic shuttling. Interestingly both termini seem to be intrinsically disordered. Less is known about the central region of the polypeptide. The region between 215-345 residues is relatively hydrophobic and it may represent a structural core. It has been proposed that this region of the protein is important for its interaction with nucleic acids in vitro, and for the proper folding of the protein; however, its impact on the viral replication cycle is not clear. The E1B can assemble a Cullin-based E3 Ubiquitin ligase complex with the viral protein E4orf6 that induces degradation of various cellular substrates and seems to be required for viral late mRNA export. However, the contribution of the known protein's structural features to either of these activities is not known. In this work we have constructed adenovirus recombinants with substitutions in the hydrophobic core region of the E1B 55kDa protein, to determine its role in viral replication cycle. Specifically, we determined its contribution in p53 and Mre11 degradation and its impact on viral progeny production. Our results show that these mutations have different effects on p53 and Mre11 degradation suggesting that this region may be determinant in substrate recognition.

"Heterologous insertions in Parvovirus B19 VP2 protein: effect on virus-like particles assembly in vitro". Areli del Carmen Morán García, Ismael BustosJaimes.Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, UNAM.Circuito Interior, Ciudad Universitaria. C.P. 04510. México D.F. Tel. 55-56232259. ismaelb@unam.mx.

Virus-like particles (VLPs) are complexes of the capsid proteins without genetic material that mimic the natural structure of virions. VLPs have antigenicity similar to whole virus andtheir structural components allow the insertion of foreign antigenic epitopes to producechimeric VLPs with new antigenic properties.Human parvovirus B19 (B19) is a single-stranded DNA virus. Its genome is contained in icosaedralcapsids of 18-26 nm of diameter. B19 capsids are composed of 60 subunitsof VP2(95%) and VP1(5%)proteins. There are several reports of the display of heterologous proteins on the external surface of B19 VLPs when they are produced *in vivo*.In addition, our group recently reported the *in vitro* assembly of VP2-VLPs.

In this research we analyzed whether or not the *in vitro* self-assembly of VP2 is affected by the addition of a foreign sequence at its N-terminus. For this purpose, we chose 2 antigenic regions of the Respiratory syncytial virus (RSV). RSV is the leading cause of respiratory infections in children and elderly people. RSV vaccine trials did not prevent the infection and caused a more acute disease. Neutralizing monoclonal antibodies target epitopes called site II and site IV, these epitopes have been mapped on a linear region in RSV F protein. Therefore, chimeric genes coding for the amino acid sequence corresponding to the sites II and IV of the F protein from RSV were merged with the sequence coding for the N-terminus of the VP2 protein. These genes were expressed and the chimeric proteins were purified. The proficiency of FII-VP2 and FIV-VP2 chimeric proteins, alone or with VP2, to bring VLPs was examined.

DLS, ultracentrifugation, and TEM analysis of assembly samples showed particles very similar in size (diameter of 21-24 nm), density (1.33g/mL) and morphology to wild-type B19 virus. Protease digestion analyses demonstrate that the heterologous peptides are displayed on the surface of the particles. The immunogenic potential of these VLPs and stability were also tested. Our results indicate that these particles are immunogenic although this does not imply protection against VSR.

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