XXII CONGRESO

DE LA RAMA DE BIOENERGÉTICA Y BIOMEMBRANAS

SOCIEDAD MEXICANA DE BIOQUÍMICA

PROGRAM

OCTOBER 17–21, 2021

Hacienda Cantalagua, Michoacán, México

Organizing Committee

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AGRADECIMIENTOS

Al Dr. David René Romero Camarena, Presidente de la Sociedad Mexicana de Bioquímica, por el valioso apoyo brindado durante su gestión en la mesa directiva 2019-2021.

A la Dra. Soledad Funes Argüello, Secretaria - Tesorera de la Sociedad Mexicana de Bioquímica, por el valioso apoyo brindado durante su gestión en la mesa directiva 2019-2021.

Al Dr. Miguel Costa Basín, Secretario Académico de Investigación y Posgrado de la Facultad de Química, Universidad Nacional Autónoma de México por el apoyo económico brindado para un programa de becas.

Al Instituto de Fisiología Celular por la difusión del congreso en sus redes sociales.
A la Universidad Autónoma de Querétaro.

Al Centro de Investigación Investigación en Alimentación y Desarrollo, A.C.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por el apoyo a la Sociedad Mexicana de Bioquímica mediante el proyecto número 318661 ”Apoyo para la organización de los Congresos Nacionales de la Sociedad Mexicana de Bioquímica, A.C.” dentro de la convocatoria “Fortalecimiento de Actividades vinculadas con la Promoción, Difusión y Divulgación de las Humanidades, Ciencias, Tecnologías y la Innovación: Academias y Sociedades Científicas 2021”.

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DRA. ADRIANA MUHLIA

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BIENVENIDA

La rama de Bioenergética y Biomembranas fue la primera rama de la Sociedad Mexicana de Bioquímica con registro en 1979, siendo los Doctores Armando Gómez Puyou, Carlos Gómez Lojero y Antonio Peña Díaz quienes forjaron el sueño y capitalizaron la idea de que estas disciplinas son parte de la esencia de la ciencia en México y en todo el mundo. Después de 42 años y 21 Congresos bianuales ininterrumpidos llegamos a la XXII Reunión Científica de la Rama, posiblemente una de las que más retos ha representado por múltiples razones. Después de más de un año y medio de lidiar con las consecuencias de una pandemia mundial, los esfuerzos continúan y los trabajos en los laboratorios no han cesado a pesar de las limitaciones presenciales con las que nos hemos encontrado.

El grupo de científicos de diversas instituciones que actualmente conforman el núcleo académico de la Rama de Bioenergética y Biomembranas es ya una familia académica de muchos años, con nuevos miembros y representación en el centro de la República Mexicana y en varios estados del Norte. La comunidad académica en esta ocasión se reunirá de manera híbrida, tanto en forma presencial como de modo virtual dadas las restricciones de la pandemia, pero con el ánimo de continuar con la labor de generar conocimiento, de formar a las nuevas generaciones de investigadores y de establecer vínculos y colaboraciones que refuercen los lazos.

El programa diseñado para esta reunión cuenta con varios investigadores de talla internacional que impartirán 7 Conferencias Magistrales de primer nivel; además, por primera vez se realizará un Simposio de Biofísica dentro del Congreso con la idea de incluir dentro de esta Rama a todos aquellos investigadores y estudiantes de la comunidad científica mexicana que realizan investigación en esta disciplina, que se propondrá para ser incluida dentro de nuestra rama, compartiendo temas relacionados estrechamente con la Bioenergética y las Biomembranas.

A su vez contaremos con ponencias orales que impartirán investigadores consolidados y estudiantes de pre y posgrado en las diversas áreas del congreso. También se contará con un concurso de videos cortos, en los que los estudiantes que inician su investigación puedan presentar sus propuestas ó los primeros resultados de la misma y en su caso, lograr obtener uno de los premios que serán otorgados por un comité evaluador ex profeso.

Bienvenidos participantes a la Hacienda Cantalagua ó a la ventana de su monitor, en donde con mucho gusto hemos trabajado para lograr unirnos y seguir trabajando.

Atentamente,

El Comité Organizador 2021.

MEDALLA “JOSÉ LAGUNA GARCÍA”

Esta medalla se otorga por la Rama de Bioenergética y Biomembranas a dos miembros que se han distinguido por la calidad de sus trabajos en investigación en el país habiendo logrado contribuciones importantes al conocimiento a nivel internacional y por su continua participación en las actividades académicas de la Rama.

Premiados en 2019
Dra. Isabel Baeza Ramírez
Dra. Xóchitl Pérez Martinez

Premiados en 2017
Dr. Oscar Flores Herrera
Dr. Salvador Uribe Carvajal

Premiados en 2015
Dr. José de Jesús García Trejo
Dr. Rafael Moreno Sánchez

Premiados en 2013
Dr. Heliodoro Celis Sandoval
Dra. Victoria Chagoya de Sánchez
Dr. Edmundo Chávez Cossío
Dr. Carlos Gómez Lojero
Dr. Armando Gómez Puyou
Dr. Antonio Peña Díaz
Dra. Marietta Tuena Sangri

Premiados en 2021
Dra. Marina Gavilanes Ruíz
Dr. Manuel Gutiérrez Aguilar

Miembros del Jurado en 2021
Dra. Soledad Funes
Dr. Carlos Gómez Lojero
Dra. Sobeida Sánchez Nieto

MEDALLA "JOSÉ LAGUNA GARCÍA"

Acuñada en el 2013.

Cuño Anverso: Efigie del Dr. José Laguna con la leyenda "JOSÉ LAGUNA GARCÍA 1921-2013"

Cuño Reverso: Formando una espiral, la leyenda "POR SUS CONTRIBUCIONES A LA BIOENERGÉTICA Y A LAS BIOMEMBRANAS". En el origen de la espiral el símbolo X~P, representando el intermediario fosforilado de alta energía de la hipótesis de E.C. Slater (1953) y en el final de la espiral, el símbolo $\Delta\mu H^+$, representando la fuerza protón-motriz de la hipótesis quimiosmótica de Peter Mitchell (1961).
Metal: Bronce
Peso: 125 g
Diámetro: 6 cm
Diseño: Miguel Gómez Counahan
Troquelado: "ARTE Y ESCULTURA FIDIAS", México.

LINEAMIENTOS PARA EL OTORGAMIENTO DE LA "MEDALLA JOSÉ LAGUNA GARCÍA"

La "Medalla José Laguna García" fue acuñada *ex profeso* en el 2013 para honrar la memoria del Dr. José Laguna, quién fue pionero de la bioquímica moderna en México, uno de los fundadores de la Sociedad Mexicana de Bioquímica (SMB) y mentor de muchos de los miembros que integraron la primera generación de investigadores que cultivaron el estudio de la Bioenergética y de las Biomembranas, generando así la corriente más activa de investigación en esta área en nuestro país.

1. Objeto del Reconocimiento

La "Medalla José Laguna García" fue concebida para preservar y estimular la investigación en el área de la bioenergética y las biomembranas. Este reconocimiento lo otorgará la Rama de Bioenergética y Biomembranas de la Sociedad Mexicana de Bioquímica (de aquí en adelante denominada como "la Rama") a miembros distinguidos de la comunidad que llevan a cabo investigación en alguna de estas dos áreas del conocimiento. Se otorga en dos modalidades, la primera a la trayectoria de científicos consolidados y la segunda como un estímulo a las contribuciones realizadas por científicos más jóvenes.

2. Académicos que pueden hacerse acreedores a este reconocimiento

En su primera edición la medalla se otorgó a investigadores que cultivaron la Bioenergética en sus inicios y que colaboraron con la creación y desarrollo de la Rama. Bianualmente se entregan dos reconocimientos "Medalla José Laguna García", una de ellas a un miembro de la Rama que cuente con una trayectoria fructífera de investigación, que haya contribuido de manera notable a profundizar el conocimiento en el campo y que haya mantenido una actividad académica sostenida en la Rama. La segunda medalla se le otorga a un miembro de la Rama menor de 50 años que haya participado con constancia en las actividades académicas de ésta y que haya realizado contribuciones relevantes en este campo.

3. Procedimiento para la concesión del reconocimiento

-No se emitirá convocatoria alguna para concursar por el reconocimiento "Medalla José Laguna García", tampoco se recibirán solicitudes institucionales o personales apoyando a un determinado candidato.

-Los merecedores de este reconocimiento honorario serán seleccionados directamente por el jurado, en función de los estudios, investigaciones y/o entrevistas que el propio jurado realice sobre la calidad de las contribuciones científicas que han llevado a cabo los miembros de la Rama, tomando en cuenta asimismo su probidad académica y moral.

-La entrega del reconocimiento "Medalla José Laguna García" que hará exclusivamente en la Reunión o Congreso bianual de la Rama. Quedará a juicio del Comité Organizador de la Reunión de la Rama escoger, dentro del programa académico, el momento apropiado en que se efectuará la ceremonia de entrega de medallas. Será prerrogativa del Comité Organizador invitar o no, a alguno de los premiados a impartir una conferencia dentro del programa científico de la reunión.

-El jurado que otorgará la medalla en su siguiente edición será elegido durante la Reunión previa de la Rama, de la misma forma en que se elige al Comité Organizador para la siguiente Reunión.

-El jurado quedará conformado por tres personas, todos ellos integrantes reconocidos y asiduos a las reuniones de la Rama. Al menos uno de ellos deberá haber participado

en un Comité Organizador de alguna Reunión. Los miembros del Comité Organizador de la Reunión en turno no podrán ser a la vez miembros del jurado durante ese mismo periodo.
-Los miembros del jurado desarrollarán sus actividades de forma honoraria, sin percibir retribución económica alguna. Además. Organizará sus sesiones y deliberaciones de la manera que mejor convenga a sus intereses. En caso de que algún miembro del jurado esté incapacitado para realizar sus funciones, el Comité Organizador de la Reunión en funciones será el encargado de seleccionar e invitar a algún otro integrante de la Rama para que ocupe el puesto vacante.
-Uno de los miembros del jurado deberá permanecer como integrante en el siguiente jurado, con el fin de preservar los antecedentes de los procedimientos y criterios de selección. El integrante que permanecerá en funciones en una segunda ocasión será seleccionado por mutuo acuerdo del jurado anterior. En la reunión subsecuente de la Rama se elegirán a dos nuevos miembros para integrar el siguiente jurado y así sucesivamente.
-El jurado dispone de más de un año y medio para llevar a cabo sus deliberaciones y deberá emitir su fallo al menos tres meses antes de la fecha en que se lleve a cabo la Reunión de la Rama, haciéndolo del conocimiento únicamente del Comité Organizador. Así mismo, será el propio Comité Organizador el encargado de informar a los colegas seleccionados que se hicieron acreedores al reconocimiento y convocarlos a la ceremonia de entrega.
-El fallo del jurado es inapelable.
-Ninguno de los firmantes de este documento puede hacerse acreedor al reconocimiento.
-No podrá otorgarse la medalla en forma póstuma.
-No podrá otorgarse este reconocimiento a científicos externos a la Rama que no hayan tenido una participación relevante y sostenida en la vida académica de la propia Rama.
-No se le podrá otorgar la “Medalla José Laguna García” a una persona que ya fue distinguida con este reconocimiento.
-En las memorias de la Reunión de la Rama ulteriores se incluirán en orden alfabético, por una parte, los nombres de los científicos distinguidos con la medalla, tanto de la reunión correspondiente como de las anteriores y por otra, los nombres de los miembros del jurado del año correspondiente.
- El financiamiento, la responsabilidad de la manufactura y la disponibilidad de las medallas para cada ceremonia de entrega, estarán a cargo de los abajo firmantes. Por lo tanto, la responsabilidad de elaborar las medallas no recaerá sobre los miembros del Comité Organizador de la Reunión de la Rama.
- El reconocimiento “Medalla José Laguna García” no podrá otorgarse en ninguna otra instancia académica o social fuera de la Rama.
- Estos lineamientos se publicarán, en lo sucesivo, en las memorias de cada Reunión de la Rama. Cualquier situación no contemplada en éstos será resuelta por el pleno del Jurado, consultado, si así lo consideran necesario, con los miembros del Comité de la Reunión de la Rama o con integrantes de jurados anteriores.

México D.F., Febrero de 2015.

Georges Dreyfus
Diego González Halphen

In memoriam

Dr. Heliodoro Celis

Heliodoro Celis Sandoval fue sin duda una persona carismática que dejó huella profunda entre aquellos que lo conocimos. Heliodoro era poseedor de una insaciable curiosidad que lo llevó a dedicarse a la ciencia, sin embargo, también pudo haber sido naturalista, historiador o analista deportivo; sus aficiones también incluyeron la comida, la música, la literatura y el cine. Ser su amigo era un privilegio, ya que significaba tener acceso a una conversación inteligente y amena, a una visión que, tamizada por su mente analítica, era siempre muy estimulante. Celis, como le decíamos, siempre estuvo rodeado de libros, que fueron sus verdaderos amigos íntimos e inseparables; era sin duda de otra época, muy probablemente del Renacimiento. Fue un docente muy querido por muchas generaciones de biólogos de la Facultad de Ciencias de la UNAM, que recibieron sus enseñanzas sobre la biología de los procariontes o sobre los conceptos básicos de la biofísica; lograba llenar sus grupos de clase y atraer la atención de numerosos jóvenes que, en no pocas ocasiones, se unieron a su grupo de investigación para continuar con una carrera académica. Su laboratorio en el Instituto de Fisiología Celular de la UNAM (IFC) siempre muy concurrido, era un lugar agradable donde se discutía de ciencia y también se cocinaba, eso sí, siempre rodeado de sus queridos estudiantes.

Estuvo involucrado en la bioenergética desde el principio de su carrera, durante sus estudios de doctorado en el CINVESTAV y su estancia posdoctoral en Bristol, trabajando con ionóforos, proteolípidos, fotosistemas, ATPasas y pirofosfatasas; sus trabajos siempre fueron claros y certeros. Participó con regularidad en estas reuniones ya sea como organizador o simplemente como asistente asiduo. Fue presidente de la Sociedad Mexicana de Bioquímica y participó con entusiasmo en otras labores académico-administrativas siendo secretario académico del IFC y tuvo una participación destacada en el célebre Congreso Universitario.

Sin duda Celis fue un erudito que cultivó con gran pasión diversas disciplinas, sin embargo, la bioquímica y la bioenergética fueron las dos pasiones que constituyeron su actividad académica principal.

Descanse en paz nuestro muy querido amigo y colega, siempre lo recordaremos con gran afecto.

Georges Dreyfus

CONCURSOS Y PREMIOS

Armando Gómez Puyou Award

El concurso se llevará a cabo los días Martes 19 y Miércoles 20 de Octubre durante las sesiones 1 y 2 “My Thesis in a Video”. Se ha seleccionado un comité evaluador de profesores renombrados que calificarán todos los aspectos de cada video. Los resultados se darán a conocer durante la ceremonia de premiación y cierre del Congreso el día Jueves 21 de Octubre.

1er. lugar. Termociclador Mini 8® (MiniPCR)

2o. lugar. Juego de Micropipetas H-Style®

3er. lugar. Crystal Taq Master Mix® (2x) (Jena Biosciences)

Concurso de Ponencias Orales para Estudiantes

El concurso se llevará a cabo durante cada una de las sesiones orales. Los participantes deberán ser estudiantes de pre- y posgrado. Para la evaluación se invitó a un comité de profesores renombrados que calificarán todos los aspectos de las presentaciones. Los resultados se darán a conocer durante la ceremonia de premiación y cierre del Congreso el día Jueves 21 de Octubre.

1er. lugar. Pipeta Multicanal Pipetman® M P8X20M USB (Gilson)

2o. lugar. REDTaq® PCR Ready Mix (Merck)

3er. lugar. PCR Cooler® – Pink y Gradilla para tubos (Eppendorf)

El premio incluye diplomas con valor curricular para las y los ponentes seleccionados, así como una liga para acceder a una colección de 500 títulos de Ciencia y Tecnología. Será decisión de futuros Comités Organizadores del Congreso de la Rama de Bioenergética y Biomembranas de la S.M.B. el continuar o no con la entrega de este premio o algún premio y/o reconocimiento similar.

AGRADECIMIENTOS

El Comité Organizador de la XXII Reunión de la Rama de Bioenergética y Biomembranas expresa su profundo agradecimiento a:

Equipo de Trabajo integrado por:

María Teresa Castillo, por su apoyo en la Coordinación y Organización del Evento, tanto en la modalidad virtual, como en la presencial

Lic. Diana Cordero por el apoyo en la parte administrativa y financiera

Biól. Andrea Ortiz por su apoyo en la logística del evento

A los estudiantes:

Dr. Cintya Alejandra Nevárez López

Lic. Microbiología Amairani Chávez Vega

Q.F.B. Emilia Refugio Gutiérrez Mireles

Por su apoyo en la logística y desarrollo del Congreso en la modalidad presencial.

XXII CONGRESO DE LA RAMA DE BIOENERGÉTICA Y BIOMEMBRANAS
October 17- 21, 2021.
MEETING PROGRAM

Sociedad Mexicana de Bioquímica A.C.

TIME	SUNDAY 17	MONDAY 18	TUESDAY 19	WEDNESDAY 20	THURSDAY 21
7:30 - 9:00		Breakfast	Breakfast	Breakfast	Breakfast
9:00 - 10:30		Oral Session I	"Biophysical Symposium" Conference Elizabeth Jonas Yale School of Medicine	Alfredo Cruz Ramírez LANGEBIO Oral Session VI	Plenary Lecture V Roberto Carlos Muñoz Garay Instituto de Ciencias Físicas, UNAM
10:30 - 11:00		Coffee Break	Coffee Break	Coffee Break	Coffee Break
11:00- 12:00	Registration 11:00-17:00	Plenary Lecture I Paolo Bernardi Università degli Studi de Padova	Conference Tamara Rosenbaum IFC, UNAM	Plenary Lecture III Michelangelo Campanella University of London	*In memoriam* Dr. Heliodoro Celis Sandoval
12:00- 12:30		Coffee Break	Coffee Break	Coffee Break	Coffee Break
12:30- 14:00		Oral Session II	Conference Luis Bagatolli INIMEC- CONICET	Oral Session VII	Armando Gómez Puyou Awards and Meeting Closure
14:00- 16:00		Lunch	Lunch	Lunch	
16:00- 17:00	Opening Ceremony	Plenary Lecture II Alvaro Marín Hernández Instituto Nacional de Cardiología	Oral Session V 16:00 – 17:00	Plenary Lecture IV Elizabeth Murphy NHLBI, National Institutes of Health	
17:00- 18:00	Cultural Conference José Luis Punzo Díaz **Centro INAH-Michoacán**	Oral Session III	Conference Iván Ortega Blake Instituto de Ciencias Físicas, UNAM	Session 2 "My thesis in a video" 17:00 – 19:00	
18:00- 19:00	Opening Lecture Salvador Uribe Carvajal Instituto de Fisiología Celular, UNAM	Coffee Break 18:00- 18:30	Session 1 "My thesis in a video" 18:00 – 20:00		
19:00- 20:00	Jose Laguna´s Medal Award Ceremony	Oral Session IV 18:30-19:30		Gala Night	

SUNDAY 17th

11:00 - 17:00 **Registration**
14:00- 16:00 **Lunch**
16:00-17:00 **Opening Ceremony**

Dra. Teresa Hernández Sotomayor
SMB President

Dra. Bertha María Josefina González Pedrajo
SMB Secretary

Organizing Committee:
Dra. Adriana Muhlia
Dr. Manuel Gutiérrez
Dr. Carlos Saldaña

17:00- 18:00 **Cultural Conference**
Nuevas metodologías para la investigación arqueológica, el caso de Michoacán
Dr. José Luis Punzo Díaz
Centro INAH-Michoacán
Chair: Dr. Carlos Saldaña

18:00- 19:00 **Opening Lecture**
Thriving in Oxygen: no two systems are created equal
Dr. Salvador Uribe Carvajal
Instituto de Fisiología Celular, UNAM
Chair: Dra. Adriana Muhlia
Dr. Manuel Gutiérrez

19:00- 20:00 **José Laguna´s Medal Award Ceremony**
Chair: Dr. Diego González
Dr. Georges Dreyfus

CULTURAL CONFERENCE

Dr. JOSÉ LUIS PUNZO DÍAZ
Instituto Nacional de Antropología e Historia

jose_punzo@inah.gob.mx

Nuevas metodologías para la investigación arqueológica, el caso de Michoacán

Arqueólogo egresado de la Escuela Nacional de Antropología e Historia en la Ciudad de México, maestro en ciencias y humanidades con terminación en historia por la UJED. Doctor en arqueología por ENAH.
El Dr. Punzo ha sido director del Museo de las Culturas del Norte en Paquimé, Chih. Actualmente es investigador en arqueología adscrito al Centro INAH-Michoacán, ha sido director de múltiples proyectos arqueológicos en los estados de Durango y Michoacán. Ha sido responsable de las zonas arqueológicas de la Ferrería en Durango y Tingambato y Tzintzuntzan en Michoacán.Ha publicado dos decenas de artículos científicos y de divulgación y cinco libros de divulgación e investigación científica.
Las principales líneas de investigación están relacionadas con la arqueología del occidente y norte de México, arquitectura de tierra, metalurgia prehispánica y las aplicaciones computacionales en arqueología.

OPENING LECTURE

Dr. SALVADOR URIBE CARVAJAL
Instituto de Fisiología Celular, UNAM

suribe@ifc.unam.mx

Thriving in Oxygen: no two systems are created equal

Oxygen and its toxic partial reduction products, the Reactive Oxygen Species (ROS) are strong drivers of evolution. O_2 and ROS vary widely in different habitats, forcing each species to evolve to deal with ROS while using O_2 as an electron acceptor. Physiological uncoupling of oxidative phosphorylation (OxPhos) prevents free radical accumulation by sustaining a high rate of electron flux, regardless of ATP synthase activity. Branched respiratory chains, uncoupling proteins (UCP) and unspecific channels promote physiological uncoupling.

Another mechanism to avoid ROS is excluding O_2. Early attempts at excluding oxygen were bacterial biofilms and later, mitochondrial compartmentalization of most O_2 metabolism. A third step was multi-cellularity. Late in evolution, a highly efficient O_2-avoidance strategy was developed in the form of an O_2 impermeable epithelium in “Oxy-regulator” organisms. In oxy-regulators physiological uncoupling became less critical for survival, leading uncoupling systems to lose efficiency, drift in function, or even disappear.

In O_2-permeant cells (oxy-conformers) respiratory chains (RC) are branched. Branching is extensive in many bacteria (e.g. Staphylococcus epidermidis) and less so in eukaryotes such as yeasts, insects or crustaceans. Alternative enzymes are differentially expressed under stress or at the stationary growth phase. In oxy-controlling organisms RCs are not branched.

UCPs are proton sinks present in eukaryotes. In unicellular organisms and in crustaceans UCPs are differentially expressed in response to stress or variations in [O_2]. Other UCP functions are observed in oxy-regulators,: e.g., in brown fat tissue UCP1 specializes in thermogenesis and in plants UCP activity promotes secondary metabolite evaporation.

The mitochondrial permeability transition (PT) is another physiologic OxPhos uncoupling mechanism with different roles in each species. In Saccharomyces cerevisiae, PT is promoted by high ATP. This results in oxidation of the NAD^+ pool and O_2 depletion. Also in yeast, Ca2+ closes the S. cerevisiae pore, likely signaling the need for high ATP in events such as cell mating. In mammals PT pores do open in response to stress briefly protecting cells against damage. However, mammalian PT seems to be fragile, as extended opening of the pore soon becomes irreversible and cell death ensues. In oxy-conformers PT seems to be less fragile than in oxy-regulators. Further research in this field will be aided when the structural identity of the PT pore is defined for each species of interest.

In short, in each species [O_2] and ROS are strong drivers of evolution due to both, the high efficiency of aerobic metabolism and their toxicity.

MONDAY 18th

7:30 - 9:00 **Breakfast**

9:00- 10:30 **ORAL SESSION I "Mitochondrial Proteins"**

Chair: Adriana Muhlia Almazán
CIAD, A.C.

9:00 - 9:15 A positive cluster of amino acids in the N-terminus of selected mitochondrial proteins is relevant for their co-translational import mediated by αβ2-NAC

María Clara Avendaño Monsalve and Soledad Funes
Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

9:15 - 9:30 The jellyfish *Stomolophus meleagris* mitochondrial adaptive response to thermal stress

Cintya Nevárez-López, Arturo Sánchez-Paz, Adriana Muhlia-Almazán. Centro de Investigación en Alimentos y Desarrollo, A.C. (CIAD). Centro de Investigaciones Biológicas del Noroeste, S.C (CIBNOR)

9:30 - 9:45 The Cox1 carboxyl terminal end is essential for efficient mitochondrial function in yeast

Itzel Abil García-Cordero, Ana Paulina Gutiérrez-Alejandre, Yolanda Camacho-Villasana, Xochitl Pérez-Martínez.
Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

9:45 - 10:00 The respiratory chain of *Rhodotorula mucilaginosa*

Paulina Castañeda-Tamez, Natalia Chiquete-Félix, Salvador Uribe-Carvajal. Department of Genetics and Molecular Biology, Institute of Cellular Physiology, UNAM

10:00 - 10:15 Revisiting accessory subunits and assembly of mitochondrial complex I from *Yarrowia lipolytica*: a complexome profiling approach

Alfredo Cabrera-Orefice, Madhurya Lutikurti, Ulrich Brandt.

	Radboud Institute for Molecular Life Sciences, Radboudumc. Netherlands
10:15 - 10:30	Rat liver versus *Saccharomyces cerevisiae*: comparison of some effectors on the mitochondrial permeability transition pore Ricardez-García Carolina, Morales-García Lilia and Salvador Uribe-Carvajal. Department of Genetics and Molecular Biology, Institute of Cellular Physiology, UNAM
10:30 - 11:00	**Coffee Break**
11:00 - 12:00	**PLENARY LECTURE I** "Defining the molecular mechanisms of the mitochondrial permeability transition" **Dr. Paolo Bernardi** Department of Biomedical Sciences, University of Padova, Italy Chair: Dr. Diego González Halphen Instituto de Fisiología Celular, UNAM
12:00 - 12:30	**Coffee Break**
12:30 - 14:00	**ORAL SESSION II "ATP-synthase & Cytochrome Oxidase"** Chair: Dra. Soledad Funes Instituto de Fisiología Celular, UNAM
12:30 - 12:45	Effect of heavy metals on the ATPase activity of the V2 and V1 F_1F_O-ATP synthase from *Ustilago maydis* Giovanni García-Cruz, Mercedes Esparza-Perusquía, Federico Martínez, Juan Pablo Pardo, Oscar Flores-Herrera. Depto. Bioquímica, Facultad de Medicina, UNAM
12:45 - 13:00	Deletion of the Inh1 subunit increases ATPase activity and reduces the stability of the F1FO-ATP synthase dimer in *Ustilago maydis* Mercedes Esparza-Perusquía, Lucero Romero-Aguilar, Juan Pablo Pardo, Federico Martínez and Oscar Flores-Herrera* Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México.

13:00 - 13:15	The critical length of the N-terminus of the ζ subunit to inhibit the F1FO ATPase of *Paracoccus denitrificans* Gilberto Garduño Javier, Francisco G. Mendoza Hoffmann, Raquel Ortega, Miguel Ángel Cevallos Gaos, José J. García Trejo. Facultad de Química, UNAM. Center of Genomic Sciences CCG, UNAM
13:15 - 13:30	The cytochrome b carboxyl-terminal end is a central regulator of the bc1 complex biogenesis in *Saccharomyces cerevisiae* Daniel Flores-Mireles, Yolanda Camacho-Villasana, Alfredo Cabrera-Orefice, Madhurya Lutikurti, Ulrich Brandt, Aldo E. García-Guerrero, Guadalupe Lozano-Rosas, Victoria Chagoya, Emma Bertha Gutiérrez-Cirlos, Andreas Carlström, Martin Ott, Xochitl Pérez-Martínez. Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM
13:30 - 13:45	Are the two small cytochromes of *Bacillus subtilis* cytochrome part of the b6c-caa3 supercomplex? Emma Berta Gutiérrez Cirlos Madrid, Gerardo Ignacio Picón Garrido, Ana Paula García García. Laboratorio de Bioquímica y Bioenergética. FES Iztacala.
13:45- 14:00	Cytochrome c oxidase activity decreases along with the mitochondrial respiratory function in the lesser grain borer, *Rhyzopertha dominica* exposed to hypoxia and hypercapnia Víctor A. Levy-De la Torre, Adriana Muhlia-Almazán, Francisco J. Cinco-Moroyoqui , Alonso A. López-Zavala, Marina Ezquerra-Brauer, Oliviert Martínez-Cruz. Departamento de Investigación y Posgrado en Alimentos. Departamento de Ciencias Químico-Biológicas. Universidad de Sonora. Centro de Investigación en Alimentación y Desarrollo, A.C.
14:00 - 16:00	**Lunch**

16:00 - 17:00	**PLENARY LECTURE II.** Kinetic modeling of central glucose metabolism in cancer cells **Dr. Alvaro Marín Hernández** Instituto Nacional de Cardiología Chair: Dra. Cecilia Zazueta Instituto Nacional de Cardiología
17:00 - 18:15	**ORAL SESSION III "Mitochondrial Pathologies"** Chair: Dra. Emma B. Gutiérrez- Cirlos Fes, Iztacala, UNAM
17:00 - 17:15	IL-4 role in the development of the lupus mouse model induced by non-bilayer phospholipid arrangements Carlos Wong Baeza, Claudia Albany Reséndiz Mora, Christian Irene Nevárez Lechuga, Anahi Sotelo Rodríguez, Giovanna Barrera Aveleida, Isabel Baeza Ramírez. Escuela Nacional de Ciencias Biológicas, IPN.
17:15 - 17:30	Respirasome prevents ROS production during its inactivation by heavy metals Jaime Abraham de Lira Sánchez, Mercedes Esparza Perusquía, Ricardo Jasso Chávez, Juan Pablo Pardo, Federico Martínez, Oscar Flores Herrera. Departamento de Bioquímica, Facultad de Medicina, UNAM.
17:30 - 17:45	Hormetic intervention of metformin and tBHQ in conjunction with exercise improves mitochondrial function in the liver of obese old rats Stefanie Paola López-Cervantes, Rafael Toledo-Pérez, Mercedes Esparza-Perusquía, Mina Konigsberg-Fainstein, Oscar Flores-Herrera. Departamento de Bioquímica, Facultad de Medicina, UNAM. Posgrado en Biología Experimental UAM, Iztapalapa.

17:45 - 18:00	Analysis of mitochondrial function in pancreatic islets and insulin production in two diet-induced obesity (DIO) models Corazón de María Márquez Álvarez, Eduardo Martínez Abundis, Nancy P. Gómez Crisóstomo, Erick N. De la Cruz Hernández. Laboratorio de Investigación en Enfermedades Metabólicas e Infecciosas, División Académica Multidisciplinaria de Comalcalco, Universidad Juárez Autónoma de Tabasco.
18:00- 18:15	Renal tumors in Wistar rats with omega 3 fatty acids supplement: Mitochondrial respiration and fatty acid composition Avendaño Briseño Karla Alejandra, Figueroa García María del Consuelo, Mejía Zepeda Ricardo Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Iztacala.
18:15 - 18:30	**Coffee Break**
18:30 - 19:30	**ORAL SESSION IV "Bioenergetics from Photosynthetic Organisms"** Chair: Dra. Miriam Vázquez Acevedo Instituto de Fisiología Celular, UNAM
18:30 - 18:45	Far-red photoacclimation in Synechococcus PCC 7335 Carlos Gómez-Lojero, Priscila Herrera-Salgado and Lourdes Elizabeth Leyva-Castillo. Departamento de Bioquímica, Cinvestav IPN.
18:45 - 19:00	Unique far-red light harvesting antenna complex involved in state transition in *Euglena gracilis* Héctor Miranda-Astudillo*, Félix Vega de Luna, Arshad Rameez, Charles Counson, Wojciech Nawrocki, Pierre Morsomme, Lukáš Nosek, Hervé Degand, Denis Baurain, Roman Kouřil, Pierre Cardol, UNAM

19:00 - 19:15	Antares I, modular photobioreactor suitable for photosynthesis and bioenergetics research Mónica Rodríguez-Bolaños[1], Pedro Miranda-Reyes[2], Héctor V. Miranda-Astudillo[1]*. [1] Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, UNAM. [2] Departamento de Química, IPN, México.
19:15 - 19:30	IL-1β Promotes Glucocorticoid Receptor Stability in the Onset of Glucocorticoid Hypersensitivity in Hepatocytes Leobarda Robles-Martinez, Daipayan Banerjee, & Mariana Nikolova-Karakashian. Department of Physiology, University of Kentucky, College of Medicine.

TUESDAY 19th

BIOPHYSICAL SYMPOSIUM

7:30 - 9:00	**Breakfast**
9:00 - 10:30	**CONFERENCE I** "ATP synthase c-subunit leak channel and mitochondrial permeability transition". **Dr. Elizabeth Jonas** Yale School of Medicine Chair: Dr. Manuel Gutiérrez Facultad de Química, UNAM
10:30- 11:00	**Coffee Break**

11:00- 12:00	**CONFERENCE II** "The modes of action of endogenous activators of TRPV channels" **Dr. Tamara Rosenbaum** Instituto de Fisiología Celular Chair: Dr. Carlos Saldaña Universidad Autónoma de Querétaro
12:00- 12:30	**Coffee Break**
12:30- 14:00	**CONFERENCE III** "The cell as a gel: materials for a conceptual discussion" **Dr. Luis Bagatolli** Instituto de Investigación Médica Mercedes y Martín Ferreyra INIMEC-CONICET-Universidad Nacional de Córdoba, Argentina. Chair: Dr. Carlos Saldaña Universidad Autónoma de Querétaro
14:00 - 16:00	**Lunch**
16:00- 17:00	**ORAL SESSION V "Membrane´s Biophysics"** Chair: Dr. Carlos Saldaña Universidad Autónoma de Querétaro
16:00 - 16:15	High-throughput fluorescent assays for identifying new Kv10.1 channel modulators Jennifer Erzsebet Olvera Martínez, Mirsha Asaret Gómez Herrera, Edgar Milo, Arlet Loza-Huerta, Enoch Luis Instituto de Fisiología Celular, UNAM
16:15 - 16:30	Biphasic effects of BL-1249 on voltage-gated ion channels Israel Armando Estrada Garrido, Oswaldo Valencia Aguilar, Esteban Gutiérrez García, Oliver Cesar Lara-Figueroa, Enoch Luis. Instituto de Fisiología Celular, UNAM

16:30 -16:45	*In silico* study of the target of the toxin Killer (K1) in the potassium channel Tok1 Jimena R. Villarreal, Verónica Morales-Tlalpan, A. González-Gallardo, C, Molina Vera, Roberto Ferriz-Martínez, Carlos Saldaña. Universidad Autónoma de Querétaro. Unidad de Proteogenómica. Instituto de Neurobiología. Campus Juriquilla, UNAM
16:45 -17:00	*In silico* determination of the binding-site affinity of 4-aminopyridine and derivatives upon the Kv ion channels Sofia Rodríguez-Rangel, Marco A. Dorantes-Ramos and Jorge E. Sánchez-Rodríguez. Departamento de Física, CUCEI, Universidad de Guadalajara
17:00 - 18:00	**CONFERENCE IV** "Structural and dynamics of bilayer and its role in transmembrane transport" **Dr. Iván Ortega Blake** Instituto de Ciencias Físicas, UNAM Chair: Dr. Carlos Saldaña Universidad Autónoma de Querétaro
18:00 - 20:00	**SESSION I "My Thesis in a Video"** - Structures - Respiratory Chains, Transport and Biotechnology - ATP Synthases

WEDNESDAY 20th

7:30 - 9:00	**Breakfast**
9:00- 10:45	**ORAL SESSION VI "New Research Models and Cardiovascular Diseases"** Chair: Dr. Oscar Flores Herrera Facultad de Medicina, UNAM
9:00- 9:45	**CONFERENCE** "Unraveling metabolic reprogramming events during Axolotl (*Ambystoma mexicanum*) limb regeneration" **Dr. Luis Alfredo Cruz Ramírez** LANGEBIO
9:45- 10:00	Contractile response in skeletal muscle of a mouse model with heart failure with preserved ejection fraction Cielo Maritza Martínez Martínez, Rocío del Carmen Montoya Pérez, Noemí García Ramírez. Universidad Michoacana de San Nicolás de Hidalgo
10:00- 10:15	Nanoencapsulated resveratrol and ciclosporine A improves cellular viability and calcium retention capacity in a cardiac hypoxia/reoxygenation model Omar Lozano García, Paulina Hernández-Fontes, Christian Silva-Platas, Gerardo de Jesús García Rivas. Escuela Nacional de Medicina, Tecnológico de Monterrey. Centro de Investigación Biomédica, Hospital Zambrano-Hellion, Tecnológico de Monterrey
10:15- 10:30	The cell culture medium with low glucose and an AMPc increase, prevents periportal hepatic dedifferentiation Elida Amaya Vicente, Genaro Vazquez Victorio. Laboratorio Nacional de Soluciones Biomiméticas para el diagnóstico y terapia (LaNSBioDyT), Facultad de ciencias UNAM

10:30- 10:45	The effect of cholesterol or ergosterol on the structure and dynamics of membrane domains Arturo Galván-Hernández* and Iván Ortega-Blake. Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México. Av. Universidad s/n, Col. Chamilpa, Cuernavaca, Morelos, 62210, México.
10:45 - 11:00	**Coffee Break**
11:00- 12:00	**PLENARY LECTURE III.** "Pharmacology of mitochondria and their social habits in health and disease" **Dr. Michelangelo Campanella** University of London Chair: Dra. Adriana Muhlia CIAD, A.C.
12:00- 12:30	**Coffee Break**
12:30- 14:00	**ORAL SESSION VII "Biomembranes"** Chair: Dra. Sobeida Sánchez Nieto Facultad de Química, UNAM
12:30 - 12:45	Rapamycin induces morphological and physiological changes without increase in lipid content in *Ustilago maydis* Lucero Romero Aguilar, Juan Pablo Pardo Vázquez and Guadalupe Guerra Sánchez Facultad de Medicina, UNAM
12:45- 13:00	Coupling between ordered and disordered phases in asymmetric lipid bilayers studied by AFM-Force spectroscopy Romina F. Vázquez, Erasmo Ovalle-García, Armando Antillón, Laura S. Bakás, Iván Ortega-Blake, Carlos Muñoz-Garay and Sabina M. Maté. Universidad Nacional de La Plata (UNLP), Argentina. Instituto de Ciencias Físicas, UNAM. Centro de Investigación en Proteínas Vegetales, UNLP, Argentina

13:00 - 13:15	Design and biophysical characterization of 3 chimeric membranolytic antimicrobial peptides Adriana Morales-Martínez, Brandt Bertrand, Jesus Silva-Sánchez, Carlos Muñoz-Garay. Instituto de Ciencias Físicas, UNAM. Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública. México
13:15 - 13:30	Physicochemical properties that determine membrane activity and selectivity of the antimicrobial peptide ascaphin-8 Brandt Bertrand, Adriana Morales-Martínez, Jesus Silva-Sanchez, Carlos Muñoz-Garay. Instituto de Ciencias Físicas, UNAM. Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública. México
13:30- 13:45	Differential distribution of sphingolipids in the plant plasma membrane regions: Possible roles of glicosilinositolphosphoceramides Laura Carmona-Salazar, Rebecca E. Cahoon, Jaime Gasca-Pineda, Ariadna González-Solís, Edgar B. Cahoon and Marina Gavilanes-Ruíz. Depto. de Bioquímica, Facultad de Química, UNAM. Center for Plant Science Innovation & Department of Biochemistry, University of Nebraska-Lincoln, USA. UBIPRO, Facultad de Estudios Superiores Iztacala, UNAM
13:45- 14:00	Molecular dynamic insights of the interaction of ascaphin-8 and 3 variants in two different compositions of lipid model membranes. Adriana Morales-Martínez, Juan Manuel Hernández Meza, Ramón Garduño Juárez, Brandt Bertrand, Carlos Muñoz-Garay. Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México (ICF-UNAM).
14:00 - 16:00	**Lunch**

16:00 - 17:00	**PLENARY LECTURE IV** "Role of mitochondrial calcium in cardiac cell death" **Dr. Elizabeth Murphy** NHLBI, National Institute of Health, USA Chair: Dr. Gerardo García Rivas Tecnológico de Monterrey
17:00 - 19:00	**SESSION II "My Thesis in a Video"** -Biomembranes and Biophysics - Oxidative Stress - Chronic Diseases
19:00 -	**GALA NIGHT**

THURSDAY 21th

7:30 - 9:00	**Breakfast**
9:00 - 10:30	**PLENARY LECTURE V** "Physical properties of biological membranes and their implication in the activity and specificity of antimicrobial peptides" **Dr. Roberto Carlos Muñoz Garay** Instituto de Ciencias Físicas, UNAM Chair: Dra. Adriana Muhlia CIAD, A.C.
10:30 - 11:00	**Coffee Break**
11:00 - 12:00	***In Memoriam*** Dr. Helidoro Celis Sandoval Dr. Georges Dreyfus Dr. Diego González Halphen

12:00 - 12:30	**Coffee Break**
12:30 - 14:00	Armando Gómez Puyou Awards & Oral Session Contest **Meeting Closure Ceremony**

ORAL SESSION I “Mitochondrial Proteins”

A positive cluster of amino acids in the N-terminus of selected mitochondrial proteins is relevant for their co-translational import mediated by $\alpha\beta_2$-NAC

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The origin of eukaryotic cells raised a big challenge for intracellular protein targeting: How to deliver nucleus-encoded proteins to the proper organelle? Mitochondrial proteins developed signals that direct them to the pore of translocation TOM, being MTSs the most studied, which are amphipathic editable alpha-helices in the N-terminus of proteins. Nevertheless, the initial steps of the import of mitochondrial proteins have not been studied in detail.

In general, it is accepted that mitochondrial proteins are synthesized in the cytosol and then are imported by a group of complexes in the mitochondria. There is evidence that mitochondrial proteins can be imported in a co-translational manner, where the translation and import of the substrate occur simultaneously. NAC (Nascent Associated-polypeptide Complex), a cytosolic heterodimeric chaperone, has been described with a crucial role during the co-translational import of mitochondrial proteins. NAC binds to the exit tunnel of ribosomes and nascent polypeptides at the same time, and in doing so, avoids nascent polypeptides from acquiring improper conformations. NAC is a heterodimer formed by the subunits α and β but, in the baker’s yeast *Saccharomyces cerevisiae,* the β subunit is duplicated, resulting in two different NAC complexes: $\alpha\beta_1$-NAC and $\alpha\beta_2$-NAC.

In a previous study, we reported that the $\alpha\beta_2$-NAC complex and the mitochondrial outer membrane protein Sam37 have a genetic and physical interaction. Furthermore, the elimination of both components in a $\Delta\alpha\beta_2\Delta$*sam37* mutant decreases the steady-state levels of selected mitochondrial proteins, such as Oxa1, Sod2, Nfs1, and Tom40. At this point, we do not know if there is a signal on mitochondrial proteins that are recognized by $\alpha\beta_2$-NAC. To address this question, we engineered chimeras by fusing the MTS of Oxa1 or Mdm38 with GFP (MTS^{Oxa1}-GFP or MTS^{Mdm38}-GFP, respectively) and evaluated their subcellular localization. While both chimeras are in the mitochondria, the level of MTS^{Oxa1}-GFP is diminished in the triple mutant $\Delta\alpha\beta_2\Delta$*sam37*. Importantly, we found that the first ten amino acids of Oxa1, previously shown to follow an import mediated by $\alpha\beta_2$-NAC, have relevant information for the recognition by this complex. In this short region of Oxa1’s MTS we found a cluster of positive amino acids (KRR) important for the co-translation import of Oxa1. Our results support the notion that NAC recognizes the first amino acids in some mitochondrial proteins to assist their co-translational import with the help of Sam37.

The jellyfish *Stomolophus meleagris* mitochondrial adaptive response to thermal stress

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The cannonball jellyfish, *Stomolophus meleagris* populations have increased significantly, associated with the increase in seawater temperature. This phenomenon has been of worldwide interest, promoting scientific efforts to understand the metabolic and energy responses of the species to environmental changes. This research addresses the study of the bioenergetics of *S. meleagris* exposed at 18, 23, and 28 ºC. The respiratory chain was characterized, and changes in mitochondrial function of adult jellyfish were determined. Mitochondrial oxygen consumption and transmembrane potential showed that the respiratory chain is active and coupled even at low temperatures. The canonical complexes from the respiratory chain and ATP synthase were identified. In addition, four alternative enzymes (two type II NADH-dehydrogenases, one mitochondrial GAPDH, and one alternative oxidase (AOX), were detected. These accessory enzymes remain active even when the organisms are under heat stress. These results suggest that the cannonball jellyfish possesses a branched mitochondrial respiratory chain and a physiological adaptation to overcome environmental changes that may negatively affect the organism's fitness.

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The Cox1 carboxyl terminal end is essential for efficient mitochondrial function in yeast

Itzel Abil García-Cordero, Ana Paulina Gutiérrez-Alejandre, Yolanda Camacho-Villasana, Xochitl Pérez-Martínez.
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The electron transport chain couples redox reactions to the creation of an electrochemical gradient that leads to the formation of ATP in a process called oxidative phosphorylation that occurs in mitochondria. In *Saccharomyces cerevisiae* complex IV or cytochrome *c* oxidase is formed by 12 subunits, most of which are encoded in the nuclear genome and imported into the mitochondria, except for three subunits, Cox1, Cox2 and Cox3, which are encoded in the mitochondrial genome, and form the catalytic core of the enzyme. Cox1 has 12 crosses through the inner mitochondrial membrane. In our group the carboxyl terminal end of Cox1 regulates the synthesis and stability of Cox1, and it is important for the regulation of the assembly of supercomplexes (Shingú-Vázquez, et al. 2010; García-Villegas, et al. 2017). Interestingly, mutants of this region are able to grow on respiratory media, indicating that Cox1 and cytochrome *c* oxidase are functional. We asked how the loss of this Cox1 regulation affects the cell? To answer this, we carried out a characterization of the cellular phenotype as well as the mitochondrial function in mutant strains of the Cox1 C-terminal end. One mutant has a deletion of the last 15 amino acids of the Cox1 C-terminal end (Cox1ΔC15) and a second mutant in wich prolines 521 and 522 were replaced by alanines ($Cox1^{P521A\ /\ P522A}$). Both mutants lost the ability to regulate Cox1 synthesis, and in addition the Cox1ΔC15 mutant affected accumulation/formation of supercomplexes. We observed that the Cox1ΔC15 mutant was sensitive to high temperatures in respiratory media (lactate and ethanol/glycerol) while the activity of complex IV in gels decreased at room temperature. In contrast, the $Cox1^{P521A\ /\ P522A}$ mutant did not exhibited any phenotype in response to various growth or stress conditions. In addition, blue native electrophoresis indicated that the Cox1ΔC15 mutant accumulated an excess of Cox1 even though in gel activity was dramatically reduced. Therefore, we conclude that the Cox1 C-terminal end is important to regulate cell growth at heat stress conditions on respiratory medium, and activity of complex IV is dramatically reduced in the Cox1ΔC15 mutant, even though Cox1 protein and supercomplexes are accumulated, indicating that they are not functional.

The respiratory chain of *Rhodotorula mucilaginosa*

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Rhodotorula mucilaginosa, is a *Basidiomycota* along with *Rhodosporidium*, *Sporobolomyces* and others. This oleaginous yeast can be found in a wide variety of environments under conditions that are unfavorable for growthin most other organisms. It can thrive at high pressures, high salt concentrations and in heavy metal polluted environments. *R. mucilaginosa* has even been isolated from permafrost soils that are approximately 150 thousand years old, making this yeast an organism with great metabolic plasticity and resistance to environmental variations. *R. mucilaginosa* produces pigments, which give it a characteristic pink-orange color. These fat-soluble carotene derivatives are torularodin, torulene and β-carotene, which behave as scavengers of reactive oxygen species. Research on this microorganism has mostly focused on improving the production of both, pigments and fatty acids by subjecting cells to many types of stress of varying intensity. In our hands, this yeast survived hosting the intracellular obligate endosymbiont *Wolbachia* sp, found colonizing insects, thus demonstrating the great resilience of this species and opening the possibility of using it as an artificial host for obligate parasites/endosymbionts.

Interestingly, *R. mucilaginosa* is an obligate non-fermentative aerobe, so the energy for production of biomass, carotenes and lipids comes mainly from its mitochondrial respiratory chain (RC). Still, its RC has not been examined in detail, so we decided to undertake this project. The *R. mucilaginosa* mitochondrial electron transport chain does contain the multi-subunit Complexes I to IV and ATP synthase first reported in mammalians and these exhibit the same sensitivity to specific inhibitors. In addition, our results suggest that RC is branched, as it contains a type II NADH dehydrogenase which is sensitive to flavones, plus an alternative oxidase, sensitive to propyl-gallate. These alternative enzymes probably contribute to the remarkable survival ability observed for *R. mucilaginosa*. Expression of these enzymes is enhanced at the stationary growth phase. Also, all RC components increase in concentration in the presence of a non-fermentative carbon source. The possible role of carotene derivatives in cell survival is still to be considered in the near future.

Revisiting accessory subunits and assembly of mitochondrial complex I from *Yarrowia lipolytica*: a complexome profiling approach.

Alfredo Cabrera-Orefice, Madhurya Lutikurti and Ulrich Brandt
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Mitochondrial complex I (CI) is the largest and most complicated enzyme of the respiratory chain, which couples the generation of proton-motive force to the transfer of electrons from NADH to ubiquinone. CI contains 14 central subunits and ~30 accessory subunits. Specific roles of accessory subunits of CI are not yet fully understood; particularly in those located at the membrane arm. The recent elucidation of the complete assembly pathway of human complex I has not only confirmed a modular mounting but also the specific proteins mediating this process; i.e. assembly factors. Since some orthologs for accessory subunits/assembly factors of CI are absent in other taxa as well as presence of fungi- and plant-specific subunits has been described, direct extrapolation of the assembly steps is not straightforward. Here, we have studied the impact of deleting several accessory subunits of the membrane arm on the assembly and stability of CI from the yeast *Yarrowia lipolytica*. To shed light on the participation of these subunits, we applied complexome profiling to define the sub-assemblies resulting from this intervention. Complexome profiling is a state-of-the art approach that combines separation of native complexes by Blue Native-PAGE, mass spectrometry identification and hierarchical clustering analysis. Absence of fungal-specific subunit NUXM led to a total loss of CI; thus, it confirmed its essentiality in this species. In contrast, absence of subunit NUNM/NDUFB5 did not prevent the assembly of a fully functional enzyme. Lack of different P distal (PD) module-located subunits consistently resulted in formation of a functional sub-complex containing modules N, Q and PP. This sub-complex retained ~30% of the NADH:ubiquinone oxidoreductase activity; however the proton-pumping efficiency was lower. As predicted, accumulation of specific assembly intermediates was observed and helped identify putative assembly factors. Up to 9 assembly factor candidates were noticed and at least half of them were caught interacting with stable CI sub-complexes. Two novel proteins did also cluster with intermediates containing the fungi-specific assembly factor CIA84, potentially suggesting a role in CI assembly. Besides, interactions of some of these sub-complexes co-migrated with the signals of respiratory complexes III and IV, which proposes the formation of partially assembled respirasomes. Our results provide a better overview on the assembly pathway of CI in *Y. lipolytica* and also suggest alternative routes to form intermediates that apparently do not occur in human mitochondria.

Rat liver versus *Saccharomyces cerevisiae*: comparison of some effectors on the mitochondrial permeability transition pore.

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Mitochondria play an essential role in bioenergetics and cellular homeostasis, ATP synthesis and death. In aerobic organisms oxidative phosphorylation (OxPhos) is vital for ATP production. In mitochondria, an unspecific channel located in the mitochondrial inner membrane (MIM) has large effects on the efficiency of mitochondria to produce ATP. The physiological role of this channel seems to vary with each species. The yeast *Saccharomyces cerevisiae* mitochondrial unspecific channel (*Sc*MUC) does share some similarities with the mammalian permeability transition pore (mPTP). When these channels open, ion and proton gradients across the inner mitochondrial membrane are depleted, which leads to deficient mitochondrial ATP synthesis. In some mammalian tissues this is associated with Ca^{2+} homeostasis and cell protection against stress (e.g., cardiomyocytes and myocytes), although once irreversibly opened it may lead to cell death. In contrast, *Sc*MUC supports fermentative activity by oxidizing the NAD^+ pool. Also, *Sc*MUC is closed by high [Pi] or Ca^{2+}. To explore a possible difference in physiologic roles for each channel, the reversible opening and closing of *Sc*MUC and mPTP from rat liver was compared in the presence of different effectors (ATP/ADP and Ca^{2+}/EGTA respectively) at different incubation times. We monitored the rate of O_2 consumption, mitochondrial swelling, and the transmembrane potential. It was observed that *Sc*MUC remained open for minutes and opening was reversible, while mPTP reversibility was lost early. This enforces the idea that each species expresses a mitochondrial permeability pore with different activities, sensitivity to effectors and even role in metabolism.

PLENARY LECTURE I

PAOLO BERNARDI, MD
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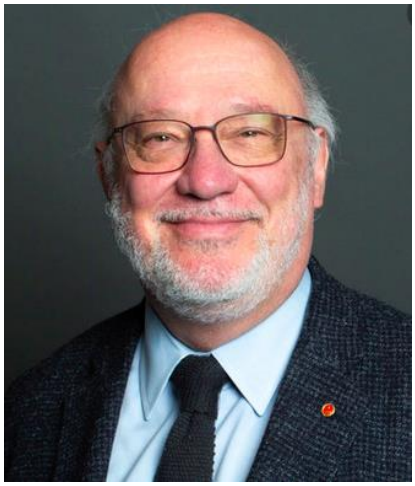

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Defining the molecular mechanisms of the mitochondrial permeability transition

Isolated mitochondria can undergo a permeability increase to solutes leading to mitochondrial swelling, which was first described nearly 70 years ago. As chemiosmotic principles of energy conservation became generally accepted, with few exceptions this Ca^{2+}-dependent inner membrane “permeability transition” (PT) was considered as an in vitro artifact of little or no physiological significance. Inhibition of the PT by cyclosporin A through mitochondrial cyclophilin D allowed to assess its role in pathophysiology, yet the nature of the channel (the PT pore, PTP) remained a mystery. F-ATP synthase and the adenine nucleotide translocator (ANT) are leading candidates as mediators of the PT but the mechanism(s) leading to channel formation remain undefined. To shed light on the structural requirements for PTP formation we tested its activity in cells with point mutations in the β, e, g and OSCP subunits of F-ATP synthase; in cells ablated for subunits g, OSCP and b; and in ρ^0 cells lacking subunits a and A6L. Taken together, our findings indicate that the PT can be mediated by two cyclosporin-sensitive channels formed by the F-ATP synthase (F-PTP) and by the ANT (A-PTP), respectively. Existence of two PTPs provides a convincing explanation for the persistence of Ca^{2+}-dependent permeabilization in the absence of an assembled F-ATP synthase and opens new perspectives to our understanding of the PT in physiology and pathology.

ORAL SESSION II "ATP-synthase & Cytochrome Oxidase"

Effect of heavy metals on the ATPase activity of the V_2 and V_1 F_1F_O-ATP synthase from *Ustilago maydis*

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Continuous exposure to heavy metals, a product of environmental pollution, is the cause of some diseases associated with the mitochondrial overproduction of reactive oxygen species (ROS), causing a decrease in ATP synthesis. F_1F_o-ATP synthase produces about 90% of cellular ATP, and its dimeric state is related to the folding of mitochondrial cristae, resulting in greater bioenergetic efficiency. In this work, the effect of heavy metals on the ATPase activity of the monomer (V_1) and dimer (V_2) of the F_1F_o-ATP synthase from *Ustilago maydis* (strain FB2) was determined. The V_1 and V_2 were solubilized with digitonin (2 g digitonin/1 g protein) and isolated as described by ATPase activity was determined by the slope of the spectrophotometric trace of the oxidation of NADH ($\sum_{340\ nm}$ = 6.22 $mM^{-1} \oplus cm^{-1}$) coupled reaction. The effect of $CuSO_4$, $CdCl_2$, $HgCl_2$, $ZnSO_4$, and $NaAsO_2$ on ATPase activity was determined using a dose-response curve (0.1–1500 µM). A significant decay in the ATPase activity of V_1 in the presence of Hg^{2+}, As^{3+}, Cu^{2+}, and Cd^{2+} with an IC_{50} of 2, 10, 23, and 76 µM, respectively and a slight resistance to Zn^{2+} was observed. The V_2 presents a greater resistance, being sensitive only to Hg^{2+}, Cu^{2+}, and Cd^{2+} with an IC_{50} of 11, 74 µM and 2.4 mM, respectively; while for As^{3+} and Zn^{2+} no decrease in activity was observed, even at 1 mM. Metal dilution (1:10) showed total reactivation of the enzyme, suggesting that the interaction of the heavy metal with the enzyme, or its modification, was not permanent. The results suggest that the monomer-monomer interaction in the dimer confers resistance to inhibition by metals, particularly with Cd^{2+}. In this sense, Steed, P., *et al.,* showed that Cd^{2+} interacts with residues R-210 and D-61 from subunits a and c, respectively, inhibiting ATPase activity of V_1. These residues are preserved in *Saccharomyces cerevisiae* and *U. maydis*, and the structure of *S. cerevisiae* V_2 showed that these residues are protected, suggesting that dimerization of complex V promotes the folding of mitochondrial cristae and confers resistance against some heavy metals, particularly Cd^{2+}, allowing ATP synthesis to be preserved.

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Deletion of the Inh1 subunit increases ATPase activity and reduces the stability of the F_1F_O-ATP synthase dimer in *Ustilago maydis*

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The F_1F_O-ATP synthase is an energy-transducing enzyme which produces ATP, from ADP and inorganic phosphate, coupled to the proton electrochemical potential ($\otimes\lceil_{H+}$) across the specific membrane of almost all eubacteria, thylakoids or mitochondria. The reverse reaction is prevented by the regulatory subunit IF_1 (Inh1 in fungi). Additionally to regulatory role of subunit IF_1 it has been suggested its participation in dimerizing complex V (V_2) which is involved in mitochondrial cristae folding. However, Nakamura (2013) demonstrated that the deletion of the IF_1 gene in the mouse had no effect on growth and no changes in the architecture of crista and the mitochondrial network were observed. Although IF_1 does not play a role in the dimerization of F_1F_O-ATP synthase, no studies on ATPase activity of the V_2 have been performed. For this, we eliminate the Inh1 gene in the basidiomycete *Ustilago maydis* and study the mitochondrial metabolism and crista architecture. The results show that in the Inh1Δ strain the cell growth, glucose consumption and biomass production were not affected. Ultrastructure and fluorescence analysis show that the size, the shape of the cristae, the mitochondrial network and the distribution of the mitochondria was similar to that of the wild-type strain. The $\otimes\rfloor_m$, ATP synthesis, and oxygen consumption in wild-type and Inh1Δ strains had similar values. Kinetic analysis of ATPase activity of complex V in permeabilized mitochondria showed similar values of V_{max} and K_m for both strains, and no effect of pH was observed. Interestingly, the dimeric state of complex V occurs in the mutant strain, indicating that this subunit is not essential for dimerization. ATPase activity of the isolated monomeric and dimeric forms of complex V showed a V_{max} values 4-times higher for the Inh1Δ strain than for the WT strain, suggesting that the absence of Inh1 subunit increased ATPase activity, supporting a regulatory role for this protein; however, no activation as pH increase was observed. ATPase activity of WT oligomers was stimulated several times by dodecyl-maltoside (DDM), probably by removal of ADP from F_1 sector, while DDM induced an inactive form of the mutant oligomers suggesting that IF1 could play a stabilizing role of the F_1F_O-ATP synthase structure.

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The critical length of the N-terminus of the ζ subunit to inhibit the F1FO ATPase of *Paracoccus denitrificans*

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The ATP synthase is an ubiquitous nanomotor that fuels the three domains life using the energy stored in electrochemical gradients across the energy transducing membranes to synthesize ATP, but it can also hydrolyze it during anaerobiosis, or lacking an alternate final electron acceptor. In our laboratory a new inhibitory subunit of the F_1F_O-ATPase of α-proteobacteria (ζ subunit) was discovered and characterized (1-2). ζ acts as a pawl-rachet to block the counterclockwise turnover of the rotor without affecting the clockwise rotation, thus stopping ATP hydrolysis while allowing ATP synthesis (3). To inhibit ATP hydrolysis, ζ folds its inhibitory N-terminus (NT) to form an α-helix that interacts with the α, β (stator) and γ (rotor) subunits. Previous studies from our laboratory showed that removal of the first 14 amino acid residues abolished the inhibitory function of ζ (2). nevertheless, the minimum number of residues involved in inhibition of the PdF_1-ATPase remains unknown. This work aims to find out which is the critical length of the inhibitory N-terminal α-helix of ζ to stop the counter-clockwise F_1-ATPase turnover. To do so, we are constructing 3 truncated ζ subunits, by removing progressively 4, 8, and 12 codons, i.e., 4, 8, and 12 amino acid residues, or ≈1, 2, and 3 α-helix turns respectively, in the direction from NT- to C-terminus in each mutant. This was carried out by PCR directed mutagenesis and amplicons were then cloned in pJET1.2 (maintenance plasmid) and pT7-7 (expression plasmid). To purify the NT truncated ζ´s, cells were induced with IPTG, harvested, lised, and differential protein precipitation was carried out with $(NH_4)_2SO_4$. The protein pellet containing ζ was then resuspended, desalted by dialysis and applied to two consecutive anion exchange and size exclusion columns, respectively. The ζ truncated mutant lacking 8 AA (ζT2-R9del) was purified and its inhibitory activity assayed in sub-bacterial particles (SBP) of the null ζ mutant of *Paracoccus denitrificans* (PdΔζ). The results show that this mutant cannot inhibit the PdF_1F_O-ATPase activity in SBP, hence it seems that the first 8 amino acids of the ζ subunit are responsible for the inhibitory activity. We are currently purifying the remaining truncated mutants to determine the critical length of the ζ´s N-terminus to exert PdF_1F_O-ATPase inhibition. The results will resolve the key inhibitory interactions of the ζ´s N-terminus α-helix with the rotary γ and α_{DP}/β_{DP} interface residues of PdF_1. Particularly, the oncoming functional results will show if the ζ-γ interactions blocking γ rotation are enough, or not, to inhibit fully the PdF_1-ATPase, or if the ζ-α_{DP} and ζ-β_{DP} interactions are also necessary for such inhibition.

The cytochrome *b* carboxyl-terminal end is a central regulator of the *bc₁* complex biogenesis in *Saccharomyces cerevisiae*

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Mitochondrial *bc₁* complex is composed of 10 different subunits, only Cytochrome *b* (Cyt*b*) is encoded in the mitochondrial genome and contains two heme *b* groups. Cyt*b* is a hydrophobic protein with 8 helices transmembrane, however the carboxyl-terminal end is facing the mitochondrial matrix. For Cyt*b* synthesis and assembly are required two chaperones Cbp3 and Cbp6 (Cbp3/6). These chaperones promote Cyt*b* synthesis and interact with newly synthesized Cyt*b* to stimulate its hemylation. Once hemes are added to Cyt*b*, Cbp3/6 are dissociated from Cyt*b* and subunits Qcr7 and Qcr8 associate to form an assembly intermediate.

The lack of Qcr7 and/or aberrant hemylation, reduces Cyt*b* synthesis through an assembly-feedback mechanism and Cbp3/6 are sequestered together with Cyt*b*. The crystallographic model of *bc₁* complex shows that Cyt*b* C-terminal end interacts with Qcr7 subunit. We propose that Cyt*b* C-terminal end is important for its hemylation and assembly-feedback mechanism, also for correct *bc₁* complex.

We created a mutant lacking the last 13 and 8 amino acids from Cyt*b* C-terminal end. Mutants were unable to respire, because Cyt*b* was not hemylated. Cyt*b* C-terminal end is involved in assembly-feedback regulation of translation and it is essential for correct *bc₁* complex assembly.

Are the two small cytochromes of *Bacillus subtilis* part of the b_6c-caa_3 supercomplex?

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Bacillus subtilis is a Gram-positive bacterium that lives in the soil or in water. It can form and endospore that gives high resistance to the organism through layers of proteoglycans. This bacterium has been used to express human proteins and has also been studied to understand the process and regulation of endospore forming. The respiratory chain is located in the only cellular membrane and contains several electron donors complexes as well as different terminal oxidases. The bacterium can grow aerobically or anaerobically. The respiratory chain can form supercomplexes of high molecular mass (such as the b_6c:caa_3 supercomplex) and complexes that are not associated. *B. subtilis* contains two small cytochromes, one with a transmembrane helix and the other with a lipid anchor. Although it is supposed that both transfer electrons between the b_6c and the caa_3 oxidase either cytochrome appears in low amount in clear native electrophoresis and mass spectrometry analysis. Mutants from each cytochrome do not show changes in growth with minimal medium. Here we show the analyses of *B. subtilis* grown in LB, 3% succinate and minimal medium. Membranes were isolated from these bacteria and analyzed with visible spectrometry, Clear native electrophoresis (CNE), in gel activity and mass spectrometry. The WT was transformed with plasmid pHP13 containing the gene *cccA*. We tested the construct growing the transformant in 3% succinate, with LB medium and in minimal medium. The mutant showed and increase of 3 times in cytochrome c_{550} compared to the WT. There were no differences in growth with the three media, compared to the WT. There were no differences in growth with the three media, compared to the WT. Mutants LUT1 (Δ cyt $_{550}$) and L16205 (ΔC_{551}) were also grown in LB and 3% succinate to compare the respiratory chain complexes analyzed with CNE. The mutant lacking c_{550} showed activity of the b_6c and caa_3 complexes, but with masses lower than 500 kDa in contrast with the WT. The mutant lacking c_{551} showed no bands of activity for these two complexes. We conclude that cytochrome c_{550} is important in supercomplex assembly but c_{551} is even more important. Our conclusion so far is that both cytochromes are needed in the respiratory chain of *B. subtilis*.

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Cytochrome c oxidase activity decreases along with the mitochondrial respiratory function in the lesser grain borer, *Rhyzopertha dominica,* exposed to hypoxia and hypercapnia

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The lesser grain borer, *Rhyzopertha dominica,* is a very destructive insect in stored grain facilities that feeds a great variety of cereals, producing economic losses. Studies on its bioenergetics are necessary to plan different strategies to reduce its infestation with other methods than insecticides. Modified atmospheres (MA) technology, where insects are exposed to low O_2 and/or high CO_2 concentrations, can be utilized as an alternative to alter their respiration. The present research aimed to evaluate the mitochondrial respiratory function (MRF) and cytochrome c oxidase (COX) activity *in vitro* of *R. dominica* isolated intact mitochondria. Insects were exposed to three MA treatments: (1) hypoxia (5% O_2); (2) hypercapnia (10% CO_2); (3) hypoxia-hypercapnia (5% O_2 and 10% CO_2), and were compared with a control group (normoxia). Intact mitochondria from the whole insect were isolated. Lactate concentration increased in all MA treatments compared with normoxia, which confirms *R. dominica* switched its metabolism to anaerobic. MRF decreased in all MA treatments up to 50% versus normoxia because of the decreased O_2 environments. All MA treatments caused mitochondrial uncoupling compared with normoxia, possible due to uncoupling protein mechanisms. Along with the MRF, COX activity decreased up to three-fold in all MA treatments compared with normoxia possible due to a reduce /inhibition of determinant metabolites, enzymes or coenzymes that directly supply the electron transport chain.

PLENARY LECTURE II

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Kinetic modeling of central glucose metabolism in cancer cells

Pathways of central glucose metabolism (glycolysis and glycogen metabolism) are essential to provide precursors for synthesizing macromolecules and may contribute to the ATP supply required for the constant and accelerated cellular duplication in cancer cells. In consequence, inhibition of these pathways has been considered an anti-cancer therapeutic option. However, the challenge is the establishment of selective therapeutic strategies that mainly affect cancer cells and minimize the toxic effects on non-cancer cells.

Kinetic modeling and control analysis of a metabolic pathway may identify the steps with the highest control in tumor cells and low control in normal cells, which can be proposed as the best therapeutic targets. Furthermore, the enzymes with the highest control can be proposed as the most suitable sites for therapeutic intervention since their inhibition will have greater adverse effects on the functioning of the pathway than the inhibition of those with low control. In this sense, our group has built kinetic models of glycolysis and glycogen metabolism in cancer and non-cancer cells, which are based on the experimental determination of the kinetic parameters (*Km, Ki, Ka and Vm*) of each of the enzymes/transporters and the concentration of the intermediate metabolites involved of the pathway.

Modeling predictions indicate that hexose-6-phosphate isomerase (HPI) and phosphoglucomutase (PGM) exert significant control on glycolysis and glycogen synthesis fluxes in cancer cells (AS-30D cells) but not in non-cancer cells (hepatocytes), suggesting that glycolytic and glycogen synthesis fluxes could be strongly decreased when HPI and PGM are simultaneously inhibited in AS-30D cells but not in hepatocytes. Experimental validation of these predictions showed that both the glycolytic and glycogen synthesis fluxes of AS-30D cells were inhibited by oxamate by inducing increased levels of Fru1,6BP, a competitive inhibitor of HPI and PGM. This data suggests that kinetic modeling permits establishing suitable therapeutic targets to inhibit the glucose metabolism in cancer cells specifically.

ORAL SESSION III "Mitochondrial Pathologies"

IL-4 role in the development of the lupus mouse model induced by non-bilayer phospholipid arrangements

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Non-bilayer phospholipid arrangements (NPAs) are lipid molecular associations that are different from the bilayer and are transient. However, if they become stable, they can trigger a disease in mice that is similar to human systemic lupus erythematosus. NPAs can be stabilized in liposomes formed by cylindrical and conical lipids or in cell membranes by drugs such as procainamide or chlorpromazine. These drugs produce a disease similar to lupus called drug-induced lupus as a side effect in people who are treated with them. In wild-type BALB/c mice, the lupus model has been developed by stabilizing NPAs by three different methods. The first one is by administering liposomes with NPAs induced and stabilized with procainamide or chlorpromazine, the second by administering the drugs alone, and the third one by administering the monoclonal antibody H308; these last two procedures stabilize the NPAs in the membranes of mouse cells. Anti-NPAs, anti-cardiolipin, anti-histones, and anti-coagulant auto-antibodies have been detected in the serum of these mice; in addition, the mice present facial lesions similar to the malar rash of patients with lupus and alterations in the skin and kidney. Here we evaluated the participation of the T_H2 response, through its hallmark cytokine IL-4, on the development of the lupus-like disease in mice. Wild type or IL-4 knockout BALB/c mice received liposomes bearing drug-induced NPAs, the drugs alone, or an anti-NPA monoclonal antibody (H308) to induce the lupus-like disease. IL-4 KO mice showed minor disease manifestations, compared to wild-type mice, with decreased production of anti-NPA IgG antibodies, no anti-cardiolipin, anti-histones, and anticoagulant antibodies, and no kidney or skin lesions. In these mice, H308 was the only inducer of anti-NPA IgG antibodies. These findings indicate that IL-4 has a central role in the development of murine lupus-like disease induced by NPA stabilization.

Respirasome prevents ROS production during its inactivation by heavy metals

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It has been proposed that mitochondrial respiratory supercomplexes functions include a low electron leak and reactive oxygen species (ROS) formation. To probe this hypothesis, we use heavy metals to induce a direct stress on respirasome due to modification or inhibition of proteins. Cd^{2+}, Hg^{2+} and Cu^{2+} are accumulated in mitochondria through a Ca^{2+} uniporter by the trans-membrane potential, and have the capacity to substitute protein cofactors, increasing redox reactions favoring ROS production; or can oxidize catalytic residues inducing a conformational change, protein malfunction or inactivation. The aim of this work was to evaluate the effect of heavy metals as oxidative stress inducers on *Ustilago maydis* respirasomes. Respirasomes and free-complex I were solubilized with digitonin and isolated as described by Esparza-Perusquía *et al.* 2017. NADH:DBQ oxido-reductase activity from respirasomes or free-complex I was evaluated spectrophotometrically at 340 nm according to Reyes-Galindo *et al.* 2019 in presence of 200 ⌈M NADH, 600 ⌈M DBQ and 16 ⌈M cytochrome c. The effect of Hg^{2+}, Cu^{2+}, AsO_2^-, Cr^{2+}, Fe^{3+}, Zn^{2+}, and Cd^{2+} was achieved with a dose-response curve (0.01–1000 ⌈M). ROS production was determined using the Amplex Red probe according to manufacturer's protocol. Our results showed that Hg^{2+} was the most aggressive metal over respirasomes, while it did not shown effect over free-complex I. Similar effect was observed with AsO_2^-. By other hand, Cu^{2+} showed a stronger effect over free-complex I than respirasomes. Respirasome showed a lower IC_{50} for Cr^{2+}, Fe^{3+}, Zn^{2+}, and Cd^{2+} than free complex I, concluding that respirasomes are more susceptible to heavy metal inactivation. Then, we determined the H_2O_2 production rate incubating the respirasomes or free complex I with the IC_{25}, IC_{50} and IC_{75} of each metal. Heavy metals induce 10-times ROS production by free-complex I than respirasomes. We conclude that the respirasome assemble prevent ROS production under heavy metal stress.

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Hormetic intervention of metformin and tBHQ in conjunction with exercise improves mitochondrial function in the liver of obese old rats.

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Aging is a natural and inevitable process characterized by the deterioration of the structural and functional capacities of the organisms. A risk factor for developing comorbidities during aging is obesity, since it generates metabolic imbalances, decreases lifespan and health. These two factors: aging and obesity modify mitochondrial function, decrease energy generation capacity and increase the reactive oxygen species (ROS) generation. Furthermore, mitochondria lose their internal membranes and reduce fatty acids oxidation. Among the interventions that have been used to reverse these effects, aerobic exercise stands out. Our working group has shown that the combined treatment metformin (MTF) and tertbutylhydroquinone (tBHQ), along with an exercise training routine prevent part of the deterioration associated with aging. So our aim was to evaluate the effect of exercise in combination with MTF and tBHQ to counteract mitochondrial damage in the liver during obesity and aging. Hence, Wistar female rats were subjected to a high-fat diet (HFD) from 21 days of age until 15 months. The treated groups performed a fartlek-type exercise and received MTF and tBHQ from 10 to 15 months of age. Liver mitochondria were isolated from these animals and the mitochondrial membrane potential, the expression of the OXPHOS complexes and the ATP synthesis were determined. Our results showed that the groups of sedentary rats + HFD present low membrane potentials, as well as a decrease in the expression of the OXPHOS complexes and the synthesis of ATP, compared with the groups that exercised or had the exercise intervention + MTF + tBHQ despite having a HFD.

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Analysis of mitochondrial function in pancreatic islets and insulin production in two diet-induced obesity (DIO) models

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Introduction: Obesity is considered the leading public health issue because of its impact on life´s span and quality; it represents the first cause for insulin and glucose dysregulation. Obesity is a consequence of chronic hypercaloric diets´ ingestion that results in a chronic inflammatory state and lipotoxicity by the excessive ectopic accumulation of lipids, which finally are causing oxidative stress and cellular damage. The beta-pancreatic cells suffer oxidative stress-associated damage mitochondrial dysfunction with altered membrane potential and opening of the mitochondrial permeability transition pore.
Objective: To evaluate the effects of high sucrose- and high fat diet-induced obesity on the beta-pancreatic cells and mitochondrial function.
Methods: Male just weaned Wistar rats were divided into three groups and feed with different diets along 12 months: Control (feed with standard chow and tap water); Sucrose (feed with 30% sucrose solution and standard chow), and Fat diet group (feed with 30% of lard in the solid chow and tap water). Bodyweight was recorded monthly. At the end of 12 months, BMI, caloric consumption, glucose, insulin levels, besides a glucose tolerance test (GTT), were determined. Rats were euthanized for blood collection and abdominal fat dissection. Langerhans islets were isolated by collagenase digestion to evaluate oxidative stress (oxygen peroxide generation), mitochondrial membrane potential, and mitochondrial permeability transition pore opening with fluorescent dyes.
Results: Body weight and caloric consumption were similar between groups with slightly higher BMI in the Sucrose group than Control. Both hypercaloric diets induced abdominal fat accumulation with fasting hyperglycemia and hyperinsulinemia. The glucose levels were higher for the two obese groups along the 120 minutes of the GTT, but the Sucrose group showed the highest values. The oxidative stress was higher only in the Sucrose group, whereas the mitochondrial permeability transition pore and membrane potential were similar between the three groups.
Conclusion: Both hypercaloric diets induced abdominal obesity, hyperglycemia, and hyperinsulinemia; however, the sucrose diet shows a greater severity in metabolic disruption, worsens the glucose metabolism, and increases oxidative stress in Langerhans islets. Our results suggest that obesity induced by carbohydrates has a more severe effect on glucose metabolism than obesity induced by high-fat consumption.

Renal tumors in Wistar rats with omega 3 fatty acids supplement: Mitochondrial respiration and fatty acid composition

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The Omega 3 fatty acids have been studied because of their supposed beneficial effects against Type 2 Diabetes Mellitus (DM2), however recently it has been proposed that high intake of these molecules increases the risk of developing cancer. Diagnosis of some type of cancer in people with DM2 has become more frequent.

It has been observed that consumption of long chain omega 3 fatty acids promotes the development of kidney tumors in Wistar rats that were experimentally induced to DM2. Diabetes and cancer have been reported to alter the lipid composition of cells membranes and their respective mitochondria. The composition determines the physicochemical properties of membranes and it has been reported that the membrane composition could affect the mitochondrial function. In this study we were interested in investigating the fatty acids composition of mitochondrial membranes and its correlation with mitochondrial respiration in renal tumors in male Wistar rats induced to DM2.

DM2 was induced in 48 hours-old newborn male Wistar Rats by an intraperitoneal injection of streptozotocin (STZ), at 125 mg/kg of body weight. The treatment with long chain Omega 3 fatty acids was administered directly into the mouth of the rats with a micropipette. When tumors were observed, the rats were killed and kidneys were removed, tissue was homogenized and mitochondria were isolated by differential centrifugation. The mitochondrial respiration was evaluated by measuring oxygen consumption and the fatty acids composition of mitochondrial membranes analyzed by gas chromatography.

A renal tumor model was successfully obtained in 50% of the experimental subjects with long chain omega 3 supplement. No tumors were developed in rats without the omega 3 supplement. Rats that developed renal tumors were found to have lower body weight gain and impaired glucose metabolism compared to the control group (glycemia between 95 and 387 mg/dL). Our data showed that in renal tumors, there was a decrease in the mitochondrial respiration in state 3 and in the state 4.

In the group that developed tumors, a significant decrease in the concentration of arachidonic acid, and an increase in palmitic acid, was found. The changes found in the proportion of these molecules shows a decrease in the membrane fluidity index (sum of unsaturated / sum of saturated fatty acids) in the experimental group. In conclusion, long chain omega 3 fatty acids are involved in tumorigenesis, in this study it was found that the administration of these molecules promoted the development of renal tumors in 50% of the population of Wistar rats that were previously induced to DM2 by STZ. Also, there are evident changes in the mitochondrial fatty acids membrane composition of tumor tissue and it appears that these changes are related to the bioenergetic function of mitochondria.

PAPIIT IN213421

ORAL SESSION IV
"Bioenergetics from Photosynthetic Organisms"

Far-red photoacclimation in Synechococcus PCC 7335

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Synechococcus PCC 7335 is a unicellular cyanobacterium isolated from Puerto Peñasco Sonora. This cyanobacterium, lives in (coarse) sand soil, which disperses the light, due to the physical properties of the light, red and far-red wavelengths become enriched when light penetrates the soil. Synechococcus PCC 7335 is adapted to the light spectrum in their habitat and performs complementary chromatic acclimation (CCA) and far-red light photo-acclimation (FaRLiP). The photosynthetic apparatus: phycobilisomes (PBS), photosystem I (PSI), and photosystem II (PSII) is adapted in light acclimation.
The PBS, the antenna in cyanobacteria, adheres to the photosynthetic membrane and contains a variety of pigments, which are expressed according to the incident light. The PBS rich in phycoerythrin appears when the cells are irradiated with green light, if the cells are illuminated with red light, the PBS is rich in phycocyanin and finally, when the cells are exposed to far-red light the PBS is rich in a new type of allophycocyanin. (Herrera-Salgado 2018). The far-red acclimatizationcclimatation of Synechococcus 7335 includes the synthesis of chlorophyll f (and chlorophyll d) and substitution of some subunits of the photosystem complexes. Chlorophyll f exists as a minority pigment (11%) and the majority pigment, 89% of chlorophyll a. Seven chlorophylls f are present in PSI and 5 chlorophylls f in PSII. Chlorophyll d is present in lower quantities, 1 chlorophyll d per 7 chlorophyll f in PSI and 1 chlorophyll d per 5 chlorophyll f in PSII. Paralogous subunits of PSI (PsaA2, PsaB2, PsaF2 PsaI2, PsaJ2 and PsaL2) and PSII (PsbA2, PsbB2, PsbC2, PsbD2 and PsbI2) have been detected by mass spectrometry except PsaI2 and PsaJ2). The photosystems participate not only in energy capture and energy transfer (proximal antenna) but also in energy trap (reaction center) in oxygenic photosynthesis. The question of the participation of chlorophyll f and d acting only as antenna[a] or if both chlorophylls perform the photochemistry in both photosystems is controversial. To solve this point we are carrying biochemical experiments to separate subunits of the reaction center from subunits of the periphery that harvest the light energy and the distribution of chlorophyll f and d in them.

[a](Chlorophyll f is assumed to play a light harvesting role needing heat from the environment (k_BT) for uphill excitation transfer to chlorophyll a)Herrera-Salgado et al. (2018) Complementary chromatic and far red photoacclimations in Synechococcus ATCC 29403 (PCC 7335). The phycobilisomes, a proteomic approach. Photosynthesis Research 138:39-56

*Main author PhD. thesis.

Unique far-red light harvesting antenna complex involved in state transition in *Euglena gracilis*

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Oxygenic photosynthesis underpins the survival of virtually all life forms by converting light energy into biologically useful chemical energy. The transfer of photosynthesis from eukaryotes that belongs to Archaeplastidae (i.e. green algae or red algae) to other non-photosynthetic organisms is a spectacular example of evolutionary innovation. This transfer occurred many times upon evolution and gave rise to highly diversified photosynthetic lineages of high ecological and/or biotechnological significance (e.g. diatoms, dinoflagelates, euglens).

In this study we characterized the light harvesting complexes from Euglena gracilis, a secondary photosynthetic unicellular eukaryote that arises from an endosymbiosis between a green alga and an ancient phagotroph euglenozoan species [1,2]. Phylogenomic, proteomic and single particle electron microscopy analyses revealed that although CP24 and CP26 minor antenna proteins are missing, PSII has a very conserved structure and can bind up to six LHCII trimers building a C2S2M2N2 PSII SC very similar to the one identified in *C. reinhardtii* [3]. In contrast, Euglena PSI SC has an unusual composition and structure, lacking several small core subunits (PSAG/H/I/K/L/O/P) and canonical LHC proteins, but being surrounded by LHCBM and specific LHCII-like antenna proteins. Some of these LHCII-like antenna associates into an unusual 250 kDa protein antenna complex which has red-shifted absorption and fluorescence spectra and is involved in state transition in low light and far-red enriched light.

Antares I, modular photobioreactor suitable for photosynthesis and bioenergetics research

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Climate change is an imminent process that will change the relations between all living organisms on Earth. Normally, more than 90% of planetary carbon is stored in algae, vegetation, and coral as biomass and organic compounds, nevertheless, the rapid growth of human population, in parallel with the increase of livestock activities and the excessive consume of fossil fuels produced the accumulation of anthropogenic gaseous CO_2 in the atmosphere. This process intensifies the greenhouse effect which has increased the average temperature of the planet during the last decades.

Oxygenic photosynthesis is responsible for the majority of atmospheric CO_2 fixation. Among the photosynthetic organisms, microalgal community can transport atmospheric carbon into biological cycles with higher efficiency rates compared with land plants. This represents an alternative to confront the actual climate change crisis. Additionally, microalgae have the potential to produce bioethanol, biodiesel, pigments, colorants, β-carotene, important long-chain polyunsaturated fatty acids and vitamins. These organisms have evolved to adapt to several environments and light qualities, as consequence, the spectral distribution of light influences their metabolism, however, development of commercial photobioreactors has evolved largely without regard to spectral optimization.

For this purpose a multi-scale modular photobioreactor fabricated from standard glass materials, *ad hoc* light circuits and small commercial devices was designed. The system is suitable to manage the principal variables of bioenergetics and photosynthesis research (temperature (15-35 °C), gas flow (CO_2/air), light intensity and spectral quality). The performance of the photobioreactor was tested by growing three evolutionary-distant microalgal species with different level of endosymbiotic relation of their plastids: Chlorophyta (*Archaeplastida*, green primary plastid), Euglenoid (*Excavata*, green secondary plastid) and Diatom (*Stramenophiles*, red secondary plastid). Our results showed an improvement of biomass production compared with the traditional flask system. The modulation of the incident spectra allowed us to enrich the specie-specific LHCE antenna from *Euglena gracilis*, and increased the biomass from the diatom specie. Taken together, these results confirm that, particularly for artificially lit photobioreactors, spectra manipulation needs to be included as a critical operational parameter to maintain optimal performance.

[#]This project was support by the Institutional Program of the Institute of Biomedical Research, National Autonomous University of Mexico: “The production of biomolecules of biomedical interest in microorganisms”.

IL-1β Promotes Glucocorticoid Receptor Stability in the Onset of Glucocorticoid Hypersensitivity in Hepatocytes

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Glucocorticoids (GC), such as cortisol, are steroid hormones secreted from the adrenal gland in response to stress. GC physiological actions such as metabolic homeostasis, cell proliferation, inflammation, development and reproduction, are mediated by glucocorticoid receptor (GR), a ligand-inducible transcription factor expressed in every cell type. In the absence of ligand, GR resides in the cytoplasm within a chaperon complex that prevent its degradation. Upon ligand binding, GR travels to the nucleus where acts as a transcription factor by binding to GC response elements (GRE). GCs are drugs prescribed for the treatment of numerous immune and inflammatory disorders, but long-term treatment with GCs is associated with impaired GC sensitivity which trigger adverse effects, impacting metabolism, and increasing susceptibility to stress and infections.

The goal of this study is to investigate how low-grade aging-associated inflammation induce GC hypersensitivity in hepatocytes and its contribution to the development of hepatic steatosis and insulin resistance. Here, we present data that IL-1β (a key pro-inflammatory cytokine) can up-regulate basal levels of GR in HepG2 hepatocytes. This up-regulation is independent of duration of IL-1β stimulation, with a rapid effect at 15 min and sustained for up to 24 h. Dexamethasone (Dex, a synthetic GC), activates GR and translocates to the nucleus where it regulates gene expression. Western blot analysis and indirect immunofluorescence confirmed GR accumulation and nuclear/cytoplasmic distribution upon IL-1β stimulation independent of Dex treatment. Moreover, 11βHSD1, an enzyme that regenerates active GC from inert 11keto forms, was induced in response to IL-1®. GR dimerization is an integral step in GC signaling, and the ubiquitin-proteasome-system is necessary to limit the duration of GC action. We found that HepG2 cells co-treated with IL-1β and MG132 (a proteasome inhibitor) delayed GR degradation indicating an operating GC-ligand dependent signaling. RNA-seq analysis of HepG2 cells stimulated with IL-1β in the presence or absence of RU486 (a GC antagonist) revealed an IL-1β/GR crosstalk related to biological processes linked to metabolism and protein synthesis/degradation.

Together these data show that IL-1® has a specific effect on GR signaling cascade by both elevating the levels of the receptor and ligand availability. Finally, our previous studies provide evidence that neutral sphingomyelinase-2 (nSMase-2) activity is increased in hepatocytes from old animals leading to IL-1β hyperresponsiveness during aging. Further studies will focus on the role of IL-1β/GR and nSMase-2 in the onset of hepatic steatosis and insulin resistance.

BIOPHYSICS SYMPOSIUM

CONFERENCE I

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ATP synthase c-subunit leak channel and mitochondrial permeability transition

Mitochondrial ATP synthase is vital for cellular energy production but may also play an important role in energy dissipation and cell death. We suggest that ATP synthase c-ring contains a leak channel that forms the main pore-forming unit of the mitochondrial permeability transition (mPT) and that the channel is activated during excitotoxic neuronal insult. We also find that the stoichiometric relationship of the ATP synthase FO with the F1 complex determines whether the ATP synthase c-subunit leak channel (ACLC) has an increased probability of opening into a large multi-conductance, voltage-gated ion channel. We show that the purified reconstituted c-subunit is inhibited by the addition of ATP synthase F1 subcomplex. In contrast, dissociation of F1 from FO occurs during excitotoxic neuronal death suggesting that the F1 constitutes an endogenous channel gate. mPT is known to dissipate the osmotic gradient across the inner membrane during cell death. We show that ATP synthase c-subunit knock down (KD) prevents this osmotic change in response to high calcium and KD also eliminates large conductance, Ca2+ and CsA sensitive channel activity of mPT. These findings elucidate the gating mechanism of the ATP synthase c-subunit leak channel (ACLC) and confirm that interaction of cyclophilin D with ATP synthase F1 subcomplex increases risk of death in stressed neurons.

CONFERENCE II

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The modes of action of endogenous activators of TRPV channels

Transient Receptor Potential (TRP) channels play diverse functions in physiology. TRP channels are polymodal proteins that allow us to respond to several stimuli. The TRPV1 and TRPV4 channels are two of the better studied members of this family and have been linked to several pathologies, which include the generation of pain. We have studied the roles of biologically relevant lysophospholipids that produce pain. Our results show that these molecules activate some TRPV channels and that this may lead to the presence of conformationally-different open states with different conductance levels, as compared to other agonists. Differential regulation of the opening of these proteins can result in fine tuning the electrical properties of cells and of pain responses.

CONFERENCE III

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The cell as a gel: materials for a conceptual discussion

In recent studies we established that glycolytic oscillations (characterized by oscillations in ATP and NADH chemical activities) in non-dividing yeasts are tightly coupled with the dynamic state of intracellular water, being this phenomenon scale independent. Specifically, we demonstrated that an optimum extent of water dipolar relaxation (which is connected with the activity of intracellular water), modulated by optimal levels of ATP and an optimally organized actin network, is crucial to the emergence of the oscillations supporting the view of a highly coherent and ordered cellular interior with properties similar to a responsive coascervate. This oscillatory behaviour was also observed for the chemical activity of intracellular K^+.

Additionally, we also found a strong coupling among temporal oscillations of thermodynamic variables such as temperature, heat flux and volume and the activity of intracellular metabolites. These results are interpreted in light of a recently proposed theoretical formalism by T. Heimburg in which isentropic thermodynamic systems can display coupled oscillations in all extensive and intensive variables, reminiscent of adiabatic waves. This interpretation is in line with the view of the cellular interior as a highly structured and near equilibrium system, where energy inputs can be low and sustain regular oscillatory regimes, challenge the notion that biological processes are essentially dissipative.

All these results, which are difficult to conceptualize using canonical cell models based in mass action kinetics and dilute solution theory, can be mechanistically depicted using an alternative theory called the Association-Induction Hypothesis (AIH), proposed by G. N. Ling in the 60's. This hypothesis, which is based in physicochemical principles of colloidal chemistry, offers a much simple mechanistic frame to explain the above reported experimental results challenging the canonical view of the cell. In fact, using this theoretical frame, we proposed that changes in intracellular water activity mediated by metabolic changes, can induce lyotropic changes in membranes, offering a different role for lipids self-assemblies compared to that offer from the canonical view of the cell.

ORAL SESSION V " MEMBRANE´S BIOPHYSICS"

High-throughput fluorescent assays for identifying new Kv10.1 channel modulators

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Ion channels are a vast family of transmembrane proteins implicated in many physio- and pathophysiological processes; therefore, it is not surprising many approved drugs target them for therapeutic purposes. For many years, patch-clamp electrophysiology has been the gold-standard technique for ion channel studies; however, it could be less helpful for the rapid discovery of active molecules on ion channels. This inconvenience has been solved thanks to non-invasive cell-based fluorescent assays that have enhanced ion channel drug discovery.
The Kv10.1 channels belong to the voltage-activated potassium channel family. The Kv10.1 channels are abnormally expressed in approximately 70% of solid tumors, where they are implicated in the tumorigenic process, making them a promising target for drug discovery modulators used in cancer treatment. To date, only non-specific inhibitors of Kv10.1 have been described. Therefore, research on the Kv10.1 has focused on searching for new and selective modulators. Here, we use two cells-based fluorescent assays to identify new Kv10.1 channel modulators that have not been previously described.
We used HEK293 wild type (HEK-WT) and HEK293 cells stably expressing the human Kv10.1 potassium channel (HEK-Kv10.1). For the cell-based fluorescent assay, we used FLIPR® potassium and FLIPR® membrane potential assay kits. FLIPR® potassium assay experiments showed that loperamide, amitriptyline, and SR33805 oxalate significantly decreased (53%, 55%, and 33%, respectively) ($p<0.0001$, one-way ANOVA) the amplitude of the fluorescence signal mediated by Kv10.1 channels. Similar results were obtained with FLIPR® membrane potential assay, showing that loperamide significantly decreased by 44% the amplitude of the responses ($p<0.05$, one-way ANOVA) associated with changes in the membrane potential of HEK-Kv10.1 but not in HEK-WT cells. Fluorescent results were confirmed by patch-clamp electrophysiology, showing that loperamide, amitriptyline and SR33805 inhibits Kv10.1 currents in a dose-dependent manner, with an IC50 of 12.8 µM, 15.2 µM and 130.8 µM, respectively. We concluded that cell-based fluorescent assays are effective and reliable methods for searching molecules with potential activity on Kv10.1 or other K^+ channels.

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Biphasic effects of BL-1249 on voltage-gated ion channels

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Ion channels are transmembrane proteins expressed in human body cells, playing a crucial role in many cellular processes, e.g., muscle contraction or neuronal communication. Pharmacological studies on ion channels, using agonists or antagonists, have been essential for understanding the biophysical properties of ion channels, and consequently, understanding their role in specific cells or systems under physiological and pathological states. In recent years, activators targeting K^+ channels have risen as new biophysical tools and purposes for treating many diseases, including cancer, diabetes, and epilepsy. BL-1249, is a TREK-1 and TREK-2 channel activator; however, the effect on other K^+ channels is unknown.
Here, we study the effect of the BL-1249 on: 1) HEK293 cells stably expressing the human Kv10.1 potassium channel and 2) endogenous ion channels expressed in the neuroblastoma cell line N1E-115. For these experiments, we performed whole-cell patch-clamp recordings. Patch-clamp studies on Kv10.1 channels revealed that BL-1249 (100 µM) increases Kv10.1 currents measured at +30 mV by 100% (n = 9, p<0.0001), shifts to the right the half-activation voltage ($V_{1/2}$) by 44.9 mV and hyperpolarizes resting membrane potential around 20 mV. BL-1249 exerted a concentration-dependent potentiation of Kv10.1 channels with an EC_{50} of 31.5 µM. BL-1249 did not have any effect on the endogenous K^+ channels expressed in HEK293 wild-type cells. We also use patch-clamp to record endogenous ion currents expressed in the N1E-115 cell line; BL-1249 (100 µM) did not affect the amplitude of the TEA-sensitive voltage-gated K^+ currents activated at +50 mV. Surprisingly, BL-1249 (100 µM) inhibited 99.7% of the TTX-sensitive voltage-gated Na^+ currents generated at 0 mV (n = 4, p=0.012). BL-1249 exerted a concentration-dependent inhibition of TTX-sensitive Nav channels with an IC_{50} of 8.1 µM. In summary, the present experiments have shown that BL-1249 is a potent inhibitor of Na^+ currents in N1E-115 cells, and for the first time, we show an opener of Kv10.1 channels. BL-1249 could be a promising tool for further studies. Grant SEP-CONACYT CB2017-2018-A1-S-13646 supported this work.

In silico study of the target of the toxin *Killer* (*K*1) in the potassium channel Tok1

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The yeast *Saccharomyces cerevisiae* is capable of synthesizing toxins. This is through a viral co-infection mediated by the satellite virus M1 and the helper virus L-A of the *Totiviridae* family. Several types of toxins *K*1, *K*2, *K*28, *K*lus exist in nature, *K*1 being one of the most studied. The phenomenon known as *Killer* is the consequence of the inhibition of the growth of yeast strains sensitive to the toxin. So far, the mechanism of action of *K*1 toxin is not known, but it is known to be mediated by two steps: 1) binding of the toxin to the 1,6 β-glucan of the sensitive yeast wall, its translocation to the plasma membrane, where 2) the toxin interacts with Kre1p, a GPI-anchored protein, which is in charge of the synthesis and structural arrangement of cell wall proteins. Once at this point, it is considered that it could have two different objectives; to embed itself directly forming pores in the membrane, causing osmotic imbalance and killing the cells or to bind to an unknown site of the Tok1 potassium channel, generating the exit of potassium until killing the cells by the same osmotic imbalance. In the laboratory it has been possible to determine, by modified Kirby & Bauer experiments, that the toxin is capable of killing sensitive *Saccharomyces* yeasts and other pathogenic microorganisms of biomedical importance such as *Klebsiella pneumoniae*. In the present work and employing *in silico* targeted mutagenesis, we plan to determine the possible binding site of the *K*1 toxin in the Tok1 potassium channel. Based on the results obtained, cysteine and tyrosine are proposed as the site of toxin-channel interaction.

In silico determination of the binding-site affinity of 4-aminopyridine and derivatives upon the K_V ion channels

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The 4-aminopyridine (4AP, also known as Ampyra® or Fampyra®) is a specific voltage-dependent potassium ion channel (K_V) drug-blocker that is clinically prescribed for the symptomatic treatment in patients with multiple sclerosis. Based in the drug-receptor mechanism of a 4AP-fluorinated analog (3F4AP) and its later synthesis as radioligand ([^{18}F]3F4AP), a new way of imaging of the central nervous system (CNS) by positron emission tomography (PET) has been proposed (P. Brugarolas, et al., 2018, Sci. Rep., 8:607). Because in development of PET tracers is useful to explore multiple derivatives and compare their physicochemical, biophysical and pharmacological properties, in this work we have implemented an *in silico* methodology to determine potential 4AP derivatives as candidates for PET tracer development for CNS imaging. We used homology modelling and molecular docking structure analysis to compute the binding site affinity of the 4AP derivatives: 3-methyl-4-aminopyridine (3Me4AP), 3-fluoro-4-aminopyridine (3F4AP), 3-methoxy-4-aminopyridine (3MeO4AP) and 2 or 3-trifluoromethyl-4-aminopyridine (3CF34AP or 2CF34AP). Ours results indicate that 4AP and derivatives binds the K_V1.2-K_V2.1 chimera channel (pdb 2R9R) and the Shaker K_V homology model (based on K_V1.2-K_V2.1 chimera and KCSA with TBA, pdb 1J95) structures by forming hydrogen bonds with the conserved threonine (Thr) 369 (Thr369) and Thr370 in the pore region. In all cases, the position of the 4AP or the 4AP derivatives are placed parallel to the pore axis of the K_V channels with the nitrogen and the amino group of the pyridine toward to the extracellular and intracellular side, respectively and the functional linked group at position 3 is oriented to the center of the pore defining the binding site of these 4AP analogs. The binding affinities computed in this work were consistent with our previous findings measured in living cells and quantified in terms of the half-maximal inhibitory concentration of 4AP or 4AP analogs (IC_{50}) producing a trend of the inhibition potency as follows: 3Me4AP > 3F4AP > 4AP > 3MeO4AP > 3CF34AP>>2CF34AP (Sofia Rodríguez-Rangel., 2020 Sci. Rep., 10 (1), 1-9). We conclude these experimental strategies (computational and functional) could be useful for the study of 4AP-based novel drugs with potential to be synthetized with ^{11}C, or ^{18}F as PET probes.

CONFERENCE IV

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Structural and dynamics of bilayer and its role in transmembrane transport

Dr. Ortega studied a Bachelor of Physics at UNAM, a Master in Mathematics at Oxford and later on, he pursued doctoral studies in Biophysics at Edinburgh and a Post-doctorate at Paul Sabatier University. He is currently a researcher at the ICF and has developed research at UNAM, UAM, UAEM and Cinvestav. His research deals with molecular biophysics and among his lines of research are studies on transmembrane transport phenomena, which have led to the proposal that the action of polyene antibiotics is affected by the structure of the cell membrane, resulting in the creation of attractive antifungals for potential therapeutic use. These derivatives have already been patented aiming to register these therepeuticals as approved medicines. He has been director of various Institutes and Faculties at UNAM, UAEM and Cinvestav. He has received various distinctions such as SNI level III, the Canifarma 2016 award and was recently awarded the Doctorate Honoris Causa by the UAEM.

ORAL SESSION VI "New Research Models and Cardiovascular Diseases"

Unraveling metabolic reprogramming events during Axolotl (*Ambystoma mexicanum*) limb regeneration

Dr. Luis Alfredo Cruz Ramírez
LANGEBIO

The Axolotl has been a model of study over 100 years since it serves as an important vertebrate model for studying regeneration and tissue repair and is the salamander species most easily bred in captivity and for which the most comprehensive genetic, genomic, and transgenesis tools have been developed. Beyond its capacity of regeneration the Axolotl has been focus of attention because it has the special characteristic of retaining the gross morphology of larvae as reproductive adults, a phenomenon known as neoteny or paedomorphosis.

Contractile response in skeletal muscle of a mouse model with heart failure with preserved ejection fraction

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Heart failure with preserved ejection fraction (HFEF) is associated with several comorbidities such as obesity, hypertension, and diabetes mellitus. This syndrome causes skeletal muscle dysfunction and is associated with reduced exercise capacity. Due to the lack of an adequate animal model that manages to recapitulate the multiple characteristics of the syndrome, its study has been obstructed. The implementation of a mouse model combining a high-fat diet + L-NAME appears to recreate the numerous systemic and cardiovascular features of human HFpEF, however, the non-cardiac mechanisms that induce skeletal muscle dysfunction are unclear. The objective of the present work was to evaluate the contractile response and the resistance to fatigue of the skeletal muscle of mice with ICFEp. Male C57BL6 mice divided into 2 groups were used: control (Ctrl) and Heart failure with preserved ejection fraction (HFEFp). A high fat (60% fat) and L-NAME (0.5 g / L) diet was administered for 8 weeks. Once the treatments were concluded, the soleus muscle was extracted to record the isometric tension. The ICFEp group presented a decrease in the force of muscle contraction over the maximum tension of the soleus muscle. We conclude that impaired physiology in skeletal muscle plays a key role in exercise intolerance in pHEFF.

Key Words: Skeletal muscle, Heart failure, Ejection fraction preserved.
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Nanoencapsulated Resveratrol and Ciclosporine A improves cellular viability and calcium retention capacity in a cardiac hypoxia/reoxygenation model

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Reactive oxygen species (ROS) one of the driving elements in cardiac ischemia-reperfusion (I-R) damage. In cells such model of exacerbated ROS production can be emulated by hypoxia-reoxygenation (H-R) conditions. This work focuses on cardiac cell protection from H-R events as a proof-of-principle strategy for *in vivo* cardioprotection. To achieve this, a model antioxidant, Resveratrol (RSV), or Ciclosporine A (CsA), which binds to cyclophilin D and retards the opening of the mitochondrial permeability transition pore, were encapsulated into poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs), a biocompatible polymer. After a physicochemical characterization of the NPs, an H-R model was performed with and were treated with either PLGA-RSV or PLGA-CsA.

The results demonstrate that, under H-R conditions, H9c2 cells viability can be preserved up to 100-fold greater administered doses of PLGA-CsA compared to CsA, and calcium retention capacity was preserved similar to untreated cells for groups treated with PLGA-RSV, PLGA-CsA and CsA groups. Such improved results by a nanoencapsulation delivery was due in part to the controlled and sustained release of RSV and CsA from the NPs. The current results underlies that nanoencapsulation delivery of ROS modulators or compounds retarding the opening of the mPTP could be a viable strategy to improve the outcome post-cardiac I-R events.

The cell culture medium with low glucose and an AMPc increase, prevents periportal hepatic dedifferentiation

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The liver is an organ with different and important functions. One of them is the glucose conversion in glycogen, which is a temporal energy reservoir, later is converted in glucose again to dosed into all cells of the body in fasting periods. The liver minimal unit is called the “hepatic lobule” and this is divided into three zones: middle, periportal, and pericentral. The last two zones have opposite and complementary metabolic activities; the pericentral zone do glycolysis and metabolizes xenobiotics while the periportal zone do gluconeogenesis and fatty acids oxidation. The identity of the hepatic zones depends on the morphogen concentration, nutrients and oxygen, as well as on mechanical signals to which the hepatocytes are exposed. On the other hand, the AMPK kinase is the main sensor of cell energy state and is activated by an increase in the amount of cAMP. Currently, primary hepatocyte cultures dedifferentiate and do not mimics the liver environment. So far, we have found that prolonged exposure of hepatocytes to a low glucose medium, prevents liver dedifferentiation and if the amount of cAMP is also increased, periportal hepatocytes characteristics are maintained.

The effect of cholesterol or ergosterol on the structure and dynamics of membrane domains

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In this talk I will present the results of the effect that sterols (ergosterol and cholesterol) have on the formation, size and mechanical response of gel-phase lipid domains that form in a bilayer composed of POPC/egg SM/sterol at different mole fractions of sterol studied by atomic force microscopy imaging and force versus distance curves. Bilayers formed by a mixing POPC/egg SM show phase segregation in the form of an almost circular domain of a thicker bilayer (gel-phase) that is around 2 μm in diameter. Addition of sterol o the mixture reduces the size of the thicker membrane domains until the bilayer looks homogeneous. The concentration of sterol needed to reach this homogeneous state is different depending on the sterol used. The relative height between the thin and thick domains is typically around 1 nm for both sterol-free and cholesterol-containing membranes that present phase-segregation and it tends to decrease as sterol is increased. For ergosterol however, its addition increases the relative height to 2 nm even for low concentrations, 5 mol%. This is conserved for 20 mol% concentration of ergosterol. This result suggests a different interaction between POPC and egg SM with these sterols. This is further supported by the rupture force histograms of the thin and thick bilayers obtained for different sterol content. Cholesterol increases rupture force of both thin and thick membrane regions (i. e. POPC and egg SM regions), whereas ergosterol only seems to affect egg SM rupture forces. Grain analysis of low- and high-resolution imaging of bilayers with 20 mol% ergosterol and 10 mol% cholesterol show a correlation between domain size and relative height. Additionally, increased relative height can be observed for small domains of ergosterol in comparison to cholesterol. The main interest in membrane models is to better understand the molecular mechanism of action of polyene antibiotics that have a slight selectivity towards fungal cells yet have a high cytotoxic effect which limits its use to critical cases. We believe that membrane properties, such as domain formation and stiffness, play a role in the action of polyenes. I will present new results that show the different effects of amphotericin B, the gold standard antimycotic, and a derivative called A21. The results show a different behavior between amphotericin B and A21 that involves lipid domains. This suggests a different interaction between the polyenes and the membrane. We are currently carrying out molecular dynamics simulations to understand the molecular interactions behind these differences between two very similar sterols and the relevance to polyene action. Results suggest that the three-dimensional structure of the sterols plays an important role in their interactions with other lipids, and this affects bilayer properties such as domain formation as seen by the analysis of the enrichment factor for each membrane system. Funding: PAPIIT-IG100920, COANCyT-Fronteras-74884

Plenary Lecture III

Dr. MICHELANGELO CAMPANELLA

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Pharmacology of mitochondria and their social habits in health and disease

Mitochondria are central to the homeostasis of mammalian cells in which they play part beyond the production of energy. By regulating both intracellular signalling and programmed cell death they are critical in the onset and progression of diseases. Surprisingly, their interaction with the surrounding environment, following failure of mitochondrial autophagy (mitophagy), remains just partially investigated limiting our capacity to detail conduits for pharmacological targeting and design innovative therapies.

In my talk I shall overview lines of research we have pursued to inform the biology and pharmacology mitochondria highlighting our ongoing work on the physical interaction between mitochondria and nucleus -actual sites of contacts which we named as Nucleus Associated Mitochondria (NAM).

The molecules involved in this route of communication will be illustrated together with the approaches devised to tackle and revert this mechanism of cellular maladaptation at the basis of chronic diseases.

ORAL SESSION VII "Biomembranes"

Rapamycin induces morphological and physiological changes without increase in lipid content in *Ustilago maydis*

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The evolutionarily conserved serine/threonine kinase TOR recruits different subunits to assemble the Target of Rapamycin Complex 1 (TORC1), which is inhibited by rapamycin and regulates ribosome biogenesis, autophagy, and lipid metabolism by regulating the expression of lipogenic genes. In addition, TORC1 participates in the cell cycle, increasing the length of the G2 phase. In the present work, we investigated the effect of rapamycin on cell growth, cell morphology and neutral lipid metabolism in the phytopathogenic fungus *Ustilago maydis*. Inhibition of TORC1 by rapamycin induced the formation of septa that separate the nuclei that were formed after mitosis. Regarding neutral lipid metabolism, a higher accumulation of triacylglycerols was not detected, but the cells did contain large lipid bodies, which suggests that small lipid bodies became fused into big lipid droplets. Vacuoles showed a similar behavior as the lipid bodies, and double labeling with Blue-CMAC and BODIPY indicates that vacuoles and lipid bodies were independent organelles. The results suggest that TORC1 has a role in cell morphology, lipid metabolism, and vacuolar physiology in *U. maydis.*

Coupling between ordered and disordered phases in asymmetric lipid bilayers studied by AFM-Force spectroscopy

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Cell plasma membranes have an asymmetric distribution of phospholipids with sphingolipids present almost exclusively in the outer leaflet of the bilayer, with consequences for the formation of lipid domains across the membrane. In this study, we explored domain formation when palmitoylsphingomyelin (SM) is incorporated into the outer leaflet of dioleoylphosphatidylcholine (DOPC)/cholesterol (Chol) supported bilayers through cyclodextrin (CD)-mediated lipid exchange, producing lipid asymmetry.

Phase segregation was assessed by AFM microscopy for different incubation conditions with CD-SM. We found that treatment with 5 mM CD-SM for 30 min produced liquid-ordered (*Lo*) domains when incubated at 37°C while more ordered domains were observed if the incubation was performed at 24°C. Less defined structures appeared with shorter times while high concentrations led to an almost continuous ordered phase in the outer leaflet.

In Force spectroscopy measurements, the force curves acquired in domains with *Lo* characteristics in the asymmetric bilayers showed two rupture events attributed to a liquid-disordered (*Ld*) proximal hemilayer that collapsed first at lower applied forces (~7.5 nN) and a *Lo* outer hemilayer that was punctured by the tip in a second rupture event at higher forces (~13 nN). The rupture depths of each event agreed with these considerations. The continuous *Ld* phase, on the other hand, showed curves with single rupture events at ~5.6 nN. The higher rupture force registered for the proximal *Ld* hemilayer in the asymmetric (*Lo*/*Ld*) domains (*Ld*~7.5 nN) compared to the continuous *Ld* phase having two opposing *Ld* leaflets (*Ld*~5.6nN) reflected a degree of interleaflet coupling when the ordered phase was present in the outer hemilayer. In addition, the mechanical properties (rupture force and Young's modulus) of *Lo* domains in asymmetric bilayers were similar to that of domains with two opposing *Lo* leaflets as measured in symmetric DOPC/SM/Chol bilayers.

These results support the idea that formation of sphingolipid-enriched ordered domains in the outer hemilayer can modify the physical properties of the inner leaflet of the plasma membrane. These coupling phenomena could play a relevant role in signal transduction across the membrane.

Design and biophysical characterization of 3 chimeric membranolytic antimicrobial peptides

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Although antimicrobial peptides have been described as an attractive alternative to conventional antibiotics, they have not yet been applied mainstream due to numerous challenges. The rational or semi-rational design of AMPs can help solve these issues and enable their use as new generation antimicrobial agents. In this study, we designed 3 chimeric peptides using previously reported potent antimicrobial peptides, namely pandinin-2, ascaphin-8 and maximin3 (isolated from an African scorpion, a North American tailed frog and a Chinese red-belly toad, respectively). Pandinin-2 and Ascaphin-8 are highly cytotoxic, while maximin 3 is not. After the multiple alignment of the three peptides we proceeded to design the 3 novel chimeric peptides, exchanging distinctive modules of each peptide.

Biophysical characterization exploring changes in membrane fluidity, release of fluorophore trapped in liposomes, light scattering and specific bioassays of the three chimeras showed interesting behaviors, in particular in liposome models mimicking different membrane types. All chimeras formed pores and modified membrane fluidity suggesting induced changes in lipid organization. Our results show that chimera 1 and 3 have a reduced activity on phosphatidylcholine-cholesterol (7:3) membranes, and thus, are membrane selective peptides with potential for clinical application. While chimera 2 was described as a super active peptide which shows no membrane selectivity, and that it has potential for only topical infections.

Physicochemical properties that determine membrane activity and selectivity of the antimicrobial peptide ascaphin-8

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The emergence of Multi Drug Resistant Pathogens has been one of the most urgent global problems over the last couple of decades, putting enormous strain on the health sector. One of the most promising alternatives to conventional antibiotics is the application of antimicrobial peptides. Ascaphin-8 is an antimicrobial peptide that was first discovered and described in 2004. It presents high activity towards clinically relevant microbial species such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The mechanism of action of this peptide until this study had not been elucidated. The hypothesis based on sequence homology to other frog skin antimicrobial peptides and biochemical properties suggests that ascaphin-8 most likely induces cell lysis affecting the cell membrane. Using liposome models to mimic different cell membranes and biophysical techniques such as liposome leakage experiments, membrane fluidity and dynamic light scattering, we demonstrated that ascaphin-8 is a pore forming peptide. We designed 3 variants modifying specific physicochemical properties to determine and describe the factors that influence the peptide´s activity and specificity, with the aim of improving its therapeutic index. A part from inducing membrane leakage, all peptides modified membrane fluidity, indicating possible changes in lipid organization. Concomitantly, we carried out antimicrobial assays against multi-drug resistant pathogenic bacterial strains to verify if the tendencies in the liposome models extrapolated to the more complexed natural lipid membranes.

Differential distribution of sphingolipids in the plant plasma membrane regions: Possible roles of glicosilinositolphosphoceramides

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The plasma membrane (PM) is essential for cell life, since it encloses the cellular content, handles transmembrane transport and perceives the external environment. Its structural organization involves the presence of nanodomains, which are enriched in sterols and sphingolipids. However, information on the precise content, distribution and functions of sphingolipids in plant PM and their domains is scarce. Hence, it is essential to describe the sphingolipidome of the PM and its domains in order to understand their functions in the membrane dynamics. Addressing this question in Arabidopsis leaves, PM were purified and their detergent-resistant membranes (DRM) were isolated. We identified and quantified the sphingolipids present in these membranes by HPLC-ESI-MS/MS. We approached the analysis using informatics tools consisting of univariate, multivariate and clustering analyzes to evaluate the distribution of the species among the membranes under study. Here, we detected and quantified 84 molecular sphingolipid species belonging to the four classes of sphingolipids: ceramides, hydroxyceramides, glucosylceramides, and glycosylinositolphosphoceramides (GIPC). Our results revealed that both types of membrane sources showed the same sphingolipid species. However, every membrane had a different distribution in terms of abundance and number of predominant species, indicating a differential assortment of sphingolipids. GIPCs were the most abundant class, where DRMs showed a 6-fold enrichment as compared to PMs. We identified the predominance of 7 GIPC especies in DRM compared to PM and these species showed saturated LCBs and VLCFA. This is consistent with the lipid raft model and supports the use of DRM as a preparation that collects the type of lipids that are present in ordered membrane domains. Recent works showed that GIPCs increase the thickness and electronegativity of model membranes. This abundance of GIPC species in the PM and in their domains suggest that they could be major participants in the biophysical properties of the membrane. GIPCs have been found to recognize microbial toxins and also exhibit the ability to sense salt through direct binding of Na^+. Thus, it is possible that the GIPCs clustered in nanodomains, may act as surface receptors to sense signals during biotic or abiotic stresses. Moreover, t18:0-based sphingolipid species have been implicated in the binding to specific proteins. It remains to be explored whether t18:0-GIPCs, which are enriched in the PM nanodomains to PM may recruit specific proteins as those involved in signal sensing.
The PM and DRM sphingolipidomes here described pave the ways for future sphingolipid research that contributes to the elucidation of their functional significance in plant membranes.

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Molecular dynamic insights of the interaction of ascaphin-8 and 3 variants in two different compositions of lipid model membranes.

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Important advances in the field of membrane active peptides have been made through in silico molecular dynamics (MD), accurately capturing peptide binding (generally through electrostatic interactions), folding (for example from an unordered state to alpha helix conformations) and insertion into lipid bilayers; all this at the atomic and temporal nano scale resolution. Thus, MD studies are currently one of the most popular strategies for getting insight on the molecular mechanisms of lipid-peptide interaction[1], pore forming peptides, and subsequently pore formation and pore dimension[2].

Ascaphin-8 an antimicrobial peptide that was first discovered and described in 2004[2] presents high activity towards clinically relevant microbial species such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*[3]. Thus far, there has been no reports on the description mechanism of action of this peptide. In fact, this is the first attempt to understand the factors that govern this peptide´s activity and specificity using MD simulations. Additionally, variants were designed to enhance activity and improve selectivity. In order to relate the activity changes in membranes of 3 ascaphin-8 variants with their organization and interaction with membrane lipids, MD simulations were performed.

The MD simulations were carried out as follows:1) the 3-dimensional structures of Aascaphin-8 and three variants were predicted using pep-fold3, minimized in Chimera, analyzed and visualized in PyMOL. 2) The systems were prepared using Charm-GUI, in POPC:POPG (8:2) and POPC:Chol (7:3) with a single monomer embedded in the lipid bilayer. Each system was solvated with TIP3P and 0.15M NaCl and the Charm36m forcefield was used. At least three simulations of each peptide were performed using the supercomputer Miztli from UNAM. The systems were minimized, equilibrated and the production ran in GROMACS 2019.6 for 250 ns at 305K obove the T_m, the pressure was maintained using semi-isotropic coupling. 3) The MD analyses included the changes in the secondary structure, radial diffusion coefficient, density and tilt angle. Preliminary results show that Ascaphin-8 and its variants can differentiate between lipid compositions of systems POPC:POPG and POPC:Chol and this could confer peptide selectivity.

Plenary Lecture IV

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Role of mitochondrial calcium in cardiac cell death

Dr. Elizabeth Murphy received her PhD from the University of Pennsylvania in Biochemistry, followed by postdoctoral studies in Physiology and then an assistant research professor at Duke University Medical Center. Before joining the NHLBI in 2006 as the head of the Cardiac Physiology Section, she was the head of the Cell Biology Group at the National Institute of Environmental Health Sciences. She became a Fellow of the American Heart Association in 2001 and a Fellow of the International Society for Heart Research in 2007; she received the NHLBI Award for Outstanding Mentorship in 2011. Dr. Murphy has authored or co-authored more than 200 papers and reviews. Dr. Murphy is a member of the American Heart Association-Council of Basic Cardiovascular Research, American Physiological Society, and International Society for Heart Research. She served as president of the International Society for Heart Research from 2016 to 2019. She serves as Deputy Editor of Circulation Research. She is also the North American Coordinator of a Leducq Transatlantic Network of Excellence on Targeting Mitochondria to Treat Heart Disease. She received the Peter Harris Research Achievement Award from the International Society for Heart Research in 2020.

Plenary Lecture V

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Physical properties of biological membranes and their implication in the activity and specificity of antimicrobial peptides

The lack of access to the information that is encoded in the lipid composition of a cell membrane was a great limitation to grasp, in its veritable proportion, the physiological relevance of the membranes and the lipids themselves. In the last decade, substantial advances have been made in the knowledge of the properties of membranes thanks to the development of biophysical techniques. There is also a better understanding of the elements that participate in lipid homeostasis thanks to genomics, proteomics and lipidomics. Currently, the challenge is to elucidate the vast information contained in biological membranes. The lipid composition and its interactions clearly involve a level of molecular organization comparable to a nucleotide sequence or the order of amino acids in a protein; sequences that in themselves encode the properties and physiological capacities of the macromolecule. In the case of the membrane, the lipids type, proportion, and the nature of their interactions between them determines the properties of the lipid membrane and their functions. In the study of antimicrobial peptides, their mechanism of action and development for application as new generation antibiotics, the use of "membrane models" has gained relevance by allowing the establishment of relevant physical parameters of the membrane, which are determinants in the insertion and specificity of peptides. The information obtained in these membrane models in most cases has been correlated with the loss or gain in the activity of peptides in cellular membranes, which are the target of these peptides.

SESSION 1 “My thesis in a video”

Section “Structure”

VIDEO 1.

Potential role of membrane-voltage on the structure and function of Dopamine Receptor D1 (DRD1)

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G-protein coupled receptors (GPCR) localized at the plasma membrane of cells are activated by extracellular ligands that trigger signaling pathways with different physiological functions. The DRD1 is a post-synaptic receptor that binds G proteins in order to trigger metabotropic signals such as the increase of adenylate cyclases activity and AMPc accumulation. DRD1 is a membrane protein with 446 amino acids, with seven transmembrane (TM) domains and eight topological domains, which are respectively divided into four extracellular and four cytoplasmic. Its molecular weight is 49 kDa, and its expression includes primarily neurons, but it’s also expressed in smooth muscle. The functions of DRD 1 include activation of adenylate cyclase activity, adult walking behavior, cellular response to dopamine and catecholamine stimulus and it has been recently involved as a stress factor associated with breast cancer.

On the other hand, recent discoveries have revealed that membrane voltage effects go beyond the regulation of voltage-gated ion channels (VGIC). Okamura et. Al (2018) have described the existence of a Voltage Sensor-containing Phosphatase (VSP) in the plasma membrane of cells that is able to regulate its enzyme activity through membrane voltage changes. This findings force us to re-evaluate the effect of membrane voltage on many other membrane proteins in which the transmembrane domains suggest either a sensor-like structure or at least voltage-sensitive amino acids that contain a charge and might react to voltage changes.

Taking the latter into consideration, we looked into the transmembrane domains of the DRD protein family in order to search for charged amino acids, i.e. glutamate (E), aspartate (D), lysine (K), histidine (H) and arginine (R). Until now, no study has evaluated the effect of membrane voltage on the structural and functional properties of DRD proteins.

Our search revealed that three transmembrane domains (TM 2, TM 3 and TM 7) contained charged amino acids. TM 2 had glutamate, aspartate and lysine at the 79, 94 and 90 positions respectively. TM 3 aspartate at the 112 position and the TM 7 contained two aspartate amino acids in the positions 323 and 345. Even if not a proper voltage sensor sequence was found, we believe that the presence of charged amino acids might cause structural or functional changes dependent on membrane voltage.

VIDEO 2.

A new perspective of Voltage Sensing Phosphatases' role in skeletal muscle contraction

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Voltage-Sensing Phosphatases (VSP) are a group of proteins first discovered in the marine invertebrate *Ciona intestinalis* and are now known to be widely expressed on different entities that range from unicellular organisms to higher vertebrates and in eukaryotic cells genome. VSP gene expression has been found in spermatocytes and ovaries, gastrointestinal and mesonephric epithelium, nervous system, and blood cells of several animal species, including humans. VSPs are conformed by a Voltage Sensor Domain (VSD), that shares molecular architecture with voltage-gated ion channels (VGIC), which consists of a NH2-terminal transmembrane region arranged in four helical segments: S1 to S4. A VSD-PD linker that possess a proximal VSP unique region (PVR) and a distal phosphoinositide binding motif
like COOH terminal portion that is critical for coupling between the VSD and the enzymatic region that contains a Phosphatase Domain (PD) and a C2 domain. It has been noted that the phosphoinositide phosphatase region of VSPs is also shared among Phosphatase and tensin homolog (PTEN) in humans and rodents (TPIP, TPTE and PTEN2).

VSPs are depolarization-activated phosphatases that play an important role in the regulation of ion channels, transporters, and receptors through the interaction with phosphoinositides (PIPs). When the membrane depolarizes, it induces motion in the VSD, activating the enzymatic region that will act on phosphatidylinositol-4,5-biphosphate [PtdIns(4,5)P2], phosphatidylinositol-3,4,5-triphosphate [PtdIns(3,4,5)P3], and phosphatidylinositol-3,4-biphosphate [PtdIns(3,4)P2].

There have been studies using VSP to elucidate the PtdIns(4,5)P2 dependent cellular function in excitation-contraction coupling of skeletal muscle, showing that Cav channels and ryanodine receptor activation requires PtdIns(4,5)P2. Nevertheless, there have been no further studies linking the VSP function in excitation-contraction of fast and slow skeletal muscle fibers. The objective of our study is to assess the effect of VSP depletion in the mechanical properties of isolated extensor digitorum longus (EDL) and soleus muscles. This will pave the way for further studies explaining the importance of VSP's voltage-sensitive enzymatic activity not only in tissues that express it, but also in PIPs function in biological membranes and cellular signaling in eukaryotes.

VIDEO 3.

Heterologous expression of *S. cerevisiae* aquaporins in breast cancer cells: A study of biomembranes and their implication in the regulation of cell volume.

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Aquaporins (AQP) are transmembrane proteins that are responsible for transporting water and small molecules through cell biomembranes, therefore, they play an important role in homeostasis. Currently, 13 aquaporins have been identified in mammals and different expression patterns of these have been related to diseases, mainly cancer (01). Recent studies have demonstrated the importance of the overexpression of AQP3, AQP5 and AQP1 in the proliferation, migration and invasion of breast cancer cells in different cell types in vitro (02, 03) and tumor growth in vivo (03). Why this overexpression occurs and how it impacts the cell cycle is still unknown.

Aquaporins have been identified in other non-mammalian organisms, such as *Saccharomyces cerevisiae*, which has only two aquaporins (AQY1 and AQY2), which are homologous to *Homo sapiens* aquaporins. In a study carried out in our laboratory it was determined that *S. cerevisiae* is not only capable of remaining viable after an osmotic shock by reducing its volume, but that it can also return to its normal state once osmotic conditions return to normal, recovering its volume (04). The adaptability of *S. cerevisiae* to osmotic changes may be due to its aquaporins, which in preliminary results obtained in the laboratory, present a differential role for the entry and exit of water from the cell.

The proposal of this work is a heterologous mammalian-yeast expression system, in which the impact of the exchange of aquaporins between mammalian breast cancer cells and yeast cells can be evaluated, how this could affect volume regulation, the cell cycle and the metabolic capacity of both cultures. The results obtained will help us to understand some more the role that aquaporins play in breast cancer and how it can be reversed or modified by replacing them with homologous aquaporins.

VIDEO 4.

Potential role of membrane-voltage on the structure and function of Frizzled Receptor 4 (FZD4)

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Frizzled receptors (FZD) belong to the G-protein coupled receptor (GPCR) family of the F class. They participate in Wnt signaling pathways including Wnt/beta-catenin and Wnt/Ca^{2+} cascades. FZD4 is the fourth of ten isotypes and contains 537 amino acids with a molecular weight of 59kDa. The cysteine-rich domain (CRD; aa 40-61) binds the Wnt protein and is followed by seven transmembrane domains from aa 161 to 221. FZD4 expression includes brain, ovary, liver, pancreas, colon, heart, skeletal muscle, endothelial cells, endometrium, bone marrow, prostate, spleen, and mammary gland. It is located at the plasma membrane of cells and can be internalized by endocytosis. Wnt pathways have been associated with the development of a variety of tumors including melanoma, acute myeloid leukemia, chronic myeloid leukemia, glioblastoma, prostate, mammary, and liver carcinoma. Therefore, findings involving the regulation of Wnt signaling cascades represent important targets for cancer classification and treatment.

Recent discoveries have revealed that membrane voltage effects go beyond the regulation of voltage-gated ion channels (VGIC). Okamura et. al (2018) have described the existence of a Voltage Sensor-containing Phosphatase (VSP) in the plasma membrane of cells that can regulate its enzyme activity through membrane voltage changes. These findings force us to re-evaluate the effect of membrane voltage on many other membrane proteins in which the transmembrane domains suggest either a sensor-like structure or at least voltage-sensitive amino acids that contain a charge and might react to voltage changes.

Taking the latter into consideration, we looked into the transmembrane domains of the FZD protein family to search for charged amino acids, i.e. glutamate (E), aspartate (D), lysine (K), histidine (H), and arginine (R). Until now, no study has evaluated the effect of membrane voltage on the structural and functional properties of FZD proteins. Our search revealed that four transmembrane domains (TM 1, TM
2, TM 4, and TM 6) contained the following aa residues; R213, K216, E217, D220, E252, R253, R272, H337, E338, E341, H343, H348, K358, R432, K436, and E458. Even if not a proper voltage sensor sequence was found, we believe that the presence of charged amino acids might cause structural or functional changes dependent on membrane voltage. This bibliographic and structural review represent the preliminary step towards deep modeling analysis and experimental confirmation of how membrane voltage may affect the function of FZD4 in Wnt signaling.

VIDEO 5.

Description of the action mechanism of the killer toxin K1 produced by *Saccharomyces cerevisiae* against *Staphylococcus aureus*

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In recent years we have observed an accelerated increase in antibiotic resistance mechanisms, resulting in multiresistant bacteria strains, for this reason we need to develop new alternatives to the use of antibiotics (2). One of these alternatives is the use of killer toxins. The first killer toxin was reported in 1963, it is produced by *Saccharomyces cerevisiae* (Somers and Bevan, 1969), subsequent to this discovery they have reported more killer toxins produced by yeast of many genera (1).

However, the study of the purification, inhibitory capacity, production and application against microorganisms with clinical and industrial importance have developed slowly since their discovery. According to the data obtained in our lab group, we have proved that the killer toxin *K*1 produced by *Saccharomyces cerevisiae* can inhibit the growth of one of the most clinical and food industry important bacteria; *Staphylococcus aureus*.

S. aureus is a Gram + bacteria, can produce enterotoxins, which has caused many sprouts of staphylococcal gastroenteritis in populations around the world, also is responsible of many cases of nosocomial diseases because it has many virulence factors (3).

After a bibliographic review about the killer toxins inhibitory mechanisms against sensible eukaryotic cell, we hypothesize that the *K*1 toxin can act against *S. aureus* by one this mechanisms: membrane damage, increase in membrane permeability to ions, use of a specific channel or receptor on the membrane (2). In this study we will prove through bioinformatic techniques, molecular biology experiments and microscopy, which is the possible molecular diana of killer toxin *K*1 and their mechanism by which it can inhibit the growth of *S. aureus*.

It its known that Killer toxins have a biophysics effect on diverses biomembranes and our analysis are based on this information, but these effects have been study in eukaryotic cells biomembranes, like yeast biomembranes or tumoral cells biomembranes, even we know the details about the interactions between the killer toxin and the sensible cells, while in prokaryotic organisms like bacteria, are not well studied yet, which is very important to develop new alternatives to treat infectious diseases and know more about this microorganisms.

VIDEO 6.

Effect of pH on *K*1 killer toxin: An update to the molecular mechanism of action

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Killer strain is the name given to a select group of yeasts, capable to produce a viral origin killer toxin. Nowadays, four variants of those killer toxins in *S. cerevisiae* (*K*1, *K*2, *K*28, and *Klus)* have been identified (Rodríguez-Cousiño et al., 2017). In the immature state, the *K*1 toxin is constituted by two subunits: α and β. The aim of this study was to validate the variables involved in toxin production and activity over the channel TOK1. Plate inhibition zone assays were performed to identify the role of the pH, media composition, and growth density. The presence of mRNA of *K*1 killer toxin was evaluated at different growth conditions (pH 4, 4.5, 4.7, 5, and 6, also YPD and inductor media), and cellular densities of the sensitive strain. It is shown that in those cultures grown under low pH (4.5-4.7) and inductor media, the production, activity, and the presence of the mRNA of *K*1 increases. Interestingly, at pH 6, the amount of *K*1 toxin was the highest, although the activity of the *K*1 was undetectable. Contrary to expectations, the activity of *K*1 over high and low cellular densities was very similar; suggesting that the amount of sensitive strain influences the toxin production. The present work shows an update to the previous molecular mechanism of action involved in the union of the *K*1 killer toxin of *S. cerevisiae* and its target, the two-pore domain outward-rectifier potassium channel TOK1 (Sesti et al., 2001). This actualization is based on the importance of the pH at the previous maturation step of pptox, in which, at pH near 6 the *K*1 pptox processing increases, reaching the maximum rate at 16 hrs.; during this period, the mature state of *K*1 (α β) links to the receptor 1-6-β-D-Glucan. The high cellular density triggers the acidification of the media (pH 4.5-4.7) by both killer and sensitive strains, and finally, under these acidic conditions, the *K*1 dimer α-β is separated, leaving the subunit α able to recognize and destabilize the channel TOK1.

SESSION 1 “My thesis in a video”

Section Respiratory Chains, Transport and Biotechonology

VIDEO 7.

Evaluation on inhibition effect of malonate prodrugs in bovine liver isolated mitochondrial succinate dehydrogenase

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Succinate dehydrogenase (SDH) is an enzyme that catalyzes the oxidation of succinate to fumarate in the Krebs cycle and a critical integral component of the electron transport chain. During ischemia, succinate accumulates and its oxidation by SDH drives reactive oxygen species production that underlies ischemia/reperfusion injury (I/R). Therefore, the inhibition of this enzyme represents a therapeutic potential for the treatment and prevention of damage due to reperfusion due to ischemia. Malonate-derived ester prodrugs, such as dimethyl malonate (DMM) and diethyl malonate (DEM), have been described as cell-permeable substances capable of inhibiting SDH. Despite the fact that these compounds have been used in various studies for the above purpose, no tests have been carried out to prove such an inhibitory effect at the mitochondrial level. Therefore, in this study, a comparison was made of DMM and DEM effect on the enzyme of isolated mitochondria; based on oxygen consumption and spectrophotometric measurement of the enzymatic activity, using the malonate as inhibitor 10 mM (control). The results obtained based on the measurement of the oxygen consumption of the succinate oxidase (Complexes II-IV) showed that the two substances studied did not act inhibitory at concentrations equal to those of malonate. In addition, the assays based on the reduction of Dichlorophenolindolephenol (DCIP) in a time course and the non-linear least squares adjustment, allowed to obtain kinetic parameters for traces without drug (C), with DMM and DEM. In turn, using molecular docking, it was possible to know the binding mechanisms of these molecules to the enzyme and relate this to their effects. Another important factor is the size of the acyl group linked by the ester bond of the two prodrugs, which is relatively small, so the esterases were unable of hydrolyzing these molecules so that they cannot bind to the malonate site of action into SDH. In conclusion, both DMM and DEM do not act as inhibitors while as non-essential activators of SDH. Therefore, the role they play in the treatment and prevention of ischemia/reperfusion must be reformulated.

VIDEO 8.

Looking for the mitochondrial uncoupling protein's function in the white shrimp *Litopenaeus vannamei*

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The white shrimp mitochondria have two functional uncoupling proteins LvUCP4 and LvUCP5. Both proteins are activated in the presence of free fatty acids, which promote an uncoupled mitochondrial state that results in increasing mitochondrial oxygen consumption and proton transport, a loss of ΔΨm, and ATP synthesis ceases. It has been proposed that in ectothermic organisms such as shrimp, these proteins regulate the mitochondrial production of reactive oxygen species (ROS) by uncoupling mitochondrial function. Thus, when shrimp are exposed to hypoxia/reoxygenation conditions, their UCPs activation could represent a physiological uncoupling mechanism of protection against ROS. In this study, we determined the role of LvUCP4 and LvUCP5 in the redox balance through their transcripts silencing. A preliminary bioassay was carried out with adult shrimp to determine the appropriate doses of double-stranded RNA (dsRNA) to be injected. In addition, the post-injection time of transcripts silencing was determined. A total of 36 juveniles of the species *L. vannamei* were injected intramuscularly with 15 and 30 µg/µL of dsRNA. After 12- and 24-hours post-injection, the organisms were sacrificed and the pleopods dissected. Results demonstrate that the uncoupling proteins present in the shrimp may be silenced using dsRNAs. Additionally, was demonstrated that silencing of LvUCPs generated changes in the levels of reactive oxygen species. This suggests that LvUCPs may be a mild uncoupling mechanism aim at preventing reactive oxygen species overproduction and oxidative damage in shrimp.

VIDEO 9.

Characterization of a chloroplast isoform of the mitochondrial calcium uniporter*

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Chloroplasts carry out bioenergetic processes through chemiosmotic mechanisms in similar ways to mitochondria. They have highly permeable stromal membranes and much less permeable thylakoid membranes. Thylakoid membranes harbor electron transport chain components as well as several ion and metabolite transporters. A recent report has unveiled the presence of a chloroplast isoform of the mitochondrial calcium uniporter relevant in metabolic and signaling reactions in plants upon drought stress. In this project, a calcium transport system was characterized in isolated chloroplasts of *Spinacea oleracea* and *Arabidopsis thaliana*, by means of an assay that allows us to measure the entry of this divalent cation indirectly, with the help of a fluorescent indicator. The experiments were implemented to establish the ideal conditions for the detection of a chloroplastic calcium transporter (cMCU).

The results obtained indicate the presence of a chloroplastic isoform of the mitochondrial calcium uniporter due to the sensitivity of this transport activity to Ruthenium Red.

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VIDEO 10.

Properties of the mitochondrial calcium uniporter complex in *Arabidopsis thaliana*

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Calcium ions regulate several processes of pathophysiological relevance in plants. Under optimal conditions, the spatial and temporal variations in the concentration of this cation affect plant growth and development. Calcium can also constitute an effective signal indicating either biotic or abiotic stress conditions. Under both conditions, calcium transport across the inner mitochondrial membrane of chloroplast envelope membrane plays a key role in the oxidative activity of these organelles. A recently identified family of six isoforms of the Mitochondrial Calcium Uniporter (MCU) mediate intramitochondrial and intrachloroplastic calcium transport in *Arabidopsis thaliana.* In addition, the transport regulator AtMICU1 constitutes a sensor controlling calcium access to MCU. Previous evidence has linked the presence of specific MCU isoforms with the resistance to drought conditions in *A. thaliana.* Plants lacking AtMICU1 have increased intramitochondrial calcium levels and deranged mitochondrial ultrastructure. Nevertheless, the reported evidence shows no obvious developmental phenotype in AtMICU1 knockout plants. Here we show a proposed MCU-AtMICU1 complex in addition to uncharacterized properties of *A. thaliana* plants lacking AtMICU1. Our results indicate that AtMICU1 is required for regulated intraorganellar calcium transport in isolated protoplasts and regulates seed development.

VIDEO 11.

Degradation of polycyclic aromatic hydrocarbons by yeasts.

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Pollutants such as polycyclic aromatic hydrocarbons (PAHs), like benzopyrene (BaP), are usually found in mixtures, they are ubiquitous and extremely toxic, so studying their effect on the physiology of various yeasts will allow them to be proposed as a mycoremediation strategy.In this work, the effect of BaP on *Candida albicans, Debaryomyces hansenii, Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* was evaluated. It was first found that temperature is a factor that affects their growth in the presence of BaP, in a range from 15 ºC to 37 ºC.After 10 days of growth in the presence of 100 ppm BaP, the four yeast strains were able to degrade more than 70 % of BaP without affecting their viability.

Using piperonyl butoxide (PPB), which inhibits cytochrome P450 monooxygenase (CYP), the enzyme complex responsible for the first stage of PAH metabolism, prevented BaP degradation in the four yeasts assayed. *DIT2* gene was identified by bioinformatics studies the as the one responsible for the metabolism of BaP and other PAHs in *Saccharomyces cerevisiae*; in this work, *DIT2* was inserted into the pYES2 plasmid to be overexpressed in the same strain, trying to obtain a robust strain that degrades PAH more efficiently. Using the *wild-type* of each, *Candida albicans, Debaryomyces hansenii, Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae,* only after a short incubation time, they were able to degrade on average 35 % of BaP, *S. cerevisiae DIT2Δ* strain, degrades about 8 % BaP and its complementation results in the recovery of the phenotype, while the strain transformed and overexpressing this gene, was able to degrade more than 40 % of BaP.

VIDEO 12.

Analysis of the interaction of *Wickerhamomyces anomalus* against phytopathogenic fungi that infect postharvest products

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The post-harvest loss of agricultural products is due to infections of phytopathogenic fungi that cause the rotting of fruits and vegetables. With climate change and globalization, post-harvest products are prone to structural damage as they reach the market and the consumer. Different chemical methods to control the growth of phytopathogenic fungi have been used, although with effects on human health. The analysis of the antagonistic interaction between organisms offers the opportunity to use this knowledge to elaborate new control strategies that are friendly to the environment and not toxic. To address this, we use three phytopathogenic fungi that commonly infect export fruits, such as avocado, pineapple, mango and cocoa, among others, and were confronted against *Wickerhamomyces anomalus*, a yeast of great biotechnological potential traditionally used in the agro-food sector, as a biopreservation agent, suitable to improve feed and food safety. The phytopathogenic fungi were *Thielaviopsis paradoxa*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. PDA plaque mycelial growth was compared between the phytopathogens and the antagonist. Yeast showed differences in the control of mycelial growth. During cocultures in liquid medium, the presence of the antagonist prevented the aggregation of phytopathogens. An analysis of the interaction in microcultures revealed the association of yeast with the surface of the filament of the fungi under study. The presence of the antagonist did not control the dispersal of fungal growth but did control the development of spores. These studies corroborate the capacity of *W. anomalus* as a biocontrol microorganism.

SESSION 1 "My thesis in a video"

Section ATP-synthases

VIDEO 13.

Modelling, docking and molecular dynamics analysis of the ζ subunit of the F_1F_O-ATP synthase from *Paracoccus denitrificans*

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The F_1F_O-ATP synthase is an ubiquitous nanomotor whose main function is the production of ATP. It does so by coupling the energy from an electrochemical gradient to the rotation of the central rotor of the enzyme. In the clockwise direction, the enzyme functions as ATP synthase, while counter clockwise (CCW) the enzyme works as a protein pumping ATPase[2]. In order to prevent this wasteful activity, several inhibitory proteins of the CCW rotation have evolved, similarly to the IF_1 and ε subunit in mitochondria and bacteria, respectively. Furthermore, a novel inhibitory protein has been described in α-proteobacteria, denoted as ζ subunit. This subunit has a different structure compared to the canonical inhibitors, although all of them inhibit in a similar way, introducing it's inhibitory domain in the $\alpha_{DP}/\beta_{DP}/\gamma$ interface. The ζ subunit's N-terminus, which is intrinsically disordered, works as the inhibitory domain by adopting an α-helix structure. Additionally, an ADP/ATP binding site of low affinity has been identified, and seems to regulate the inhibitory activity of the ζ subunit. This binding site diverges from other nucleotide binding sites previously described. Moreover, the binding of ADP or ATP causes conformational changes in ζ. In spite of this, a proper description of the binding site and how it regulates the zeta activity has not been elucidated. In this context, we want to characterize the nucleotide binding site of the ζ subunit of *Paracoccus denitrificans* (Pdζ) by performing docking and molecular dynamics (MD) on the Pdζ, thus seeking to identify the main residues involved during the nucleotide binding and evaluate the conformational changes related to the binding, and its relation to the biological activity of the protein. In summary, we have identified the residue fingerprint of the interaction between the nucleotide and Pdζ. Also, the MD shows that in the presence of the ligand, the conformational dynamics of Pdζ diverge from that shown in its absence.

VIDEO 14.

MPK6 revealed as a regulator of the plasma membrane H^+-ATPase MPK6 during cold acclimation

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Cold acclimation is a natural process developed by some plant species to withstand freezing temperatures. In the course of this process, multiple changes such as cell wall modifications, changes in plasma membrane (PM) composition, increase of cryoprotectants, osmolytes and antioxidants and cytoskeleton stabilization are carried out, preceded by signal transduction and gene expression changes. The PM H^+-ATPase is a key enzyme in plant cells since it generates an electrochemical proton gradient used for secondary transport and pH homeostasis. Its regulation is provided by several mechanisms included phosphorylation by some kinases, but no mitogen-activated protein kinases (MAPK) have been described so far. These are proteins involved in intracellular signaling pathways that respond to different stresses. MPK3, MPK4 and MPK6 are activated in the cold response. We have found that H^+-ATPase activity diminished during cold conditions in wild type (wt) plants, but this effect was lost in MPK6-lacking mutants. With the aim of elucidating the mechanism underlying the MPK6 role on the H^+-ATPase activity, we determined the gene expression of the *AHA1*, *AHA2* and *AHA3*, which encode the main PM H^+-ATPase isoforms, and the levels of the H^+-ATPase and 14-3-3 protein in MPK3 or MPK6 knock-out mutants. No absolute correlation was found between gene expression or proteins levels with the H^+-ATPase activity changes, neither in the wild type plants or the mutants. Alternatively, the influence of PM fluidity was investigated. Results from H^+-ATPase activity and PM fluidity of non-acclimated and cold acclimated wt, *mpk3* and *mpk6* plants were integrated in a Pearson plot. Acclimation increased the PM fluidity but diminished the H^+-ATPase activity in wt and *mpk3* plants. Notably, no changes were found in *mpk6* after acclimation. Therefore, we inferred that MPK6 is involved in a control of membrane fluidity that affects the activity of the ATPase. This opens the possibility of a new form of regulation of the enzyme via the lipid environment mediated by a MAP kinase cascade. In addition, other kinases but not MAPKs have been reported as H^+-ATPase regulators so far.

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VIDEO 15.

Expression of ATP6 synthase subunit from *Crassostrea gigas* facing infection and silencing of the Ostreid herpes virus type 1

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Pacific oyster *Crassostrea gigas* culture, has been introduced in several countries due to its rapid growth and great adaptation to environmental conditions. However, the production is highly vulnerable to infectious agents such as viruses, being the principal threat the Ostreid herpesvirus type 1 (OsHV-1), which can causes massive mortalities in a short time. Virus has the ability to alter host biochemical metabolism influencing the functionality of organelles, such as mitochondria. It is well known that mitochondria have their genome, that encodes proteins involved in processes such as electron transport and oxidative phosphorylation. One of the mitochondrial genes is the ATP6 gene, whose protein is part of F_0 domain of ATP synthase, participating in the translocation of protons from the intermembrane space to the mitochondrial matrix and vice-versa, for synthesis or degradation of ATP. To date, there is insufficient information to understand the pathophysiological effects that OsHV-1 causes in *C. gigas*. Therefore, it is important to use new techniques to counteract the effects of different viruses on their hosts, such as gene knockdown mediated by RNA interference. For this reason, and since some viruses can alter mitochondrial gene expression, affecting functions such as reactive oxygen species production and ATP synthesis, the absolute expression of the ATP6 gene in oyster tissue was evaluated by RT-qPCR. Changes in expression levels were observed in control, OsHV1- infected, and infected and silenced organisms with a shRNA targeting viral DNA polymerase, at different hours post-infection. Suggesting that OsHV-1 has the ability to modulate the expression of genes whose proteins participate at the mitochondrial level to replicate and proliferate, while silencing of the virus could allow recovery of mitochondrial function in surviving organisms.

VIDEO 16.

Phyletic distribution and natural history of accessory subunits e and g that participate in dimeric/oligomeric F_1F_O ATP synthase arrangements

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F_1F_O ATP synthase complex is largely conserved across bacteria, mitochondria and chloroplast. In mitochondria, this complex can be found as a dimer/oligomer formed through highly divergent accessory subunits. The dimers of mitochondrial ATP synthase are unique because they organize into long rows that induce membrane invaginations named cristae. However, a wide diversity at mitochondrial cristae morphology can be found among Eukaryotes.

Five different dimeric/oligomeric F_1F_O ATP synthase arrangements have been reported in eukaryotes: ovine, bovine and *Saccharomyces cerevisiae* (type I), *Chlamydomonas reinhardtii* (type II), *Paramecium tetraurelia* and *Tetrahymena thermophila (type III)*, *Euglena gracilis (type IV)*, and finally *Trypanosoma Brucei*. At each case, different subunit protein and lipids seem to be involved in dimer formation and oligomerization.

To get insights about how these subunits were recruited into the ATP synthase complex, we performed a phylogenetic analysis of accessory subunits e and g involved in dimer/oligomer type I stabilization. Amino acid sequences were retrieved either from Pfam (http://pfam.xfam.org/), or using BlastP searches against the complete genomes available at NCBI's RefSeq genome database (ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq). Progressive multiple amino acid sequence alignments were performed with ClustalX (http://www.clustal.org/clustal2/). Phylogenetic analyses were conducted using the MEGA_X (http://www.megasoftware.net).

Accessory subunit e and g were found only in eukaryotes; therefore, these proteins originated after the last eukaryotic common ancestor. Both subunits were found in animals, fungi, and some protists. Subunit g was found in some plants, but subunit e is absent in plants. Interestingly, the motive GXXXG and other key positions to stabilize dimer type I, are highly conserved. Results suggests a broad distribution of dimeric type I ATP synthase in animals and fungi. Different dimer/oligomeric arrangements of ATP synthase probably evolved independently several times through the evolution of eukaryotes. This work was supported by UNAM-PAPIIT grant IN218819.

VIDEO 17.

Evaluation of mutants lacking plasma membrane H^+-ATPase Pma1 or Pma2 from the corn smut basidiomycete *Ustilago maydis*

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Plasma membrane H+-ATPases of fungi, yeast and plants act as proton pumps to generate a proton electrochemical gradient essential for nutrients secondary transport and intracellular pH maintenance. *Saccharomyces cerevisiae* has two genes (pma1 and pma2) that coded H+-ATPases. The pma1 gene is the predominant one in the fungal physiological functions. Plants have a larger number of genes that code for proteins with structural and functional similarity to the previous ones. Ustilago maydis is a biotrophic basidiomycete that infects corn and teozintle, where the presence of two H+-ATPase-encoding genes UmPma1 (UMAG_02851) and UmPma2 (UMAG_01205) with high identity to fungi and plants, respectively, has been described. Unlike S. cerevisiae, these two genes are expressed jointly in U. maydis yeast. To study the implication of these enzymes in fungal metabolism and physiology, we generated strains lacking one of these two proton pumps (ΔPma1 and ΔPma2) and characterized with biochemical and microscopy assays. Mutant strains could survive with only one functional pump, even each enzyme showed different Vmax and KM values. Our results indicated that both H+-ATPases work jointly for the membrane potential maintenance and the electrochemical proton gradient generation.

VIDEO 18.

Importance of DELSEED loop electrostatic interactions in a peptide based on the thermophilic F1-ATPase b subunit

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ATPase has a very important role in cellular metabolism, it is responsible for the synthesis of adenosine triphosphate, which is a key molecule for most of cellular reactions. To understand the rotational mechanism during catalysis, many researches have focused on the role played by the interactions of b-g subunits in the torque transmission.1 At first, the mechanism proposed as responsible for the torque transmission was through the salt bridges that the highly conserved DELSEED loop forms with the g subunit.2 However, different studies showed that the specific electrostatic interactions between DELSEED sequence and the g subunit are not necessary for the torque transmission.3 Thus, the role of the charged residues in DELSEED loop still needs to be clarified. On the other hand, both the conformational changes and pH can induce differences in the protonation states and therefore in the charge density of proteins.4 As a consequence, these factors can cause attractive or repulsive electrostatic interactions to be favored.

In this work a peptide, called DEL, composed of the two a-helix connected by the DELSEED loop isolated from thermophilic organism Bacillus PS3, was used to tackle the role that electrostatic interactions have in its intrinsic flexibility. Through constant pH molecular dynamics simulations (pH-MD), it was possible to achieve a conformational equilibrium, as well as charge density probability of the protonable residues of peptide DEL. This research supports that inner flexibility is considerably affected by pH. Furthemore, the electrostatic potential of the DEL peptide exhibits a large difference compared when it is in the context of b subunit. This work can give notions of the relationship that the charges in DELSEED have with the neighborhoods of b subunit.

SESSION 2 “My thesis in a video”

Section Biomembranes & Biophysics

VIDEO 19.
Development of Refined Interaction Potentials for POPC Bilayer Simulations

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Severe fungal infections kill more than 1.5 million people per year worldwide. Currently, amphotericin B (AmB) is the most effective antifungal medication to treat serious fungal infections. The mechanism of action of AmB involves binding with membrane sterols, resulting in cellular disruption and death, thus affecting not only fungi, but also human cells, producing toxicity and serious side effects. Eukaryotic cell membranes are composed of phospholipid molecules [mainly phosphatidylcholine (POPC)], membrane proteins, and sterols such as ergosterol in fungi or cholesterol in mammals. Thus, finding a molecule as effective as AmB but highly selective towards ergosterol is one key milestone to design novel treatments for fungal infections. The Biophysics group of the Institute of Physical Sciences of the UNAM is focused on finding a similar molecule to AmB displaying specific selectivity for ergosterol and no toxicity to humans. Since sterols highly interact with POPC in the cell membrane, the aim of this thesis was to describe the properties of a POPC bilayer by using computational simulations. The resulting description contributes as a reference model that can be used as a foundation to perform simulations including either ergosterol or cholesterol, and to further include the interaction with AmB. Molecular Dynamics (MD) simulations were performed for POPC bilayers of 512 lipid molecules, hydrated with 22984 TIP3P water molecules, and no ions were added. MD simulations were performed with GROMACS version 5.1 calculating a trajectory of 10 ns length. The systems were subjected to energy minimization and then equilibrated to NTP at 150 K and 303 K. Semi-isotropic pressure coupling was carried out using the Berendsen and Parrinello-Rahman barostat for equilibration and for production cycling, respectively. The following properties were calculated; Area and Volume per lipid, Bilayer Thickness, Compressibility Modulus, and Order Parameters, which were compared with experimental data from literature. The novelty of the obtained system relies on the refinement of the force field parameters, resulting in reduced deviations between the calculated values and the experimental ones, validating the quality of the developed simulation model. Beyond, the established system resembles the liquid-crystalline phase of the bilayer, which is the one of actual biological relevance. The presented strategy demonstrates that performing simulations with the refined parameters offers results that are closer to the experimental values, therefore, to biological systems. Nevertheless, despite good results were obtained from fitting the quantum calculations, further refinement of the potential as well as longer simulations are needed to obtain a more reliable description of the bilayer.

Acknowledgements: DGAPA PAPIIT 100920 CONACyT FRONTERAS 74884.

VIDEO 20.

Interaction of Ovalbumin with DMPC and DMPC/sphingomyelin/Cholesterol bilayers

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In living systems, cells are separate from the external environment with a lipid bilayer. In this bilayer, phospholipids and proteins play relevant roles between the outside and inside the cell [1]. Understanding lipid-protein interactions membrane-related is fundamental to unpuzzle their relationship to the cell's functions, being the plasma membrane, a site where lipids and proteins interact. One way to study lipid-protein interactions is to know the energetic state of lipid membrane models. In this work, we present a calorimetric study exploring how changes in the properties of the membrane affect the interaction of proteins with the lipid bilayer. We used synthetic membranes models (large unilamellar vesicles, LUV) as an alternative to recover information about membrane systems. To this, we study the interaction of ovalbumin (OVA) with DMPC and DMPC/sphingomyelin/cholesterol (MSC) membranes by using Differential Scanning Calorimetry (DSC) [2] and Dynamic Light Scattering (DLS). Lipid composition, the size of liposomes -that implicate curvature changes-, and the membrane state are determinants to induce nonspecific lipid-protein interactions at the level of the surface of the bilayer. Cholesterol and sphingomyelin rule the thermodynamic properties and the cooperativity of lipid/protein membranes. Our results contribute to the understanding of the driven forces in the lipid-protein interactions membrane-related.

VIDEO 21.

Potential role of membrane-voltage on the structure and function of β1 adrenergic Receptor (β1AR)

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Introduction: Voltage Sensor Domains (VSD) confer voltage sensitivity to voltage-gated ion channels, in this domain, multiple amino acids residues with positive and negative charges play key roles in sensing electric field changes across the cell membrane and are known to regulate voltage-gated ion channels (VGIC). In the last decade the perspective of membrane protein regulation was widen, since the first Voltage Sensor-Containing Phosphates (VSP) was described in many species. This represent a new paradigm in which voltage membrane needs to be taken into account as a regulator of membrane protein function. G-protein coupled receptors (GPCR) represent one of the most abundant membrane protein family characterized by 7 Transmembrane domains (TMD). The β adrenoceptors (βAR) are part of the A family of GPCRs and are expressed in kidney cells and adipocytes, with the highest expression level in cardiac tissue. The family contains three isotypes. βAR mediate the activation of adenylyl cyclases, which convert ATP into cAMP that binds to PKA (cAMP-dependant protein kinase A). PKA then phosphorylates calcium channels to increase calcium influx. High intracellular calcium concentrations are needed for proper firing of sinoatrial and atrioventricular nodes to obtain a normal heart rate. In the kidney, βAR are involved in renin release and in adipocytes they upregulate lipolysis.

Objective: The aim of this work is to propose that membrane voltage may play a key role in the regulation of non-VGIC membrane proteins such as βARs.

Methodology: We reviewed the sequence of all TMD of the three beta adrenergic receptors looking for the presence of charged amino acids such as glutamate (E), aspartate (D), lysine (K), histidine (H) and arginine (R) if three or more charged amino acids were found in at least one TMD we then categorized the protein as: potentially voltage regulated.

Results: We found the β1AR to be the best candidate of our search and classified it as “potentially voltage regulated”. B1AR contains 477 amino acids and after analyzing all 7 TMD we found the following:

- TMD 4 (173-196): A**R**A**R**GLVCTVWAISALVSFLPILM
- TMD 5 (320-349): Q**K**AL**K**TLGIIMGVFTLCWLPFFLANVV**K**AF
- TMD 7 (353-477): P**DR**LFVFFNWLGYANSAFNPIIY

Conclusion: By identifying charged amino acids at the TMD mentioned above, we propose that β1AR is a good candidate to be analyzed by protein modelling and experimental approaches in order to determine the functional and structural effects of membrane voltage.

VIDEO 22.

Sporulation in *Saccharomyces cerevisiae* as a strategy to survive the selection by the *K*1 toxin: Follow up the killer effect by alteration of biomembranes through fluorescent probes.

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The killer phenomenon is widely diversified in yeast fungi, where from the production, and secretion of killer toxins by resistant strains, inhibition of the growth of non-immune strains is promoted. Today it is known that this phenomenon is the result of the persistent cytoplasmic coinfection of two double-stranded RNA viruses (M virus and L-A virus), with each of the phenotypes of yeast strains presenting autoimmunity to the toxin produced. The importance of understanding, and knowing the dynamics of interaction, and competition, as well as the evolutionary processes that underlie this biological system, has potential ecological implications, since killer yeasts are widely distributed in natural environments[3].

The objectives that compose this research are to generate fluorescent strains K^+ (42300; haploid *α*), and K^- (5x47; diploid *a*/*α*) of *Saccharomyces cerevisiae*, through the insertion of disruption *cassettes* directed to the URA3 gene, using the technique of PCR and homologous recombination, to achieve the expression of fluorescent proteins in the new mutant phenotypes. As part of the objectives, it also seeks to analyze with greater clarity the evolution process, and probable coevolution in culture, emphasizing the sporulation mechanism that we speculate has not only implications as a resistance strategy in the presence of the *K*1 toxin, but also which could also function as a coexistence and survival strategy by enabling recombination between *α* cells from sporulation of the K^- phenotype, and those from the division of the K^+ phenotype, resulting in recessive heterozygous cells for the sensitive phenotype, and therefore, heterozygous dominant to the killer phenotype.

VIDEO 23.

Interaction of *Pseudomonas aeruginosa* and the killer toxin produced by *saccharomyces cerevisiae*

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Pseudomonas aeruginosa is a gram negative bacteria, generally located in soil and aquatic environments, opportunistic and multiresistant (1, 2); it possesses toxin secreting systems and hydrolytic enzymes that can attack the host after infection and it is the cause of chronic lower respiratory tract infections contributing to complications in patients with cystic fibrosis causing significant deterioration of their pulmonary function having a significant impact on morbidity and mortality in these patients (1, 3). It has been reported that *P. aeruginosa* strains in patients with cystic fibrosis have the ability to recombine their genome with other strains not related to this disease, favoring their genetic diversity and favoring resistance to different antibiotics (1,3). *K*1 killer toxin is produced by different species of *Saccharomyces* and has been shown to have an antifungal effect against sensitive species of the same genus (4), having its main effect by direct interaction with the biomembranes. In our research group we have observed that this killer effect by *Saccharomyces cerevisiae* is equally efficient on different prokaryotic microorganisms of great biomedical importance, presenting variety in the action mechanism in each microorganism. Since *P. aeruginosa* represents an important multiresistant agent, it is of our interest to study the interaction of the killer toxin produced by *Saccharomyces cerevisiae* with *P. aeruginosa*, hypothesizing that it will generate an inhibition effect on the growth of *P. aeruginosa*. We expect to evaluate the inhibition time, a probable population recovery effect and the competitive effect between the killer toxin of *s. cerevisiae* and the toxins secreted by *P. aeruginosa*. Finally, we hope to be able to identify by microscopy the molecular interaction of the toxin with *P. aeruginosa*, hypothesizing that it may be through some molecular target or through membrane permeability.

SESSION 2 “My thesis in a video”

Section Oxidative Stress

VIDEO 24.

Effect of three different exercise protocols on lipid peroxidation and glutathione redox status in skeletal muscle of obese rats

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Obesity is a pathological state that deteriorates the body's functioning; skeletal muscle is a tissue that is severely affected by this condition. The accumulation of fat in the muscle eventually causes a malfunction, increased oxidative stress, mitochondrial dysfunction and muscle fatigue. Treatment with a regular exercise protocol has been documented to improve skeletal muscle function. At present, exercise has been implemented as an efficient method to maintain an optimal state of health, avoid cell damage, and improve the body's general functioning; the effects obtained from exercise will be largely dependent on the intensity, duration, and type of exercise. The work aimed to explore the best effect of three different exercise protocols on lipid peroxidation and glutathione redox status of the skeletal muscle of obese rats. Male Wistar rats of 250-570 g were used; 5 groups were managed: control, obese, obese with low-intensity exercise, obese with moderate-intensity exercise and obese with high-intensity exercise. Obesity was induced by a high-fat diet / 8 weeks and the exercise protocols were applied for 8 weeks at the three different intensities. Once the protocols were concluded and after the sacrifice, the skeletal muscles were extracted, which were homogenized and the lipid peroxidation levels were analyzed by the thiobarbituric acid reaction and the glutathione levels by the glutathione reductase enzymatic method. The results showed a decrease in lipid peroxidation levels with applying the three different exercise protocols to obesity. However, the low and moderate-intensity protocols were the ones that presented the best results, showing a decrease in the levels of 37.8 and 32.4% respectively, regarding the redox status of glutathione, low-intensity exercise was the one that presented the most significant effect, with an increase in levels of 223%. These results show that implementing an exercise protocol improves muscle function during obesity, with protocols ranging between low and moderate-intensity being more efficient when applied in this pathology.

VIDEO 25.

Evaluation of the physiological response of *Ustilago maydis* yeasts that accumulate melanin by random mutation

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During the Ustilago maydis ΔPMA1 proton pump deletion in the FB2 strain, a random mutation occurred in the 5' end and 3' end of *UmPMA1* gene. This mutation was morphologically characterized by TEM, showing large intracellular electrodense bodies suggesting melanin accumulation. Cells carrying the mutation showed brown colonies, and when they were in solution, the media becomes brown after six days of culture. The intracellular aggregates were extracted by n-butanol: acetic acid: water (70:20:10) solution; the U.V, and red spectra were obtained demonstrating the same spectrum of commercial Eumelanine. The physiological response of these cells containing melanin accumulation was compared with the wild-type strain. The growth of the wild-type (FB2) and mutant strain (ΔPMA1), reached the exponential phase in the first 12 h of the culture with rates of 0.281 ± 0.004 and 0.221 ± 0.003 h^{-1} with duplication times of 2.46 and 3.13 h for FB2 and ΔPMA1, respectively. Glucose consumption did not show a significant difference during the growth of the strains. The analysis of proton pumping showed a significant difference, due strain ΔPMA1 pump more protons than the wild strain. In oxygen consumption, there were no significant differences in both strains. The analysis of osmotic, oxidative, and nitrosative stresses showed no significant difference as compared to the wild-type strain. To evaluate the production of melanin in the mutants, Tyrosinase activity was measured in the crude extract of wild-type, and mutant cells using L-Dopa as substrate. In cells that accumulated melanin, the tyrosinase activity was double concerning the wild-type cells. Our results suggest that the presence of melanin is in response to aleatory mutation with no changes in the physiologic response, helping cell functionality due to its multifunctional character against oxidizing agents.

VIDEO 26.

Moringa oleifera glucosinolates prevent the aggregation of maturase K and ovalbumin through their interaction by glycosylation

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Protein aggregation is a phenomenon by which proteins, under different types of stress, adopt conformations rich in beta sheets, which promotes the formation of amyloid structures, which are present in various pathologies. Among the factors that can favor this phenomenon are oxidative stress and some types of electromagnetic radiation, which, through limited protein denaturation, increase the formation of aggregates, in addition to promoting protein glycation. *Moringa oleifera* has been studied for its multiple medicinal properties, and, in addition, among its phytochemicals there are different molecules such as glucosinolates capable of binding by glycation to proteins serving as protective molecules of these. Using FoldAmyloid and Amylpred2 servers, the regions responsible for the aggregation of various animal and plant proteins were determined. The results indicate that ovalbumin has 13 regions in its amino acid residue sequence as potentially amyloidogenic and maturase K presented 28 regions. Therefore, it was decided to use these proteins to evaluate the inhibition capacity of amyloid aggregates by glucosinolates from the *M. oleifera* extract.

Both *M. oleifera* maturase K and ovalbumin were subjected to oxidative stress and microwave radiation to reach an aggregated or amyloid state, and the formation of these aggregates was quantified by means of the thioflavin T fluorescence signal. The analyzes showed that both tert-butyl hydroperoxide and microwaves are effective methods to induce aggregation regardless of the protein nature, in addition to the fact that the samples in the presence of *M. oleifera* glucosinolates were able to inhibit the formation of up to 65%. The analysis by SDS-PAGE showed how the banding pattern of the samples is modified, forming bands of high molecular weight. Our results suggest that *M. oleifera* glucosinolates are molecules that protect proteins against oxidative stress conditions that can trigger the aggregation phenomenon. In addition, through molecular modeling, a glucosinolate binding site was found in maturase K, which prevents protein aggregation when exposed to microwave radiation.

VIDEO 27.

Effect of Apocynin on Oxidative Stress in Diabetic Rat Hearth Muscle

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Diabetes mellitus is a group of hyperglycemic syndromes resulting from the malfunction of the β-cells of the pancreas and decreased insulin sensitivity, which can damage cardiac tissue. In a hyperglycemic state, the generation of Reactive Oxygen Species (ROS) and oxidative degradation of Lipids increase. Apocynin, an active isolated from the plant Pricorhiza kurroa, is considered an antioxidant agent because it inhibits NADPH oxidase activity.

In this work, we analyzed the influence of apocynin on oxidative stress in cardiac muscle and insulin sensitivity in Wistar strain male diabetic rats. Using 4 groups: Control (C), Control+Apocynin (C+A), Diabetes (D), Diabetes+Apocynin (D+A) and once the disease was established, treatment with apocynin (3 mg/kg/day) was started for 8 weeks. Metabolic biomarkers, insulin resistance curve and cardiac tissue homogenates, oxidative stress were monitored. The results showed a significant decrease in the glycemic levels of the D+A group and the levels of ROS and Lipoperoxidation, also improving insulin resistance.

In conclusion, apocynin has significant effects on glycemic levels and insulin resistance, reducing oxidative stress by decreasing ROS concentration and Lipoperoxidation in cardiac tissue.

VIDEO 28.

Biochemical and genomic study in response to oxidative stress mediated by arsenic in the extremophile strain staphylococcus ARSCP-1

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Arsenic (As) is a widely distributed element in the earth's crust that has constant interactions with biota. Most of the prokaryotes (mainly the extremophiles) have developed the ability to metabolize and resist As using different mechanisms, highlighting the reduction of arsenate [As (V)] to arsenite [As (III)] that allows the expulsion of As out of the cell. It even turns out to be a metabolizable compound used as a terminal electron acceptor in some microorganisms. For all the above, the present project aims to evaluate the resistance mechanisms to As and the response to oxidative stress caused by exposure to arsenic in the polyextremophilic bacterial strain *Staphylococcus*. ARSC1-P isolated from the crater lake of the “El Chichón” volcano. Genomic and metabolic approaches were carried out to determine the As resistance mechanisms and how they are influenced by the oxidative stress generated.

The genome of the *Staphylococcus* ARSCP-1 isolate was sequenced, being taxonomically assigned as *Staphylococcus hominis*, additionally antibiotic resistance genes were identified such as: amoxicillin, ampicillin, penicillin, gentamicin and As resistance genes (arsABCDE). This strain can tolerate concentrations up to 200 mM As(V) or 1mM As(III). The IC50 for As(V) of the strain is up to 10 times higher in anaerobic conditions (382 mM) than in aerobic conditions (36 mM), an unusual response in microorganisms that has not reported the use of As (V) as an terminal electron acceptor.

Inverted vesicles of cells from *Staphylococcus* ARSCP-1 were prepared and the effect of As (V) on ATP synthesis was evaluated. Surprisingly, an increase in ATP production rate was determined in the presence of As (V) A $K_{0.5}$ = 3.33 mM was obtained for As (V), and a Km for ADP of 0.8mM. Several authors have determined in bacteria of the genus *Staphylococcus* the activity of the assimilatory enzyme arsenate reductase that is characterized by the ability to detoxify the element by expelling it from the interior of the cell. However, the presence of a non-assimilatory metabolism of As for the formation of ATP has not been reported so far; where As acts as a terminal electron acceptor in bacteria of the genus *Staphylococcus*. In this work, it is suggested that the reduction of As (V) possibly by the quinol pool generates a change in the delta pH of the isolated vesicles and a possible mechanism of energy production that allows a greater tolerance under anaerobic conditions.

VIDEO 29.

Study of the mitochondria-sarcoplasmic reticulum association in cardiomyoblasts under stress: A possible sulforaphane-induced reductive stress

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Sulforaphane (SFN) is found in broccoli, this molecule confers protection against oxidative stress-induced damage through the induction of Nrf2, a factor intimately related to the activation of the antioxidant response. Associations between mitochondria and the sarcoplasmic reticulum [SR, a.k.a mitochondria-associated SR membranes (MAMs)] are structures that regulate several functions in the cell, including the management of misfolded proteins and oxidative stress[1,2]. Dissociation of these structures has been linked to the establishment of different pathologies, such as cardiovascular diseases[1]. Importantly, in models of oxidative stress, MAMs have a dual role as they can either increase or decrease the associations reflecting different cellular responses[3]. Therefore, we investigated the possible preservation of mitochondria-SR contacts in cardiomyoblasts pretreated with SFN [2.5 µM for 1 h] and subjected to hydrogen peroxide (H_2O_2, 25 µM for 1 h]. We monitored the cell viability of the H9c2 cell line and determined the contacts between both organelles by electron and confocal microscopy, furthermore we evaluated oxidative stress and ER stress under the same experimental conditions.

Our results show that pretreatment with SFN at 2.5 µM decreases the distance and colocalization in both organelles compared to H_2O_2-treated cells, contributing to the decrease in oxidative stress. Regarding ER stress, immunodetection of activating transcription factor 4 (ATF4) showed a significant increase in cells treated only with SFN and in the SFN+H_2O_2 cell group, suggesting that SFN *per se* increases ER stress from early times and thus induces reductive stress, a scenario that has been described in other models[4]. When we evaluated exposure to different times of SFN at the same concentration, we appreciated that the proteins glucose-related protein 94 (GRP94), ATF4 and Ero1α increased progressively in a time-dependent manner. Our data suggested that, on the one hand, MAMs need a fine-tuning of the redox state to establish positive communication and, on the other hand, that SFN is a molecule with the capacity to induce reductive stress which is an important point to consider in the field of contactology.

VIDEO 30.

Loss of cellular anthocyanin agglomerates is associated with the Red Drupelet Reversion disorder in blackberry fruit

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Red Drupelet Reversion (RDR) in blackberry is a physiological disorder where numerous fruit drupelets regress to red from black color during post-harvest handling. The specific chemical or biochemical mechanism causing RDR is still unknown. Recent studies have revealed that RDR induced by mechanical damage was not linked to changes in anthocyanin content, but it has been suggested that this disorder could be caused by a loss of cellular anthocyanin agglomeration and a subsequent break in the interaction between anthocyanin and other cellular compounds. Based on this notion, the main goal of this study was to evaluate the relationship between cellular anthocyanin agglomeration and RDR disorder in blackberry. Blackberry clamshells *cv*. 'Tupi' (170 g) were subjected to vibration (10 Hz and acceleration of 0.5 g) during 5 min to induce RDR. Then, reverted and no reverted drupelets were separated and subjected to protoplast isolation by cell wall digestion with cellulases and pectinases. Protoplast of reverted and no reverted drupelets were observed in a light microscope, and intensity of fluorescence emission was analyzed by fluorescence microscopy. Results revealed that reverted fruit possessed anthocyanins dispersed within the cells; in contrast, no reverted fruit held anthocyanins in spherical agglomerates. Also, the anthocyanin fluorescence emission was reduced in reverted drupelet. This suggest that blackberry mechanical damage induced by vibration could break the cellular anthocyanin agglomerates decreasing anthocyanin fluorescence emission and changing anthocyanin color from black to red. To confirm this idea, no reverted protoplasts were subjected to sonication treatment. With this experiment was induced the loss of anthocyanin agglomeration; fluorescence and color analyses revealed that sonicated protoplasts show an anthocyanin emission fluorescence and color like reverted drupelets. These results suggest that blackberry RDR is associated with the loss of cellular anthocyanin agglomeration.

VIDEO 31.

Bioinformatic and Experimental Analysis of Two-Component Systems that Regulate Protease Genes Expression in Clinical Isolates of *Serratia marcescens*

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Serratia marcescens is an opportunistic pathogen with intrinsic resistance to multiple antibiotics. *S. marcescens* expresses several virulence factors, being secreted proteases particularly crucial during host interaction. To date, the metalloproteases PrtS, SlpB, SlpC, SlpD, and SlpE have been described among *S. marcescens* strains. There are few studies that address the regulation of this enzymes. However, what is known is that gene expression of some of these proteases is negatively and positively regulated by the two-component systems, CpXAR and EepSR, respectively. CpxA and EepS are membrane embedded sensor histidine kinases that become autophosphorylated in response to an unknown stimulus. Then, CpxA and EepS transfer the phosphate group to the cytosolic transcriptional regulators CpxR and EepR, respectively, leading to altered expression of its target genes.

In this work, we characterize three clinical isolates of *S. marcescens* (EB042, SM002 and HU1848), previously identified as highly proteolytic activity producer strains. By using genomic DNA from strains EB042, SM002 and HU1848 as template, the different protease encoding genes were PCR amplified and sequenced. We found that protease genes of strains EB042, SM002 and HU1848 are highly conserved compared to reported *S. marcescens* strains. The activity of secreted proteases was also evidenced by zymogram gels. Moreover, protein identity of SlpD and PrtS was confirmed from supernatant samples by LC-MS/MS. In addition, through RT-PCR assays, we found that in SM002 and HU1848 strains, *slpE* and *eepR* transcripts were over-expressed. While a low abundance of negative regulators of proteases expression such as *cpxR* and *hexS* (a new regulator gene), was revealed. On the other hand, we defined putative DNA-binding motifs by the transcriptional regulators, CpxR (16 bp) and EepR (11 bp) using the bioinformatic tool MEME. Generated motifs were incorporated into FIMO to scan *S. marcescens* SmUNAM836 and U36365 genomes. A total of 369 and 1000 hits were obtained for CpxR and EepR motifs, respectively. These hits were manually curated leading to 89 and 125 possible regulated genes for CpxR and EepR, respectively. Among the results, there were genes previously reported to be regulated by CpxR such as *mdtl, ybdG, metN, udK, phoP*, stress response proteins, and a serralysin precursor. Few reports have described genes regulated by EepR, but our results showed a possible regulation of genes involved in the synthesis and export of virulence factors and secondary metabolites, a well-known characteristic of this response regulator. Also, using the EepR motif we found a protein with a putative metalloprotease function and a metalloprotease transporter. Interestingly, *degP*, a well-described protease/chaperone regulated by CpxR, was one of the EepR results. Other possible regulated genes to highlight are membrane modification proteins, efflux pumps and porins, antibiotic modification enzymes and biofilm.

Overall, our data suggests that increased proteolytic activity displayed by evaluated strains is associated with dysregulation/activity of the transcriptional regulators, CpxR and EepR. Evaluation of its contribution to other phenotypes including biofilm formation and antibiotic resistance will be a key point to understand the virulence of *S. marcescens* clinical isolates.

SESSION 2 “My thesis in a video”

Section Chronic Diseases

VIDEO 32.

Characterization and proteomic analysis of extracellular vesicles derived from cervical cancer

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Background: Cervical cancer is a pathology with a high incidence worldwide and is one of the main causes of death in women. Extracellular vesicles (EVs) are particles released naturally by cells and are delimited by a membrane formed by a lipid bilayer. Tumor-derived EVs play a critical role in intercellular communication through the transport and exchange of biomolecules such as proteins, contributing to tumor development. Evidence indicates that HPV E6/E7 oncoproteins can modify the protein content of EVs. However, the total protein content of EVs produced by cervical cancer cells has not been described.

Methods: HeLa cell line EVs were isolated from the culture supernatant using the miRCURY exosome kit. EVs morphology and molecular characterization were examined through transmission electron microscopy, western blot and mass spectrometry analysis. Gene ontology analysis was performed on the proteins identified in the EVs using the FunRich software. Pathways and processes involving these proteins were identified through the Kyoto Encyclopedia of Genes and Genomes (KEGG) and REACTOME databases.

Results: EVs isolated showed a spheroidal or cup-shaped morphology and the presence of a lipid bilayer. The EVs marker proteins Hsp90, Alix and Flotillin-1 were identified in HeLa cell EVs. Calnexin protein was not present in EVs, showing that there was no contamination with cell debris. We identified 364 cellular proteins in HeLa cell line EVs, however, we did not identify viral proteins. In addition, we report the presence of serine protease 56 protein that had not been previously reported in EVs. Our results showed 17 proteins involved in cancer signaling pathways such as NOTCH1, WNT, PI3K/AKT, MAPK, EGFR and ERBB2; and 20 proteins involved in processes such as proliferation, apoptosis avoidance and angiogenesis.

Conclusion: Our data suggest that the membrane vesicles released by cervical cancer cells contain proteins associated with tumorigenesis and could play an important role in tumor progression through the transfer of this content.

VIDEO 33.

Detection of mitochondrial DNA and its relationship with pro-inflammatory cytokines in plasma from children with obesity and metabolic syndrome

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Introduction: It has been described that excess in fatty acids increases ROS generation in obese (Ob) and metabolic syndrome (SM) conditions (1). The high ROS levels oxidize easily mitochondrial DNA (mtDNA) and fragment it. To this respect, it has been reported that small fragments which correspond to MTND3 and MTCO1 genes can escape to the cytosol through the mitochondrial permeability transition pore (2) and subsequently to plasma (3) where can act as DAMP and bind to Toll-like-9 receptor on circulating leukocytes, for activating NF-κβ which promotes the synthesis of cytokines e.g. IL-1β (4). On the other hand, mtDNA can also bind to NLRP3 receptors to form the inflammasome which activates caspase-1 and, in turn, promotes the release of IL-1β, leading a chronic inflammatory response (5). **Objective:** To identify the presence of mtDNA in plasma and relationship it with pro-inflammatory cytokines levels in a cohort of children with obesity and metabolic syndrome. **Material and Methods:** 40 children with Ob and 40 children with SM were included in the study. DNA oxidized (8-OH-dG) was measured by ELISA. Whole plasmatic small mtDNA fragments (MTND3 and MTCO1) was detected by qPCR and large mtDNA fragment (intact mtDNA) by end-point PCR and quantified with Picogreen. Intact mtDNA were normalized with small fragments to evaluate its integrity. Cytokines were measured with flow cytometry. ANOVA and Spearman correlations analysis were performed in Prism software. $p<0.05$ was considered statistically significant. Approvals were obtained from the Ethics and Research Committees of the School of Medicine Tecnológico de Monterrey. All legal guardians gave their written informed consent. **Results:** A significant increase in 8-OH-dG levels was observed in the Ob and SM groups compared to the control group ($p = <0.001$). The presence of greater damage was observed in the Ob, SM 3F and SM 4-5F groups ($p = <0.0001$). A positive correlation was identified for MTCO1 with IL-18 ($r=0.397$, $p=0.0057$), IL-23 (0.313, $p=0.0245$), IL-33 ($r=0.2864$, $p=0.0366$) in the Ob group. In SM 3F MTCO1 had a positive and significant correlation with IL-1β ($r =0.4776$, $p= 0.0367$), IL-8 ($r=0.5594$, $p=0.0034$), IL-18 ($r=0.332$, $p=0.0421$), IL-33 ($r=0.365$, $p=0.0280$). Finally, MTCO1 fragment had a significant correlation with IL-8 ($r = 0.6000$, $p = 0.0367$) and IL-17 ($r = 0.7333$, $p = 0.0156$) in the SM 4-5F group. **Conclusion:** Obesity and MS lead to damage and fragmentation of mtDNA, which may have directly related to the activation of the immune system.

Acknowledgment: Funded by the group of Medicina Cardiovascular y Metabolómica, Tecnologico de Monterrey.

VIDEO 34.

Effect of NaCl, Diazoxide, and Exercise on Blood Pressure and Skeletal Muscle Function

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Muscle tissue represents about 40% of total body mass and muscle activity is one of the main determinants of global metabolism in basal and active states. Excess sodium plays a fundamental role in increasing blood pressure, causing endothelial dysfunction through angiotensin II. In turn, this triggers a series of responses that reduce the functionality and the percentage of skeletal muscle mass. On the other hand, diazoxide is a potent vasodilator and is considered a KATP channel opener. These channels enhance muscle contraction. Exercise leads to vasodilation and mediates endothelial dilation, thereby improving muscle contraction and metabolism. Wistar rats were subjected to different treatments to establish experimental protocols that significantly affect blood pressure and skeletal muscle performance. The dose to administer diazoxide was 35 mg/kg/day for 14 days. Rats received 8% NaCl orally. For the exercised groups, a medium-intensity exercise protocol was used. The groups were monitored for weight and basal glucose during the eight weeks of the protocol. Blood pressure was measured in conscious animals every two weeks. The soleus muscle and Extensor digitorium longus were dissected to be taken to the tension register and determine the muscular strength. As a result, an increase in the diastolic and systolic pressure was observed in the NaCl group and a decrease in the other groups. It was observed that there is better muscle contraction and greater resistance to fatigue in the groups with diazoxide, exercise, and exercise + diazoxide, an adverse situation that occurs in the group treated with NaCl. With this, we can determine which experimental protocols improve skeletal muscle performance and blood pressure.

VIDEO 35.

Short-term training leads to improvement of mitochondrial network organization in the subsarcolemmal region of skeletal muscle fibers in obese rats

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Obesity and metabolic syndrome (MS) are related to a significant reduction in mitochondrial quality. Evidence suggests that both conditions lead to unbalanced fusion and fission, the main events of mitochondrial dynamics and this is associated with alterations in mitochondrial function, mainly in highly energetic tissues such as skeletal muscle. In the treatment of metabolic disorders, adaptations resulting from moderate-intensity exercise stand out, considered to be regulated by AMPK.

This project aimed to determine changes in mitochondrial function and distribution of the two main mitochondrial subpopulations (SSM and IMF), as well as the modifications in expression of the main regulators of mitochondrial dynamics, biogenesis and AMPK content and activity in a murine model of obesity after short-term moderate exercise.

All procedures were approved by the animal use and care committee (Protocol #2019-007). 12 weeks old male Zucker fa/fa rats were randomly divided into a sedentary group and an exercise group (n=4/group). The exercise consisted of 4 weeks of swimming training for 60min/5days/week. After 48 hours of the last exercise bout, animals were euthanized and gastrocnemius and soleus muscles were isolated. Data are expressed as the mean ± standard error; unpaired t-test was performed for comparison between groups.

After four weeks of swimming training, a significant increase in fission was evidenced by changes in phosphorylation of Drp1 and AMPK (1.4 and 1.3, $p<0.01$). Subsarcolemmal mitochondria showed a more organized network in comparison with the sedentary group ($p=0.04$) and a significantly higher myofiber area ($p=0.03$) was observed, a change associated with increased biogenesis in gastrocnemius muscle. In soleus muscle, significant changes in Drp1 and Opa1 mRNA levels were observed.

An increase in fission regulated by AMPK might be segregating damaged mitochondria and enhancing its removal while activating mitochondrial biogenesis to ensure restoration of mitochondrial mass by generating a healthier population in the subsarcolemmal region. These results suggest a differential response between a muscle with a predominantly glycolytic metabolism (gastrocnemius) and a muscle with a higher oxidative metabolism.

VIDEO 36.

Mitochondrial adaptations in cadmium toxicity-mediated insulin resistance in the islets of Langerhans

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Insulin resistance a pathology whose prevalence has increased significantly has been associated recently by cadmium exposure. Previously we and others demonstrated that insulin resistance by hypercaloric diets triggers alterations on mitochondrial bioenergetics which in turn affect mitochondrial morphology. On insulin resistance by cadmium exposure oxidative stress and inflammation play a vital role on development of pathology. The islet is known by its reduced antioxidant defense capacity, so seems to be an interesting target to study mitochondria because especially in the islet, the regulation of metabolic pathways and bioenergetics its vital to the adequate insulin secretion. The objective was to evaluate the mitochondrial life cycle and function in Langerhans islets of rats with insulin resistance induced by cadmium exposure. Wistar male rats were used divided into: NCD Group (LabDiet 5012 and *ad libitum* drinking water), and Cd Group (LabDiet 5012 and *ad libitum* drinking water with $CdCl_2$ 15ppm). The treatment was given for three months, then oral glucose tolerance and insulin response curves, in vitro insulin release, western blot for mitochondrial life cycle proteins, TEM analysis and study of the activity and expression of mitochondrial complexes and supercomplexes were made. The results show the presence of hyperglycemia hyperinsulinemia, that corroborate the presence of RI. In the mitochondrial morphology analysis, a less mitochondrial number accompanied by an increase of Pink1 and Parkin expression was observed in the Cd group as indicative of possible mitophagy, further it was observed evidence of fission by increase in Fis1 and Opa-1 (s) isoform. Opa-1 also mediates the cristae junction width (CJW) and respect to NCD group it was observed an increase in CJW in Cd group. Finally, it is well known that CJW impacts on mitochondrial transport chain conformation and Supercomplexes association, in this case a major presence of Supercomplexes I+III_2+IV and III_2+IV was observed even with a reduced activity if complexes I and III_2. Results show that important adaptations take place at the level of the mitochondrial internal and external morphology and the functional organization of the same, emphasizing that even in the presence of such adaptations the insulin biosynthesis and secretory capacity of islets of Langerhans remain functional.

VIDEO 37.

Dynamics of A2A adenosine receptor modulated by different membrane mimetic systems.

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G-protein-coupled receptors (GPCRs) are cell surface membrane receptors that transduce key extracellular signals into the cell to regulate different physiological functions, this renders them important drug targets. The A_{2A} adenosine receptor (A_{2A}AR) has been extensively studied by X-ray crystallography, Cryo-EM and NMR. All these previous studies have used different membrane mimetics (detergent micelles and lipid bilayer nanodiscs). Since a previous study has shown that a strategically placed fluorine probe near the intracellular side of TMVII can render well resolved peaks in ^{19}F-NMR spectra (1), in this work we continue this line of research and now test different membrane mimetic systems to analyze how A_{2A}AR conformational ensembles are modulated by their environment in the presence of different ligands. Here we show that ^{19}F-NMR has such sensitivity that it allows us to detect the modulation of the different membrane mimetic systems on the conformers and dynamics of A_{2A}AR.

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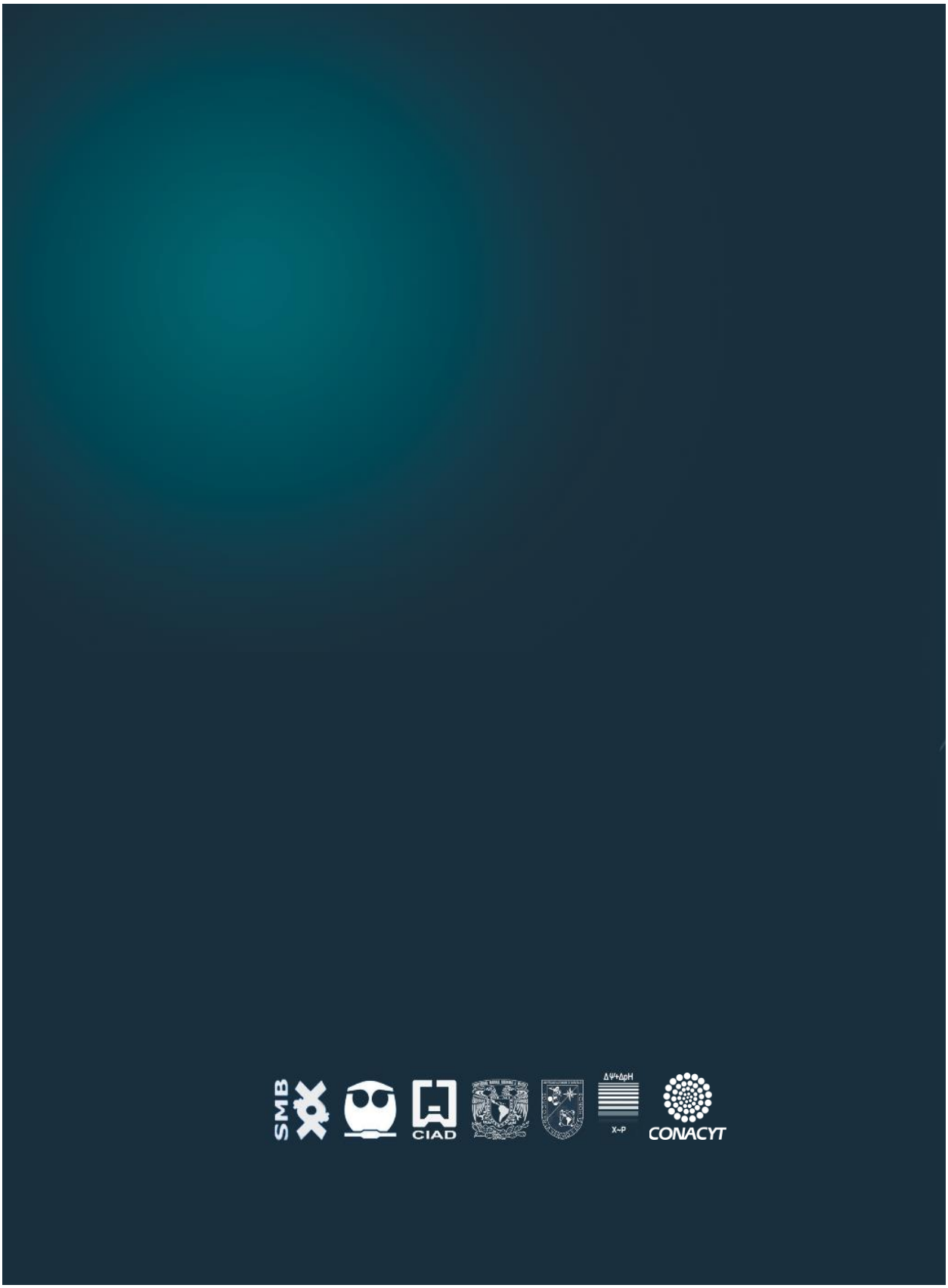
SMB
CIAD
ΔΨ+ΔpH
X~P
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