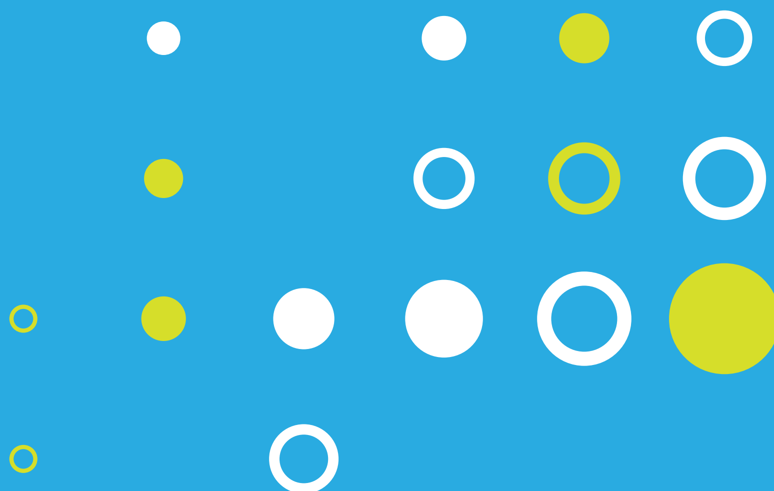
BIOCELL nº38

ISSN: 0327-9545 (print)
ISSN: 1667-5746 (online)

October 2014



SAIB

Sociedad Argentina de
Investigaciones en Bioquímica
y Biología Molecular

Vol. 38 - Suppl. 2 - 2014
Published in Argentina

ISSN 0327 - 9545 (printed version)
ISSN 1667 - 5746 (on-line version)

BIOCELL

Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

Argentine Society for Biochemistry and Molecular Biology Research

Rosario, Argentina - 11 al 14 de Noviembre de 2014

BIOCELL

An international journal of Biology

BIOCELL - Volume 38 - Supplement 2 - October 2014 - Mendoza, Argentina.

ISSN 0327 - 9545 (printed version) - ISSN 1667 - 5746 (on-line version)

This journal is included in the Life Sciences Edition Contents, the Science Citation Index, Electronic Library Project of the Institute for Scientific Information (ISI), Scisearch and Research Alert databases of Current Content; ISI/BIOMED, Index Medicus and MEDLINE available on the NLM's on line MED'LARS systems; EMBASE/Excerpta Medica; Chemical Abstracts, Biological Abstracts (BIOSIS), Index Medicus for Latin America and LATINDEX, SciELO Argentina.

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- SAIB -
50th Annual Meeting
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ACKNOWLEDGEMENTS

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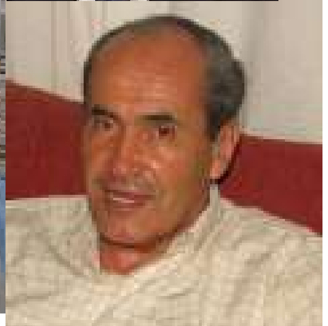


1970-1970 RODOLFO BRENNER

1965-Nace SAIB. Como ya he comentado varias veces, SAIB nació en el pequeño comedor del Instituto de Investigaciones Bioquímicas de la Fundación Campomar del Dr. Luis Federico Leloir, ubicado en la calle Vuelta de Obligado del barrio de Belgrano en Buenos Aires. Allí, Luis Federico Leloir, Andrés Stoppani, Federico Cumar y yo fijamos las normas de la Sociedad Argentina de Investigación Bioquímica. A sugerencia de Stoppani, para ser socio sólo se requiere, democráticamente, dedicarse a la Investigación Bioquímica, independientemente del título profesional y con ello eliminó competencias laborales. Ese es el ideal de SAIB, propiciar la colaboración mutua. Las áreas de investigación dependieron de las preferencias de sus desarrolladores. En el área de la Bioquímica de Lípidos fuimos varios sus cultores. En el Instituto de Investigaciones de La Plata (INIBIOLP) encaré personalmente junto a muchos otros buenos investigadores varios temas. Uno fue la biosíntesis y funciones de los recién conocidos ácidos polietilénicos esenciales requeridos en la nutrición animal. Así reconocimos la serie linoleica n-6, que lleva el ácido araquidónico de origen fundamentalmente terrestre y también la serie n-3 de origen fundamentalmente marino y cuyos componentes, los ácidos polietilénicos eicosapentaenoico (EPA, 20:3, n-3) y docosahexaenoico (DHA, 22:6 n-3), señalamos, juegan un papel fundamental en la función cerebral además de otras funciones. También demostramos sus mecanismos de biosíntesis por intermedio de diversas enzimas desaturantes, las desaturasas, así como su regulación por una serie de hormonas las SREBP-1C, los PPARs, etc. Por otro lado, demostramos los efectos en diversos lípidos tanto en su estructura como en sus funciones en las células y sus organelas recurriendo a técnicas de biología molecular. Ellos se completaron con la investigación de los efectos sobre la fisiología y la nutrición animal. Importantes fueron los estudios en colaboración con investigadores de Santa Fe dirigidos por la Dra. Yolanda Lombardo sobre su acción en la diabetes experimental. Estos logros fueron acompañados por investigaciones del metabolismo lipídico en peces, moluscos e insectos, tales como el *Triatoma infestants* (vinchuca) transmisor de la enfermedad de Chagas. Todos estos estudios fueron divulgados en el ambiente nacional en los congresos anuales de SAIB, y en el ambiente internacional en visitas y conferencias en laboratorios de América, Europa y Japón así como en múltiples congresos. Preferentemente, fueron comunicados en la International Conference on the Bioscience of Lipids (ICBL) realizados en Europa anualmente. Así se difundieron los logros obtenidos en nuestro país y la Argentina fue ampliamente reconocida en esos aspectos de la ciencia. Muchos fueron mis excelentes colaboradores que son también autores de estos trabajos. Recordaré entre ellos especialmente a: Raul Peluffo, María E de Tomás, Osvaldo Mercuri, Anibal M. Nervi, Josefa Tacconi de Alaniz, Nelva Tacconi de Gomez Dumm, Ricardo Pollero, Angel Catalá, Horacio Garda, Carlos Marra, Betina Córsico, Omar Rimoldi y Alejandra Tricerri.

Los Congresos científicos de SAIB siempre fueron de alto nivel y tuvieron gran asistencia de investigadores. Sin embargo, creo que la reunión más famosa y ampliamente difundida por la prensa fue la 6ta, realizada el 28 de octubre de 1970 en nuestra Facultad de Ciencias Médicas de La Plata. La causa fue que el Dr. Luis Federico Leloir había sido notificado de ser receptor del premio Nobel de Química. Leloir había sido el primer presidente de SAIB, así que la alegría para los socios de SAIB fue doble, dado que Leloir era muy querido. Fue un momento de euforia en todo el país pero

especialmente en La Plata. No puedo olvidar, aún hoy emocionado, el estruendoso e interminable aplauso con que fue recibido Leloir al aparecer en el salón de Conferencias. Pero también me asombró su sencillez cuando lo acompañé al comedor de los estudiantes. Él llevaba su propia bandeja para servirse. Ello provocó un nuevo y estruendoso aplauso pero esta vez de los estudiantes. En este congreso también inauguramos la costumbre de invitar a renombrados científicos extranjeros a las reuniones de SAIB. Asistieron en esa oportunidad el Dr. L.L.M. Van Deenen, holandés, especialista en las bioquímica de las membranas biológicas y el Dr Salí Wakil, iraki, investigador de la Duke University, que acababa de descubrir la función primordial que tenía la malonil coenzima A en la biosíntesis de los ácidos grasos saturados. La investigación de la Bioquímica de Lípidos fue también realizada en Argentina por otros científicos socios de SAIB. En Bahía Blanca, el Dr. Nicolás Bazán había creado el Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) y con la colaboración de Norma M. Giusto, Marta I. Aveldaño, Ana M. Pechén, Elena Rodriguez de Turco, Hugo Caldironi, Graciela Cascone y varios más, desarrolló el estudio del metabolismo de los fosfolípidos en tejidos nerviosos que llevaron posteriormente a relacionarlos con las alteraciones mentales efecto de la vejez. En Córdoba, el Dr. Ranwell Caputto formado junto con el Dr. Leloir, creó un brillante grupo de investigadores en la Facultad de Ciencias Químicas de la Universidad local constituído por su hija Beatriz Caputto, Hugo Maccioni, Juan Curtino, Bruno Maggio, Carlos Arce, entre otros, que se dedicaron a dilucidar la biosíntesis de los ganglioósidos cerebrales y sus funciones. A éste siguieron otros temas importantes. En la universidad de Tucumán el Dr. Ricardo Farías inició, con el asesoramiento del Dr. R. E. Trucco en la década del 70, una serie de investigaciones referentes a los efectos de dietas suplementadas con diferentes lípidos sobre la estructura y las funciones de las membranas biológicas. Posteriormente, sus intereses cambiaron y realizó muchísimas publicaciones en el campo de la microbiología. En Rosario, en el Instituto de Biología Molecular y en su Universidad el Dr. Diego de Mendoza ha formado un grupo científico que ha estudiado y sigue investigando la biosíntesis de ácidos grasos *en Bacillus cereus* y en *Bacillus subtilis* y su función en las membranas bacterianas.

En resumen, la investigación de la bioquímica de los lípidos ha sido y es un tema ampliamente estudiado con éxito por los grupos mencionados y otros socios de SAIB que al llegar y difundirse en el ámbito internacional provocaron, por su importancia, un reconocimiento del alto nivel científico alcanzado por nuestro país.

Rodolfo R. Brenner

1977-1977 ANTONIO BLANCO

Llegar a los cincuenta años con la pujanza con que lo hace SAIB es un acontecimiento digno de destacar y celebrar.
La sociedad nació en 1965 por iniciativa de unos pocos integrantes de los grupos de investigación bioquímica existentes en el país en esa época. El hecho de que esos grupos ya exhibían una producción de nivel excepcional, marcó la calidad que ha caracterizado a SAIB desde sus comienzos.

A través de los años hemos asistido a un crecimiento sostenido, tanto del número de investigadores como de centros altamente calificados. No sólo en Buenos Aires, donde inicialmente se concentraba la actividad y recursos, sino que fueron surgiendo otros en La Plata, Córdoba, Rosario, Tucumán, Santa Fe, Bahía Blanca, Mendoza, Mar del Plata.

Es gratificante comprobar cómo aumenta la participación de jóvenes muy meritorios en las reuniones anuales, índice de la labor de formación de discípulos que realizan esos centros, constituidos en focos de irradiación que contribuyen significativamente al desarrollo de la ciencia en Argentina.

Desde hace varios años mi salud no me permite asistir a las reuniones anuales, pero guardo el recuerdo de su constante superación. Gracias a la infraestructura hotelera existente en nuestras sierras resultaba conveniente realizarlas en Córdoba, por lo cual tuvimos la responsabilidad de organizarlas en muchas oportunidades; cada vez era necesario buscar hoteles con mayor capacidad. En una de esas reuniones se dio una situación que me impactó. Fue en la Casa Serrana de la Obra Social de Correos en Huerta Grande. A la hora del almuerzo nos dirigíamos al comedor. Para acceder a la sección reservada a los asistentes al Congreso, había que atravesar un sector ocupado por familias de afiliados de la Obra Social que se alojaban en ella. Cuando el Dr. Leloir, que ya era Premio Nobel, ingresó en el local, todos los allí presentes, unas 80 personas, se pusieron de pie e iniciaron un cerrado aplauso. Realmente me emocionó esa reacción espontánea de reconocimiento y afecto a un gran científico por parte de gente no relacionada con la ciencia.

Para mí este episodio reflejaba la consideración y respeto que la ciencia merece en buena parte de la sociedad, que sin duda comprende su importancia como motor de desarrollo e independencia del país.

La ciencia argentina tiene en la actualidad una jerarquía ampliamente reconocida como competitiva a nivel internacional; en este sentido, sin duda la investigación bioquímica, catalizada por SAIB, ha sido una de las que más han contribuido a alcanzar esa situación de privilegio.

Pese a los vaivenes y retrocesos que hemos experimentado en el pasado, los últimos años han sido favorables en cuanto al apoyo oficial brindado a la investigación

científica. Es de esperar que éste se sostenga en el futuro, independientemente de los cambios políticos.

Antonio Blanco

1980-1980 JUAN DELLACHA

Durante los años 1979 y 1980, la reuniones de la SAIB se llevaron a cabo en el Hotel "Ejercito de los Andes" en la localidad de Bermejo de la Provincia de Mendoza. En mi carácter de Vicepresidente de la Sociedad integré la Comisión de la misma con el Dr Marcelo Dankert y la Dra Susana Passerón, presidida por el Dr Luis F. Leloir, para organizar la XV Reunión Nacional de la SAIB del 21 al 24 de octubre de 1979. Cabe señalar que en dicha reunión se presentaron 135 trabajos científicos, en la forma de presentaciones orales de 10 minutos de duración y 5 minutos de discusión. Además se contó con las presencia de distinguidos invitados como el Dr Osvaldo Cori de Chile, el Dr Hugo Armelín de Brasil, los Dres Alberto Soriano, Horacio Burgos y Francisco Bertini de Argentina, quienes realizaron aportes significativos en cada una de sus conferencias.

La XVI Reunión Nacional de la SAIB de 1980 tuvo un carácter especial ya que se organizó en forma conjunta con la IX Reunión Anual de la Sociedad Argentina de Biofísica (SAB) y la IV Reunión Anual de las Sociedad de Bioquímica de Chile.

En esta reunión, en mi carácter de Presidente de la SAIB y con la valiosa colaboración de los Dres Alberto Boveris y Alcides Rega de la SAB y el Dr Joaquín Cannata y la Dra Susana Passerón de la SAIB, organizamos la reunión de las tres sociedades que se llevó a cabo del 17 al 20 de octubre de 1980. Es de destacar que este comité organizador de esta reunión, contó con la colaboración de un Comité local en Mendoza, presidido por el Dr Juan Carlos Fasciolo, cuyo apoyo fue decisivo para el buen desarrollo y éxito de este congreso conjunto.

En el acto de apertura de la XVI Reunión Nacional de la SAIB, el Dr Severo Ochoa merecedor del Premio Nobel de Medicina 1959 por haber descubierto que la enzima polinucleótido fosforilasa era capaz de sintetizar *in vitro* ARN, disertó sobre "Regulación de la biosíntesis de proteínas en eucariotes".

Las 203 comunicaciones presentadas durante los días de la reunión fueron expuestas en función de sus temas, en las siguientes sesiones: Biología Molecular; Acción Hormonal; Regulación Metabólica; Enzimología; Bioenergética; Neuroquímica; Metabolismo de Lípidos y Estructura y Función de Membranas; y Metabolismo de Hidratos de Carbono.

A los fines de conocer donde se ubicaba la frontera de la biología molecular, en el período que se realizaron estas dos reuniones nacionales de la SAIB, se puede mencionar el trabajo de David Goeddel y colaboradores, publicado en el Proceedings of the National Academy of Sciences (USA), **76**, 106-111 (1979) titulado "Expression in *Escherichia coli* of chemically synthesized genes from human insulin". Este trabajo realizado gracias al desarrollo de la biología molecular y de la ingeniería genética alcanzado en ese momento, logra la síntesis de la insulina humana que comenzó a distribuirse como medicamento a principios de los años 80.

Juan M. Dellacha

1982-1983 JOSÉ SANTOMÉ

Elección del lugar de realización de las Reuniones Anuales

La creación de la SAIB tuvo una extraordinaria influencia en el desarrollo de la investigación bioquímica en nuestro país. Hasta su creación, los escasos grupos de trabajo existentes en Argentina y en el resto de América Latina, presentaban y discutían el resultado de sus investigaciones en las reuniones de la Asociación Latinoamericana de Ciencias Fisiológicas (ALACF). En ella constituían un pequeño grupo, diluido entre numerosos fisiólogos ajenos a la bioquímica.

El principal objetivo de la SAIB fue la realización de una reunión anual, en la cual investigadores bioquímicos del país, sin considerar su título profesional, pudieran presentar y discutir sus investigaciones.

Al comienzo, las reuniones se realizaban en laboratorios constituidos por grupos sólidos de trabajo. Dada la importancia que esas reuniones adquirían en las localidades donde se realizaban, frecuentemente con la presencia y el apoyo de los gobiernos locales, se consideró importante realizarlas en laboratorios en formación, necesitados de ese apoyo. Sin embargo, no siempre fue posible cumplir con ese objetivo y, en ocasión de golpes militares, hasta fue difícil obtener autorización para realizar algunas reuniones. En 1976, por ejemplo, la XII Reunión Anual de la SAIB, en Sierra de la Ventana, se realizó con la presencia de soldados armados vigilando en los jardines.

La presentación de trabajos en las reuniones de SAIB, siempre entusiasmó a los investigadores y sobre todo a los becarios, que generalmente preparan sus presentaciones con gran dedicación. El número de trabajos presentados fue creciendo en forma sorprendente, al extremo de que fue necesario abandonar la elección del lugar de realización en función del apoyo a investigadores de distintos lugares del país. Fue necesario recurrir a centros turísticos capaces de recibir un número tan elevado de participantes.

El cambio se realizó durante mi presidencia de SAIB en 1982 y 1983. La organización de la XVIII Reunión Anual, en 1982, última de las realizadas en apoyo de grupos locales, estuvo a cargo de los investigadores de la Universidad de Rio Cuarto. La XIX Reunión, organizada por el Presidente y la Comisión Directiva de SAIB, se llevó a cabo en un hotel de Huerta Grande, provisto de un número adecuado de habitaciones y de salas para la realización de reuniones simultáneas, indispensables para presentar, en el corto tiempo disponible, el elevado número de presentaciones y conferencias programadas. Es digno de destacar la continua colaboración de los investigadores cordobeses en la organización de la reunión. A partir de la XIX, la mayoría de las Reuniones Anuales de SAIB también se realizaron en la Provincia de Córdoba, con excepciones generalmente motivadas por reuniones conjuntas con otras sociedades científicas.

José Santomé

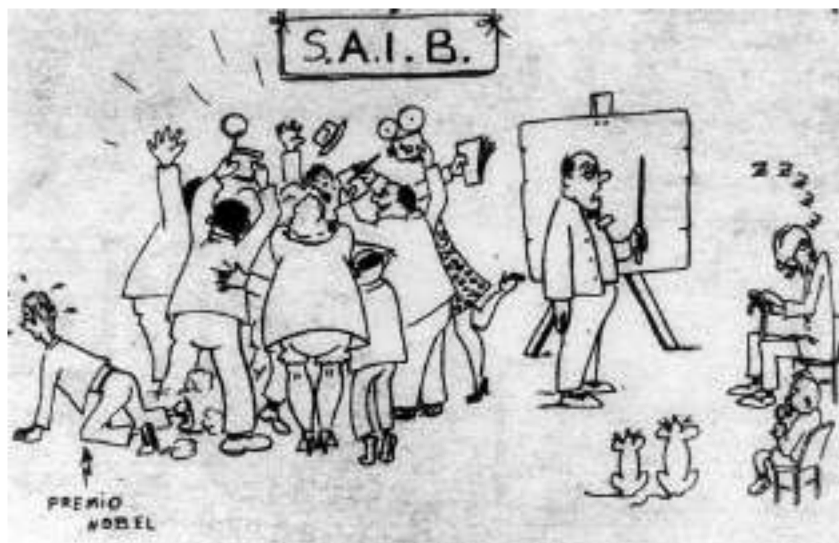
1992-1993 ARMANDO J. PARODI

Recuerdo de las primeras Reuniones de SAIB

Creo que soy de los pocos que asistimos a la primera reunión de SAIB y que todavía andamos dando vueltas por el pago. Había entrado a realizar la Tesis Doctoral bajo la dirección de Leloir en Agosto de 1965 y en Noviembre de ese mismo año fuimos convocados a la reunión que se llevó a cabo en el Departamento de Química Biológica de la Facultad de Farmacia y Bioquímica de la UBA. Durante la mañana tuvimos sucesivas reuniones con los distintos grupos de trabajo que nos explicaron lo que estaban haciendo. Posteriormente hubo algunas comunicaciones orales cortas y la conferencia plenaria a cargo de Philip P. Cohen (P.P. Cohen, el del ciclo de la urea) que por alguna razón que desconozco andaba por Buenos Aires. Todas las charlas se realizaron en el aula donde ahora se dan los seminarios del IQUIFIB. Y eso fue todo.

La segunda reunión de SAIB tiene la especial y única característica de no haber tenido lugar. En 1966 se realizaba un Congreso de Fisiología en Mar del Plata y los directivos de SAIB, seguramente agotados por el esfuerzo de haber organizado la primera reunión decidieron que dicho Congreso de Fisiología podía ser contado como segunda reunión.

La tercera reunión, en 1967, tuvo lugar en la entonces Campomar (Obligado y Monroe). Fue un tanto trabajosa para los hospedadores porque como no contábamos con un aula suficientemente grande tuvimos que sacar todos los libros, revistas y estanterías de la biblioteca para hacer lugar (y luego volver a colocarlos en su lugar, obviamente).



SAIB,

6th Annual Meeting, 1970

El 1968 la reunión (cuarta) tuvo lugar en la Cátedra de Química Biológica de la Facultad de Ciencias Químicas de la UNC. Tengo un recuerdo particularmente inusual de dicha reunión, como fue la pelea entre dos investigadoras en el estrado durante la conferencia de una de ellas.

Al año siguiente la reunión tuvo lugar en Horco Molle (Tucumán). Fue la primera donde aparecieron, como gran novedad, los posters. Varios investigadores habían concurrido ese mismo año a un Congreso de FEBS en Madrid y habían traído la novedad de que no era necesario que todas las comunicaciones fuesen orales.

Al año siguiente la reunión fue en La Plata. Tuvo especial repercusión porque pocos días antes se había anunciado el otorgamiento del Premio Nobel de Química a Leloir. Adjunto un dibujo que da cuenta de dicho alboroto. Y aquí me detengo. SAIB ha ido creciendo, de una reunión de unos pocos amigos donde todos nos conocíamos a las reuniones actuales, multitudinarias, pero que increíblemente siguen siendo aún un tanto artesanales. ¿Fue positiva la creación de SAIB? Naturalmente que sí. La Bioquímica y la Biología Molecular local han progresado mucho en estos 50 años. No tanto como hubiésemos querido pero lo importante, dadas las vicisitudes por las cuales pasó nuestro país, mucho más de lo que era dable esperar. Y mucho mérito de ello se lo debemos a nuestra Sociedad.

Armando J. Parodi

1996-1997 RICARDO BOLAND

Asumí la Presidencia de SAIB el 16 de noviembre de 1995 en la XXXI Reunión Anual. Me secundó una Comisión Directiva (CD) cuyos miembros desarrollaron un trabajo muy eficaz. Quiero mencionar la gran dedicación y valiosos aportes de los Dres. Marta Hallak (Secretaria), Alberto Kornblihtt (Prosecretario), Josefa Tacconi de Alaniz (Tesorera) y Estela Machado (Protesorera), en la organización de los eventos científicos, comunicación con los socios e implementación de trámites reglamentarios. Con ellos y demás integrantes de CD durante las reuniones mensuales que se realizaban en el INGEBI compartí, mientras se discutían distintos temas y la planificación de actividades, el placer de trabajar juntos en la resolución de problemas en un clima de cordialidad y amistad, lo cual me resultó muy gratificante dejándome recuerdos imborrables. No faltaron episodios anecdóticos especiales que prefiero omitir. Más bien deseo destacar el compromiso y el activo rol que tuvo SAIB durante mi gestión (1995-1997) en política científica, sin descuidar el cumplimiento de sus finalidades específicas. Fue un período de gran crisis y relegamiento de la ciencia nacional, caracterizado por un apoyo deficiente o inexistente en algunos casos, afectando seriamente su estructura y funciones, evidenciados por el cierre del ingreso a Carrera del Investigador, número insuficiente de Becas, otorgamiento de escasos y magros subsidios, obsolescencia del equipamiento. Frente a esta situación, SAIB efectuó una crítica severa de manera permanente, manifestándose públicamente a través de solicitadas publicadas en los principales diarios de la Capital Federal y mediante notas y entrevistas con las autoridades de la Secretaría de Ciencia y Tecnología de la Nación, reclamando la integración del Directorio del CONICET por científicos del máximo nivel y un aumento sustancial del presupuesto para corregir las deficiencias mencionadas. Actualmente la estructura y funcionamiento del sistema científico del país han mejorado sustancialmente. La actitud de SAIB en que aquellas circunstancias dejó un mensaje ético y de compromiso ante eventuales desvíos del camino trazado ahora.

En 1996 SAIB organizó conjuntamente con la Sociedad de Bioquímica y Biología Molecular de Chile el VIII congreso de PABMB, en la localidad de Pucón, Chile. El evento, que se realizó del 16 al 21 de noviembre en el Gran Hotel Pucón, contó con un destacado programa integrado por 6 conferencias a cargo de Néstor Carrillo (Argentina), Leopoldo de Meis (Brasil), Ramón Latorre (Chile), Georges Dreyfus (Méjico), Susan Taylor (USA), William Whelan (USA), 26 Simposios así como comunicaciones orales y mostraciones. La SAIB brindó ayuda económica para la asistencia de alrededor de 100 becarios y varios investigadores jóvenes, utilizando fondos propios (inscripciones, cuotas societarias), provenientes de IUBMB, Fundación Antorchas y del CONICET. Debido a los requisitos reglamentarios, la Asamblea Anual Ordinaria se efectuó en territorio nacional, en Buenos Aires, el 18 de octubre de 1996. En dicha oportunidad el Dr. John H. Exton (USA) dictó una Conferencia Plenaria sobre "Rol of G proteins in phospholipase regulation".

La Reunión Anual de 1997 (noviembre 11-14) se llevó a cabo en el Hotel de Luz y Fuerza en Villa Giardino, Córdoba, tuvo un programa científico también relevante: 10 conferencias a cargo de Joseph Batenburg (Holanda), Miguel Beato (Alemania, Bruno Chatton (Francia), Manuel Espinosa (España), Thomas Gudermann (Alemania), Khalid Iqbal (USA), Ernesto Podestá (Argentina), Jack Preiss (USA), Enrico Stefani (USA), Giuliana Zanetti (Italia), varios simposios y numerosas comunicaciones orales y mostraciones. Se otorgaron ayudas económicas para alojamiento y comidas a más de 100 becarios. Además de recursos propios, se obtuvo un apoyo significativo, luego de arduos esfuerzos pero exitosos, de empresas privadas nacionales, y en menor proporción del CONICET y la Fundación Antorchas.

Finalmente, las actividades científicas de SAIB en 1997 contaron con un broche de oro. En el mes de octubre organizó en la FCEN de la UBA una conferencia plenaria a cargo del Dr. Stanley Prusiner de la Universidad de California describiendo sus estudios pioneros sobre los priones. Pocos días después fue galardonado con el Premio Nobel de Medicina.

Ricardo Boland

2004-2005 ERNESTO PODESTA

Es un placer y una alegría para mi tener la oportunidad de presenciar los primeros 50 años de la Sociedad y poder desearles un muy feliz aniversario. Esta es una gran oportunidad también para felicitar a los “Maestros” que tuvieron la brillante idea de constituir este ámbito de discusión y presentación de los avances logrados por la comunidad Científica en el área. Estos maestros han generado una escuela que mantiene vivo el espíritu de la Sociedad y han contribuido a la formación de numerosos discípulos, que a lo largo de los años han seguido los pasos de estos pioneros, manteniendo una comunidad científica de excelencia y con un espíritu de lucha ante cualquier adversidad. La Sociedad Argentina de Investigaciones Bioquímicas y Biología Molecular está considerada entre las mejores sociedades multidisciplinarias, localmente, en la región y en el mundo y sus investigadores son reconocidos por su tesón y excelencia. El logro de mantener ese espíritu y seguir generando escuelas de ciencias son labores que tienen una envergadura y valor que no se compara aún con el mejor trabajo de investigación publicado en la revista internacional de mayor prestigio en el área. Es por ello que también felicito a todos los Ex-Presidentes e integrantes de los respectivos Consejos Directivos y a la comunidad científica por mantener viva esta piedra fundamental generada por un pequeño grupo de iluminados hace 50 años.

Ernesto Jorge Podestá

Tuesday, November 11	Wednesday, November 12	Thursday, November 13	Friday, November 14
14:00 Registration	9:00 - 11:00 **Symposia** *Room A*: Microbiology *Room B:* Cell Biology	9:00 - 11:00 **Symposia** *Room A:* Lipids *Room B*: Signal Transduction	9:00 - 11:00 **Symposia** *Room D*: Translational Science *Room B*: Plant Biochem. & Mol Biol
	11:00-11:30 Coffee break	11:00-11:30 Coffee break	11:00-11:30 Coffee break
	11:30-12:30 **Plenary Lecture** Eduardo Blumwald (*Room A*)	11:30-12:30 **"Ranwel Caputto" Lecture** Jose Luis Daniotti (*Room A*)	11:30-12:30 **Translational Sc Lecture** Gabriel Rabinovich (*Room D*)
14:00-16:00 ***Pre Congress Activity*** *25 years of Biotechnology career in Rosario* (*Room B*)	12:30 Time for Lunch	12:30 Time for Lunch	12:30 Time for Lunch
	13:00 – 15:00 **Posters Section** BT-P01 to BT-P22 CB-P01 to CB-P20 MI-P01 to MI-P27 NS-P01 to NS-P05 PL-P01 to PL-P22 SB-P01 to SB-P06	13:00 – 15:00 **Posters Section** CB-P21 to CB-P40 EN-P01 to EN-P14 MI-P28 to MI-P53 LI-P01 to LI-P10 PL-P23 to PL-P44 ST-P01 to ST-P15	13:00 – 15:00 **Posters Section** CB-P41 to CB-P63 LI-P11 a LI-P23 MI-P54 to MI-P81 PL-P45 to PL-P64 ST-P16 to ST-P32
16:00 - 18:30 **Symposium** **Education and Science** (*Room A*)	15:00-16:00 **Plenary Lecture** Robert E. Anderson (*Room A*)	15:00-16:00 **"EMBO" Lecture** Dominique Ferrandon (*Room A*)	15:00-16:00 **Plenary Lecture** Iván Matic (*Room D*)
	16:00-18:00 **Oral Communications** *Room A:* Microbiology (MI-C01 to MI-C08) *Room B:* Cell Biology (CB-C01 to CB-C08) *Room C:* Signal Transduction (ST-C01 to ST-C06) and Plant Biochem. & Mol Biol (PL-C01 and PL-C02)	16:00-18:00 **Oral Communications** *Room A:* Cell Biology (CB-C09 to CB-C16) *Room B:* Plant Biochem. & Mol Biol (PL-C03 to PL-C10) *Room C:* Lipids (LI-C01 to LI-C07)	16:00-18:00 **Oral Communications** *Room D:* Structural Biology (SB-C01 to SB-C04) and Microbiology (MI-C09 to MI-C12) *Room B:* Cell Biology (CB-C17 to CB-C19) and Neuroscience (NS-C01 to NS-C03) *Room E:* Biotechnology (BT-C01 to BT-C07)
18:30-19:00 Coffee break	18:00 – 19:00 ***Round Table:*** Argentine Science: Present and Perspectives	18:00-18:30 Coffee break	18:00-18:30 Coffee break
19:00-20:00 **Opening Ceremony** ***Tribute to Former SAIB President*** (*Room A*)	19:00-19:30 Coffee break	18:30-19:30 **"Héctor Torres" Lecture** Alberto Kornblihtt (*Room A*)	18:30-19:30 **Closing Lecture** Marcelo Rubinstein (*Room D*)
20:00-21:00 **Opening Lecture** David D. Sabatini	19:30-20:30 **"Alberto Sols" Lecture** Jesús Balsinde (*Room A*)	19:30 SAIB General Assembly	
21:00 Cocktail			21:30 Closing Ceremony & Awards Closing Dinner

ACTIVIDAD PRE-CONGRESO SAIB 50

Simposio 25 años de Biotecnología en Rosario

11 de Noviembre de 2014

Salón B

Coordinadora: Eleonora García Véscovi.

Directora Escuela de Biotecnología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR

14:00-14:15 Presentación a cargo de Esteban Serra; Decano de la Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

14:15-14:40 Hugo Menzella. *Laboratorio de Medicamentos. Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. Rosario*

"Desarrollo y producción de enzimas industriales en la Facultad de Ciencias Bioquímicas y Farmacéuticas de la UNR"

14:40-15:05 Raquel L. Chan. *Instituto de Agrobiotecnología del Litoral (CONICET-UNL), Santa Fe*

"¿Plantas tolerantes a estrés o plantas más productivas? Un dilema que no debería plantearse"

15:05-15:30 Mariano R. Gabri. *Laboratorio de Oncología Molecular, UNQ, Quilmes*

"Participación del gangliósido NGcGM3 en el proceso de transformación maligna y su uso como blanco para el desarrollo de una vacuna terapéutica para cáncer de pulmón"

15:30-15:55 Adrián Mutto. *Laboratorio de Biotecnología Reproductivas y Mejoramiento Genético Animal. UNSAM - Instituto de Investigaciones Biotecnológicas, San Martín*

"Generación de animales modificados genéticamente por medio de herramientas de edición genómica"

PROGRAM

TUESDAY, November 11, 2014

14:00-16:00 **REGISTRATION**

16:00-18:30 ***SYMPOSIUM***

EDUCATION AND SCIENCE

Chairperson: Omar Coso

16:00-16:30	***Guillermo Jaim Etcheverry***
16:30-17:00	***Gabriel Gellón***
17:00-17:30	***Mariano Sigman***
17:30-18:00	***Alberto Kornblihtt***
18:00-18:30	**General Discussion**

18:30-19:00 ***COFFEE BREAK***

19:00-20:00 ***OPENING CEREMONY***

Carlos S. Andreo

SAIB President

CEFOBI, CONICET - Universidad Nacional de Rosario, Argentina

Tribute to Former Presidents of SAIB

20:00-21:00 ***OPENING LECTURE***

David D. Sabatini

Department of Cell Biology, New York University School of Medicine, USA

"Personal recollections of a life-long affair with cell biology"

Chairperson: Luis S. Mayorga

21:00 ***COCKTAIL***

WEDNESDAY, November 12, 2014

09:00-11:00 ***SYMPOSIA***

Room A

MICROBIOLOGY SYMPOSIUM

Chairpersons: Andrea Smania and Diego Comerci

09:00-09:30 **Diego de Mendoza**
Instituto de Biología Molecular y Celular de RosarioCONICET-UNR, Argentina
"Enzymatic diversity in lipoic acid modification of proteins"

09:30-10:00 **Vanessa Sperandio**
Southwestern Medical Center, University of Texas, USA
"EHEC sings: pour some sugar on me"

10:00-10:30 **José Arguello**
Worcester Polytechnic Institute, Massachusetts, USA
"The roles of heavy metal transport ATPases in bacterial virulence"

10:30-11:00 **Darío Zamboni**
Universidade de Sao Paulo, Brasil
"Inflammasome activation in response to infection with intracellular pathogens"

Room B

CELL BIOLOGY SYMPOSIUM:
Cell organelles in health and disease

Chairpersons: Carlos Arregui and Dario Krapf

09:00-09:30 **Cecilia Alvarez**
CIBICI, CONICET – Facultad Ciencias Químicas, UN Córdoba, Argentina
"The secretory pathway and its adaptation to a high secretory demand"

09:30-10:00 **Maximiliano Gutierrez**
MRC National Institute for Medical Research, London, UK

"Intracellular trafficking of phagosomes: lessons from mycobacteria "

10:00-10:30 **Sidney W. Whiteheart**
University of Kentucky, Lexington, USA
"The Molecular Basis for Platelet Secretion"

Graça Raposo
10:30-11:00 Institut Curie, Centre de Recherche, France
"Melanosome biogenesis and mechanisms of intercellular communication in the skin"

11:00-11:30 ***COFFEE BREAK***

11:30-12:30 ***PLENARY LECTURE***

Eduardo Blumwald

Department of Plant Sciences, University of California, Davis, USA

"Cellular ion homeostasis: emerging roles of intracellular NHX-type Na^+/H^+ antiporters in plant growth and development"

Chairperson: Norberto Iusem

13:00-15:00 ***POSTER SESSION***

BT-P01 to BT-P22 **CB-P01 to CB-P20**
MI-P01 to MI-P27 **NS-P01 to NS-P05**
PL-P01 to PL-P22 **SB-P01 to SB-P06**

15:00-16:00 ***PLENARY LECTURE***

Robert E. Anderson

University of Oklahoma Health Sciences Center, USA

"Very long chain fatty acids: their role in retina and brain function"

Chairperson: Gabriela Salvador

16:00-18:00 ***ORAL COMMUNICATIONS***

Room A: Microbiology (MI-C01 to MI-C08)

Room B: Cell Biology (CB-C01 to CB-C08)

Room C: Signal Transduction (ST-C01 to ST-C06) and Plant Biochemistry and Molecular Biology (PL-C01 and PL-C02)

Room A

Microbiology (MI-C01 to MI-C08)

Chairpersons: Mónica Delgado and Federico Sisti

16:00-16:15

MI-C01

IDENTIFICATION OF A METACASPASE PROTEIN SUBSTRATE CONSERVED AMONG EARLY DIVERGENT EUKARYOTIC CELLS

Bouvier, LA[1]; Niemirowicz, GT[1]; D´Alessio, C[2]; Cazzulo, JJ[1]; Alvarez, VE[1]

[1]IIB-INTECH/UNSAM-CONICET [2]FIL-IIBBA CONICET. E-mail: lbouvier@gmail.com

16:15-16:30

MI-C02

EXPOSING THE SECRETS OF TWO WELL KNOWN DAIRY PHAGES: GENOMIC AND STRUCTURAL ANALYSIS OF J-1 AND PL-1

Dieterle, ME[1]; Bowman, C[2]; Batthyany, C[3]; Lanzarotti, E[1]; Turjanski , A[1]; Hatfull, G[2]; Piuri, M[3]

[1]Dpto. de QB, FCEN, UBA, IQUIBICEN-CONICET.[2]University of Pittsburgh.[3]Institut Pasteur de Montevideo. E-mail: medieterle@qb.fcen.uba.ar

16:30-16:45

MI-C03

BROMODOMAIN FACTOR 3 IS ESSENTIAL FOR *Trypanozoma cruzi* GROWTH AND DIFFERENTIATION

Alonso, VL[1]; Ritagliati, C[1]; Motta, MC[2]; Cribb, P[1]; Serra, EC[1]

[1]IBR–CONICET. Fac. Cs. Bioq. y Farm., UNR, Argentina [2]Universidade Federal do Rio de Janeiro, Brazil. E-mail: alonso@ibr-conicet.gov.ar

16:45-17:00

MI-C04

OLIGOTYPING ANALYSIS OF A WASTEWATER TREATMENT PLANT MICROBIOME

Pérez, V1[2]; Ibarbalz, FM[1]; Figuerola, E[1]; Erijman, L[13]

[1]INGEBI "Dr Hector N. Torres"-CONICET. [2]AySA S.A. [3]DFBMC, FCEN-UBA. E-mail: mvperez.ingebi@gmail.com

17:00-17:15

MI-C05

OUTER MEMBRANE VESICLES IN *Serratia*: PROTEOMIC ANALYSIS AND DELETERIOUS EFFECT IN AN INSECT MODEL

Bruna, R[1]; Lestradet, M[2]; Ferrandon, D[2]; García Véscovi, E[1]

[1]IBR, CONICET-UNR. [2]Institut de Biologie Moleculaire et Cellulaire, CNRS-Université de Strasbourg. E-mail: bruna@ibr-conicet.gov.ar

17:15-17:30

MI-C06

IDENTIFICATION OF A COMPOUND FROM SOYBEAN HULL PYROLYSATE WITH ACTIVITY AGAINST *Salmonella enterica*

Viarengo, G[1]; Giri, GF[2]; Furlán, RL[3]; Suárez, AG[2]; Spanevello,, RA[2]; García Véscovi, E[1]

[1]IBR-CONICET. [2]IQUIR-CONICET. [3]Lab. de Farmacognosia, Fac. de Cs Bioquímicas y Farmacéuticas (UNR) E-mail: viarengo@ibr-conicet.gov.ar

17:30-17:45

MI-C07

CO-DEPENDENCE ON COPPER EXCESS AND PERIPLASMIC STRESS TO CONTROL *Salmonella CUEP* EXPRESSION

Pezza, A; Pontel, LB; Soncini, FC

Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR, Rosario, Argentina. E-mail: pezza@ibr-conicet.gov.ar

17:45-18:00

MI-C08

IDENTIFICATION OF A *Brucella* PERIPLASMIC PROTEIN CENTRAL FOR THE ADAPTATION TO ENVIRONMENTAL CHANGES

Valguarnera, E[1]; Spera, JM[1]; Altabe, S[2]; Ugalde, JE[1]

[1]IIB-UNSAM. [2]IBR-UNR. E-mail: evalguarnera@iibintech.com.ar

Room B

Cell Biology (CB-C01 to CB-C08)

Chairpersons: José Luis Daniotti and Hugo Luján

16:00-16:15

CB-C01

A HIGH-VOLUME EXOPLASMIC HEMI-TMD IS A NOVEL SIGNAL FOR ENDOCYTOSIS AND POLARITY IN YEAST

Gonzalez Montoro A, Bigliani G, Valdez Taubas J.

CIQUIBIC, CONICET - Depto de Química Biológica, Fac. Ciencias Quimicas, Univ. Nac. de Córdoba. E-mail: jvaldezt@dqb.fcq.unc.edu.ar

16:15-16:30

CB-C02

REGULATION OF SECRETORY CAPACITY IN SPECIALIZED SECRETORY CELLS

García IA, Martinez HE, Sampieri L, Alvarez CI.

CIBICI-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. E-mail: agarcia@fcq.unc.edu.ar

16:30-16:45

CB-C03

RAB27A CONTROLS HIV-1 ASSEMBLY BY REGULATING PLASMA MEMBRANE LEVELS OF PHOSPHATIDYLINOSITOL 4,5-BISP

Pereyra Gerber P, Cabrini M, Jancic C, Banchio C, Von Bilderling C, Sigaut L, Pietrasanta L, Ostrowski M.

Instituto de Investigaciones Biomédicas en Retrovirus y SIDA, Universidad de Buenos Aires, Argentina. E-mail: pehuen87@hotmail.com

16:45-17:00

CB-C04

HEMIN INDUCES MITOPHAGY IN K562 CELLS

Salassa BN[2], Vergara AN[2], Grosso RA[1], Moor F[2], Colombo MI[1], Fader CM[1].

[1]Instituto de Histología y Embriología, UNCuyo-CONICET. [2]Facultad de Farmacia y Bioquímica, UJAM. E-mail: bnsalassa@gmail.com

17:00-17:15

CB-C05

THE GIARDIAL EPSIN-LIKE PROTEIN POSSESS A DUAL EPSIN-EPSINR ROLE IN CLATHRIN-MEDIATED TRAFFICKING

Feliziani C[1], Zamponi N[1], Gottig N[2], Rópolo AS[1], Lanfredi-Rangel A[3], Touz MC[1].

[1]Instituto Ferreyra. INIMEC-CONICET. UNC.Cordoba [2]FCBF. IBR-CONICET. Rosario [3]FIOCRUZ-BA, Brazil. E-mail: cfeliziani@immf.uncor.edu

17:15-17:30

CB-C06

ROLE OF THE GTPASE RAB22A DURING ANTIGEN CROSS PRESENTATION BY DENDRITIC CELLS

Cebrián I, Mayorga LS. IHEM (CONICET/UNCuyo) Mendoza. E-mail: ignaciocebrian@yahoo.com.ar

17:30-17:45

CB-C7

RAB24 AND ITS CONNECTION WITH THE ENDOCYTIC/LYSOSOMAL PATHWAY

Amaya C, Militello RD, Colombo MI.

IHEM- CONICET-U.N.CUYO-Mendoza. E-mail:mcolombo@fcm.uncu.edu.ar

17:45-18:00

CB-C08

IDENTIFICATION AND CHARACTERIZATION OF INHIBITORS OF HECT E3 LIGASES BY HIGH THROUGHPUT SCREENING

Rossi M[1], Rotblat B[2], Ansell K[3], Cavasotto CN[1], Ciechanover A[4], Melino G[2].

[1]IBIOBA-MPSP-CONICET. [2]MRC Toxicology Unit. [3]MRC Technology Technion-Israel Institute of Tech. E-mail: mrossi@ibioba-mpsp-conicet.gov.ar

Room C

Signal Transduction (ST-C01 to MI-C08) and Plant Biochemistry and Molecular Biology (PL-C01 and PL-C02)

Chairpersons: José Luis Bocco and Paula Portela

16:00-16:15

ST-C01

SRC IS A CONNECTING PLAYER BETWEEN PKA ACTIVY AND HYPERPOLARIZATION DURING MOUSE SPERM CAPACITATION

Stival C[1], La Spina FA[2], Arranz SA[1], Visconti PE[3], Buffone MG[2], Krapf D[1]

[1]IBR (CONICET-UNR) and Fac Cs Bioq y Farm (UNR). [2]IBYME (CONICET). [3]UMASS-AmherstMAEEUU E-mail: cintusv_87@hotmail.com

16:15-16:30

ST-C02

SPATIO-TEMPORAL CELL SIGNALING DYNAMICS: TOWARDS AN AKT SUBCELLULAR LOCALIZATION FINGERPRINT

Blaustein M, Colman-Lerner A

IFIBYNE-CONICET DFBMC-FCEyN-UBA E-mail: mblaustein@fbmc.fcen.uba.ar

16:30-16:45

ST-C03

ANALYSIS OF THE CELL CYCLE REENTRY DURING MATING PHEROMONE RESPONSE IN *S. cerevisiae*

Grande A, Altszyler E, Chernomoretz A, Colman-Lerner A

DFBMC (FCEN-UBA)IFIBYNE (UBA-CONICET) E-mail: aliciag@fbmc.fcen.uba.ar

16:45-17:00

ST-C04

ACTIVATION OF THE TERMOSENSOR DESK INVOLVES REVERSIBLE FORMATION OF A TRANSMEMBRANE SERINE ZIPPER

Cybulski LE[1], Ballering J[2], Moussatova A[3], Inda ME[1], Tieleman P[3], De Mendoza D[1], Killian A[2]

[1]-Facultad de Cs Bioquímicas y Farm-IBR CONICET [2]Utrecht University [3]Calgary University E-mail: larisa.cybulski@gmail.com

17:00-17:15

ST-C05

TUMOR SUPPRESSOR ACTIVITY OF KLF6 TRANSCRIPTION FACTOR IN ONCOGENESIS TRIGGERED BY ACTIVATED H-RAS

Trucco LD, Nicola JP, Soria G, Bocco JL

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI) CONICET-Univ. Nac. Córdoba. E-mail: ltrucco@fcq.unc.edu.ar

17:15-17:30

ST-C06

NRF2 MEDIATES HO-1 EXPRESSION TRIGGERED BY KSHV-VGPCR - ROLE OF MAPK SIGNALING PATHWAY COMPONENTS

Sapochnik D[1], Medina MV[1], Blengini ML[1], García Solá M[1], Mesri E[2], Coso OA[1]

[1] LFBM – DFBMC - FCEyN – UBA - CONICET. CABA Arg. [2] Univ. of Miami School of Medicine Miami USA. E-mail: omaracoso@yahoo.com

17:30-17:45

PL-C01

ATHB1, AN Arabidopsis HD-ZIP I TRANSCRIPTION FACTOR, IS INVOLVED IN HYPOCOTYL ELONGATION

Capella M; Ribone P; Chan RL

Instituto de Agrobiotecnología del Litoral, CONICET-UNL, CCT-Santa Fe, CP3000, Santa Fe, Argentina. E-mail: mcapella@fbcb.unl.edu.ar

17:45-18:00

PL-C02

CARACTERIZATION OF NEW GENES REGULATED BY GRF TRANSCRIPTION FACTORS

Ferela A; Debernardi JM; Beltramino M; Palatnik JF

Instituto de Biología Molecular y Celular de Rosario (IBR), Rosario, Argentina. E-mail: anto.ferela@hotmail.com

18:00-19:00 ***Argentine Science: Present and Perspectives***

Fernando Goldbaum

Presidente de la Agencia Nacional de Promoción Científica y Tecnológica

Roberto Salvarezza

Presidente del Consejo Nacional de Investigaciones Científicas y Tecnológicas

Lino Barañao

Ministro de Ciencia, Tecnología e Innovación Productiva de Argentina

Chairperson: Hugo Luján

19:00-19:30 ***COFFEE BREAK***

19:30-20:30 ***"ALBERTO SOLS" LECTURE***

Jesús Balsinde

Universidad de Valladolid, España

"Novel lipid mediators of innate immunity and inflammation"

Chairperson: Beatriz Caputto

THURSDAY, November 13, 2014

09:00-11:00 ***SYMPOSIA***

Room A

LIPIDS SYMPOSIUM

Chairpersons: Norma Giusto and Claudia Banchio

09:00-09:30 **Mario Amzel**

The Johns Hopkins School of Medicine, Baltimore, USA

"Control of the levels of PIP3: Structure and Function of the lipid kinase PI3Kalpha"

09:30-10:00 **Ariel Igal**

Columbia University, New York, USA

"New insights into the role of StearoylCoA desaturase-1 in the regulation of metabolic and signaling pathways in cancer"

10:00-10:30 **Alicia Jawerbaum**
Centro de Estudios Farmacológicos y Botánicos, Universidad de Buenos Aires, Argentina
"Lipid metabolism in maternal diabetes. Role of PPARs activation"

10:30-11:00 **Dennis Voelker**
National Jewish Health, Department of Medicine, Denver, USA
"Protein and Lipid Regulators of Lipid Transport to the Locus of Phosphatidylserine Decarboxylase 2"

Room B

SIGNAL TRANSDUCTION SYMPOSIUM

Chairperson: Matias Blaustein

09:00-09:30 **Anabella Srebrow**
IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina
"Post-Translational Modifications Influence the Splicing Machinery and Vice Versa"

9:30-10:00 **Hernán Grecco**
Departamento de Física de la FCEN-UBA, Argentina
"Which, when and where: probing spatiotemporal profiles of Epidermal Growth Factor phosphorylation"

10:00-10:30 **Marcelo Yanovsky**
Instituto Leloir, Buenos Aires, Argentina
"Regulatory Networks Controlling Daily and Seasonal Rhythms in Plants"

10:30-11:00 **Maria Balboa**
Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas, Barcelona, España
"Lipin-1 Integrates Lipid Synthesis with Proinflammatory Responses during TLR Activation in Macrophages"

11:00-11:30 ***COFFEE BREAK***

11:30-12:30 ***"RANWEL CAPUTTO" LECTURE***

Jose Luis Daniotti
CIQUIBIC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina

"Protein lipidation: Role in membrane association, intracellular trafficking and

signalling"

Chairperson: Hugo Maccioni

13:00-15:00	***POSTER SESSION***

CB-P21 to CB-P40	**EN-P01 to EN-P14**
MI-P28 to MI-P53	**LI-P01 to LI-P10**
PL-P23 to PL-P44	**ST-P01 to ST-P15**

15:00-16:00	***"EMBO" LECTURE***

Dominique Ferrandon
Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

"Study of resilience, a second dimension of host defense, in intestinal infections of the genetic model organism Drosophila melanogaster"

Chairperson: Eleonora García Véscovi

16:00-18:00	***ORAL COMMUNICATIONS***

Room A: Cell Biology (CB-C09 to CB-C16)

Room B: Plant Biochemistry and Molecular Biology (PL-C03 to PL-C10)

Room C: Lipids (LI-C01 to LI-C07)

Room A

Cell Biology (CB-C09 to CB-C16)

Chairpersons: Luis Mayorga and Mario Guido

16:00-16:15

CB-C09

GUANINE QUADRUPLEXES AND EMBRYO DEVELOPMENT

Margarit E, David AP, Domizi P, Banchio CE, Armas P, Calcaterra NB.

IBR–CONICET/UNR. Esmeralda y Ocampo, Rosario, Santa Fe, Argentina. E-mail: margarit@ibr-conicet.gov.ar

16:15-16:30

CB-C10

TREACLE DEPLETION IN *Danio rerio* INDUCES OXIDATIVE STRESS AND APOPTOSIS

Mouguelar VS, Porcel de Peralta M, Coux G, Calcaterra NB.

IBR (CONICET/UNR). Esmeralda y Ocampo - Predio CCT. Rosario, Santa Fe – Argentina. E-mail: mouguelar@ibr-conicet.gov.ar

16:30-16:45

CB-C11

OXALATE INDUCES BREAST CANCER TUMOR

Castellaro AM[1], Tonda A[2], Cejas H[3], Ferreyra H[2], Caputto BL[1], Pucci O[2], Gil GA[1] .

[1]Depto. Química Biológica, FCQ, UNC, CONICET. [2]Cat. Ginecología, HNC UNC. [3]Cat. Patología, HNC UNC. E-mail: acastellaro@fcq.unc.edu.ar

16:45-17:00

CB-C12

ACYL-COA SYNTHETASE 4: A NOVEL REGULATOR OF THE MTOR PATHWAY IN BREAST CANCER CELLS

Orlando UD, Castillo AF, Dattilo MA, Solano AR, Maloberti PM, Podestá EJ.

INBIOMED UBA-CONICET, Department of Biochemistry, School of Medicine, University of Buenos Aires. E-mail: ulises_orlando@yahoo.com.ar

17:00-17:15

CB-C13

C-FOS AS ACTIVATOR OF PHOSPHOLIPIDS SYNTHESIS: NEW TARGET IN BRAIN CANCER

Prucca CG, Velazquez FN, Cardozo-Gizzi AM, Caputto BL.

CIQUIBIC (UNC-CONICET). Universidad Nacional de Córdoba, Córdoba, Argentina. E-mail: cprucca@fcq.unc.edu.ar

17:15-17:30

CB-C14

INVOLVEMENT OF PKCα INHIBITION AND RETINOID SYSTEM INDUCTION IN THE MAMMARY CANCER STEM CELL BIOLOGY

Berardi DE, Flumian C, Diaz Bessone MI, Cirigliano SM, Bal de Kier Joffe ED, Urtreger AJ, Todaro LB.

Research Area, Institute of Oncology A.H. Roffo, 5481 San Martin Av (1417), Buenos Aires, Argentina. E-mail: damianberardi@gmail.com

17:30-17:45

CB-C15

SPHINGOMYELIN METABOLISM IS INVOLVED IN THE MAINTENANCE OF TISSUE ORGANIZATION OF COLLECTING DUCTS

Brandán YR[1], Guaytima EV[1], Favale NO[2], Pescio LG[2], Sterin Speziale NB[2], Marquez MG[1].

[1]Instituto de Investigaciones en Ciencias de la Salud Humana - UNLaR [2]FFyB – UBA, IQUIFIB–CONICET. E-mail: brandanyamila@gmail.com

17:45-18:00

CB-C16

ALTERNATIVE LENGTHENING OF TELOMERES IN HUMAN GLIOMA STEM CELLS

Silvestre DC, Jeitany M, Pineda JR, Hoffschir F,Mailliet P, Studler JM, Junier MP, Chneiweiss H, Boussin FD.

Laboratoire de Radiopathologie, CEA, Fontenay-aux-Roses, France. Email: david.silvestre@curie.fr

Room B

Plant Biochemistry and Molecular Biology (PL-C03 to PL-C10)

Chairpersons: Maria Valeria Lara and Sebastián Asurmendi

16:00-16:15

PL-C03

SEARCHING FOR THE N-END RULE PATHWAY IN CHLOROPLASTS AND ITS SUBSTRATE SELECTOR

Colombo CV[1]; Rosano GL[1]; Mogk A[2]; Ceccarelli EA[1]

[1]Instituto de Biología Molecular y Celular de Rosario. [2]Zentrum für Molekulare Biologie, Heidelberg. E-mail: colombo@ibr-conicet.gov.ar

16:15-16:30

PL-C04

EVIDENCE OF *in vivo* PYRUVATE CARBOXYLASE ACTIVITY FOR AN *Arabidopsis* NADP-MALIC ENZYME

Badia M[1]; Mans R[2]; Lis A[2]; Tronconi M[1]; Arias C[1]; Andreo C[1], Drincovich M[1]; Van Maris A[2]; Gerrard Wheeler M[1]

[1]CEFOBI, UNR, Argentina. [2]Department of Biotechnology, Delft University of Technology, Netherlands.E-mail: badia@cefobi-conicet.gov.ar

16:30-16:45

PL-C05

ATHSCB AND ITS ROLE IN PLANT IRON HOMEOSTASIS

Leaden L; Pagani MA; Busi MV; Gomez-Casati DF

CEFOBI-CONICET-UNR, Rosario, Argentina. E-mail: leaden@cefobi-conicet.gov.ar

16:45-17:00

PL-C06

DIFFERENT CONTRIBUTION OF MITOCHONDRIAL METABOLISM TO SYNTHESIS OF LIPID IN SOYBEAN AND CASTOR SEEDS

Gerrard Wheeler MC; Arias CL; Righini Aramburu S; Andreo CS; Drincovich MF; Saigo M

Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), CONICET-UNR . E-mail: saigo@cefobi-conicet.gov.ar

17:00-17:15

PL-C07

A GENOME WIDE EXPRESSION PROFILE OF YERBA MATE (*Ilex paraguariensis* A. St.-Hil.)

Debat HJ[1]; Gabriele M[2]; Aguilera PM[2]; Bubillo RE[3]; Otegui MB[4]; Ducasse DA[1]; Zapata PD[4]; Marti DA[2]

[1]IPAVE-CIAP-INTA. [2]IBS-UNaM-CONICET. [3]EEA Cerro Azul-INTA.[4] INBIOMIS-FCEQyN-UNaM, Misiones. E-mail: humbertodebat@gmail.com

17:15-17:30

PL-C08

THE COX ASSEMBLY PROTEIN ATCOX 10 IS INVOLVED IN PLANT EMBRYOGENESIS AND SENESCENCE

Mansilla N; Garcia L; Gonzalez DH; Welchen E

Instituto de Agrobiotecnología del Litoral (IAL-CONICET-UNL), Santa Fe. E-mail: nmansilla@ial.santafe-conicet.gov.ar

17:30-17:45

PL-C09

CHLOROPLAST-GENERATED REACTIVE OXYGEN SPECIES PLAY A ROLE IN LEAF DEVELOPMENT AND SENESCENCE

Mayta ML; Carrillo N; Lodeyro AL

Instituto de Biología Molecular y Celular de Rosario (IBR; CONICET-Universidad Nacional de Rosario) E-mail: mayta@ibr-conicet.gov.ar

17:45-18:00

PL-C10

AN IRON DEPENDENT DEATH PATHWAY INVOLVED IN THE RESPONSE OF PLANTS TO BIOTIC AND ABIOTIC STRESSES

Martin V [1]; Distéfano A [1]*; Dixon S2; Bellido A [1]; Soto D [1]; Córdoba JP [1]; D´Ippolito S [1]; Colman S [1] Bartoli C[3]; Fiol D[1]; Zabaleta E[2]; Stockwell B[2]; Pagnussat G[3] . *These authors contributed equally to this work*

[1]IIB-CONICET-UNMdP. [2]Dept Biological Sciences, Columbia University. [3]INFIVE- CONICET-UNLP. E-mail: victoriamartin78@gmail.com

Room C

Lipids (LI-C01 to LI-C07)

Chairpersons: Maria del Carmen Fernandez Tome and Marina Gonzalez

16:00-16:15

LI-C01

LIPIDS AS SIGNALS IN STEM CELL DIFFERENTIATION

Montaner A, Girardini J, Costa M, Banchio C.
IBR-CONICET. Ocampo y Esmeralda, Rosario, Argentina. E-mail: montaner@ibr-conicet.gov.ar

16:15-16:30

LI-C02

MOLECULAR MECHANISM OF THE PHOSPHOLIPID SYNTHESIS ACTIVATION BY C-FOS

Cardozo Gizzi AM, Renner ML, Caputto BL.
Depto. de Química Biológica., CIQUIBIC (CONICET), Fac. de Cs. Químicas, UNC,Argentina. E-mail: acardozo@fcq.unc.edu.ar

16:30-16:45

LI-C03

ENDOCANNABINOIDS IN *Caenorhabditis elegans* DAUER DIAPAUSE REGULATION

Galles C, De Mendoza D. Instituto de Biología Molecular y Celular de Rosario. E-mail: galles@ibr-conicet.gov.ar

16:45-17:00

LI-C04

SPHINGOSINE 1 PHOSPHATE CONTROLS SPHINGOLIPIDS SYNTHESIS DURING MDCK CELL DIFFERENTIATION

Santacreu BJ, Sterin Speziale NB, Favale NO.
Fac. de Farmacia y Bioquímica-Universidad de Buenos Aires - IQUIFIB-CONICET. E-mail: bsantacreu@ffyb.uba.ar

17:00-17:15

LI-C05

IMPORTANCE OF THE SPHINGOLIPIDS IN THE CILIUM FORMATION OF MDCK CELLS

Pescio LG, Favale NO, Sterin-Speziale NB.
Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. IQUIFIB - CONICET E-mail: lucilagpescio@yahoo.com.ar

17:15-17:30

LI-C06

***Haematococcus pluvialis* AS A SOURCE OF TRIACYLGLYCEROL FOR BIODIESEL AND VALUABLE CO-PRODUCTS**

Scodelaro Bilbao PG, Damiani C, Martin L, Popovich C, Salvador GA, Leonardi PI
CERZOS-INIBIBB-UNS-CONICET E-mail: pscodela@criba.edu.ar

17:30-17:45

LI-C07

NLPR CONTROLS GENES INVOLVED IN NITROGEN AND LIPID METABOLISM IN OLEAGINOUS *Rhodococcus* STRAINS

Hernández MA, Alvarez HM.
CRIDECIT, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco. E-mail: mahernandez@unpata.edu.ar

18:00-18:30 ***COFFEE BREAK***

18:30-19:30 ***"HECTOR TORRES" LECTURE***

Alberto Kornblihtt
IFIBYNE, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina
"Shedding light on alternative splicing"
Chairperson: Diego de Mendoza

19:30 **SAIB GENERAL ASSEMBLY**

FRIDAY, November 14, 2014

09:00-11:00 ***SYMPOSIA***

Room A

TRANSLATIONAL SCIENCE SYMPOSIUM

Chairpersons: *Mario Guido and Alejandro Colman Lerner*

09:00-09:30 **Joaquín Maximiliano Espinosa**
University of Colorado, USA
"Taming the p53 network for therapeutic purposes"

09:30-10:00 **Claudio Alonso**
University of Sussex, UK
"Hox gene regulation during neural development in health and disease"

10:00-10:30 **Nancy Carrasco**
Yale University, USA
"The sodium/iodide symporter (NIS): an unending source of surprises"

10:30-11:00 **Paul Michels**
University of Edinburgh, UK
"Drug target selection and drug discovery for trypanosomes; the promise of glycosomal enzymes"

Room B

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY SYMPOSIUM

Chairpersons: *Eduardo Zabaleta and Javier Palatnik*

09:00-09:30 **Paula Casati**
CEFOBI – Facultad Ciencias Bioquímicas, CONICET-UNR, Rosario, Argentina
"Role of chromatin remodeling proteins in UV-B responses in plants"

09:30-10:00 **Axel Brennicke**
Molekulare Botanik, Universität Ulm, Germany
“The world of RNA editing in mitochondria and chloroplasts in plants”

10:00-10:30 **Gabriela Pagnussat**
Instituto de Investigaciones Biológicas, CONICET-UNMdP, Mar del Plata, Argentina
“Patterning and fertilization of the Arabidopsis embryo sac in Arabidopsis thaliana”

10:30-11:00 **Javier Botto**
IFEVA – Facultad de Agronomía, CONICET-UBA, Argentina
“*Function of BBX proteins in plant light signaling*”

11:00-11:30 ***COFFEE BREAK***

11:30-12:30 ***“TRANSLATIONAL SCIENCE” LECTURE***

Gabriel Rabinovich
IBYME-CONICET, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina
"Sweetening immune and vascular signaling programs in cancer"
Chairperson: Hugo Luján

13:00-15:00 ***POSTER SESSION***

CB-P41 to CB-P63 **LI-P11 a LI-P23**
MI-P54 to MI-P81 **PL-P45 to PL-P64**
ST-P16 to ST-P32

15:00-16:00 ***PLENARY LECTURE***

Iván Matic
Université Paris Descartes, France
"Stress, mutagenesis and adaptation in bacterial populations"
Chairperson: Andrea Smania

16:00-18:00 ***ORAL COMMUNICATIONS***

Room A: Structural Biology (SB-C01 to SB-C04) and Microbiology (MI-C09 to MI-C12)

Room B: Cell Biology (CB-C17 to CB-C19) and Neuroscience (NS-C01 to NS-C03)

Room C: Biotechnology (BT-C01 to BT-C07)

Room A

Structural Biology (SB-C01 to SB-C04) and Microbiology (MI-C09 to MI-C12)

Chairpersons: Julia Cricco and Paula Vincent

16:00-16:15

SB-C01

ENHANCED CRYSTALLIZATION OF *Leptospira interrogans* HEME OXYGENASE BY DIRECTED MUTAGENESIS

Soldano A, Catalano-Dupuy DL, Ceccarelli EA.
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, UNR. E-mail: soldano@ibr-conicet.gov.ar

16:15-16:30

SB-C02

ANTIBIOTIC RESISTANCE IN *Staphylococcus aureus* BY ALLOSTERIC CONTROL OF PENICILLIN-BINDING PROTEIN 2A

Otero L[13], Fishovitz J[2], Rojas-Altuve A[1], Chang M[2], Mobashery S[2], Hermoso JA[1].
1Instituto Rocasolano, Spain. 2University of Notre Dame, USA. 3Instituto Leloir, Argentina. E-mail: lotero@leloir.org.ar

16:30-16:45

SB-C03

GAINING INSIGHT INTO THE ACTIVATION MECHANISM OF THE RESPONSE REGULATOR NTRX FROM *Brucella abortus*

Fernández I, Otero LH, Klinke S, Carrica MC, Goldbaum FA.
Fundación Instituto Leloir - IIBBA (CONICET). E-mail: ifernandez@leloir.org.ar

16:45-17:00

SB-C04

PHOSPHORYLCHOLINE PHOSPHATASE OF *Pseudomonas aeruginosa*: STRUCTURAL INSIGHTS INTO INHIBITION SITE

Boetsch C[1], Bustos-Guajardo D[2], Alzate-Morales J[2], Verara-Jaque A[2], Lisa AT[1], Beassoni PR[1].
1Depto Biología Molecular, UNRC, Río Cuarto, Argentina. 2CBSM, Univ. de Talca, Chile. E-mail: cristhian.boetsch@gmail.com

17:00-17:15

MI-C09

THE MISMATCH REPAIR PROTEIN MutS CONTROLS THE ACCESS TO REPLICATION OF THE DNA POLYMERASE IV

Margara, LM; Argaraña, CE; Monti, MR
CIQUIBIC-CONICET, Dpto. de Qca. Biol,, FCQ-UNC, Córdoba, Argentina. E-mail: lmargara@fcq.unc.edu.ar

17:15-17:30

MI-C10

DIVALENT METAL IS REQUIRED FOR LIGAND BINDING TO THE *E. faecalis* CITRATE METABOLISM REGULATOR

Blancato, V1[2]; Magni, C[2]; Lorca, GL[1]
[1]Department of Microbiology & Cell Science, University of Florida, USA. [2]IBR-CONICET-UNR, Argentina.
E-mail: blancato@ibr.gov.ar

17:30-17:45

MI-C11

CHARACTERIZATION OF ESSENTIAL ACYL- COENZYME A CARBOXYLASES OF *Mycobacterium tuberculosis*

Bazet Lyonnet, B; Diacovich, L; Cabruja, M; Gago, G; Gramajo, H

IBR, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario. E-mail: bazetlyonnet@ibr-conicet.gov.ar

17:45-18:00

MI-C12

APPLICATION OF FLUOROPHAGES FOR TB-DETECTION AND WHOLE CELL SCREENING OF ANTITUBERCULAR DRUGS

Rondón, L[1];Urdániz, E[1]; Latini, C[3]; Matteo, M[3]; Poggi, S[3]; Martí, M1; Hatfull, G[2]; Piuri, M[1]
[1]Depto. de Química Biológica, FCEyN, UBA, IQUIBICEN-CONICET, Bs As, Argentina. E-mail: lrondon@qb.fcen.uba.ar

Room B

Cell Biology (CB-C17 to CB-C19) and Neuroscience (NS-C01 to NS-C03)

Chairpersons: Marisa Colombo and Nora Calcaterra

16:00-16:15

CB-C17

HOW IS CHOLINE KINASE ALPHA REGULATED DURING RA-INDUCED NEURONAL DIFFERENTIATION?

Domizi P, Banchio C.
IBR-CONICET, Ocampo y Esmeralda, Rosario, Argentina. E-mail: domizi@ibr-conicet.gov.ar

16:15-16:30

CB-C18

E2F1 AND E2F2 INDUCTION IN RESPONSE TO DNA DAMAGE PRESERVES GENOMIC STABILITY IN NEURONAL CELLS

Castillo DS[1], Campalans A[2], Radicella JP[2], Cánepa ET[1], Pregi N[1].
[1]Depto QB, FCEN, UBA, Argentina [2]CEA, iRCM, Fontenay aux Roses, France. E-mail: ecanepa@qb.fcen.uba.ar

16:30-16:45

CB-C19

LYSOPHOSPHATIDYLCHOLINE: A SIGNAL FOR NEURONAL DIFFERENTIATION IN NEURO-2A CELLS

Paoletti L, Domizi P, Banchio C.
IBR-CONICET. Ocampo y Esmeralda, Rosario, Argentina. E-mail: paoletti@ibr-conicet.gov.ar

16:45-17:00

NS-C01

ROLE OF C-FOS DURING NEUROGENESIS

Velazquez FN[1], Prucca CG[1], Etienne O[2], D'Astolfo DS[1], Silvestre DC[2], Boussin FD[2], Caputto BL[1].
[1]CIQUIBIC-CONICET, Dpto. de Qca. Biológica, FCQ-UNC, Córdoba, Argentina, [2]CEA, IRCM, Paris, France. E-mail: fvelazquez@fcq.unc.edu.ar

17:00-17:15

NS-C02

C-FOS REGULATES BRANCHING FORMATION IN NEURONS

Rodríguez Berdini L, Ferrero GO, Cardozo Gizzi AM, Caputto BL.
Dpto. de Química Biológica, CIQUIBIC (CONICET), Fac. de Ciencias Químicas, UNC, Argentina. E-mail: lrodriguez@fcq.unc.edu.ar

17:15-17:30

NS-C03

PEA3 TRANSCRIPTION FACTORS: MEDIATORS OF BDNF-MEDIATED HIPPOCAMPAL DENDRITE GROWTH DURING DEVELOPMENT.

Fontanet PA, Alsina FC, Irala D, Bonafina A, Paratcha G, Ledda MF.
Molecular and Cellular Neuroscience Division. IBCN - CONICET. Buenos Aires University, Argentina. E-mail: paulafontanet@gmail.com

Room C

Biotechnology (BT-C01 to BT-C07)

Chairpersons: Verónica González Pardo and Juan Carlos Diaz Ricci

16:00-16:15
BT-C01
RECOMBINANT GLYCOPROTEINS AS NOVEL ANTIGENIC TARGETS FOR DIAGNOSIS OF HEMOLYTIC UREMIC SYNDROME

Melli LJ[1], Ciocchini AE[1], Caillava AJ[1], Chinen I[2], Rivas M[2], Feldman MF[3], Ugalde JE[1], Comerci DJ[1].
[1]IIB-UNSAM-CONICET [2]Servicio Fisiopatogenia ANLIS-Malbrán [3]Dept. of Biological Sciences UofA, Canada. E-mail: lucianomelli@gmail.com

16:15-16:30
BT-C02
PHOSPHOLIPASE C FOR SOYBEAN OIL DEGUMMING

Elena CE, Ravasi P, Cerminati S, Peirú S, Menzella HG, Castelli ME.
IPROBYQ CONICET, Faculta de Ciencias Bioquímicas y farmacéuticas, Universidad Nacional de Rosario. E-mail: clauelena13@gmail.com

16:30-16:45
BT-C03
HIGHLY EFFICIENT PHOSPHOLIPASE C PRODUCTION FOR INDUSTRIAL OIL DEGUMMING USING *Pichia pastoris*

Ravasi P, Elena CE, Castelli ME, Cerminati S, Peirú S, Menzella HG.
IPROBYQ CONICET, Facultad de Ciencias Bioquimicas y Farmacéuticas Universidad Nacional de Rosario. E-mail: pablo.ravasi@gmail.com

16:45-17:00
BT-C04
TRANSGENIC *Arabidopsis* PLANTS BEARING *PROMOTERHAHB11::HAHB11* EXHIBIT SIGNIFICANTLY ENHANCED BIOMASS

Giacomelli JI, Mora MC, Cabello JV, Chan RL.
IAL Instituto de Agrobiotecnología del Litoral, CONICET-UNL, Santa Fe, Argentina. E-mail: giacomelli@fbcb.unl.edu.ar

17:00-17:15
BT-C05
SENSITIVE WHOLE-CELL BIOSENSORS FOR THE DETECTION OF A BROAD-SPECTRUM OF TOXIC METAL IONS

Cerminati S, Soncini FC, Checa SK
Instituto de Biología Molecular y Celular de Rosario (IBR) CONICET-UNR, Rosario, Argentina
E-mail: checa@ibr-conicet.gov.ar

17:15-17:30
BT-C06
METABOLIC ENGINEERING FOR THE PRODUCTION OF NEUTRAL LIPIDS IN *Escherichia coli*

Menendez-Bravo SM, Comba S, Sabatini M, Arabolaza AL, Gramajo HC.

Instituto de Biología Molecular y Celular de Rosario. E-mail: menendezbravo@ibr-conicet.gov.ar

17:30-17:45

BT-C07

VARIANT-SPECIFIC SURFACE PROTEINS FROM *Giardia lamblia* AS A NEW TOOL FOR ORAL DRUG DELIVERY

Serradell MC, Martino RA, Rupil LL, Gargantini PR, Saura A, Lujan HD.
CIDIE-CONICET. School of Medicine, Catholic University of Cordoba. Argentina E-mail: marianelaserradell@gmail.com

18:00-18:30	***COFFEE BREAK***

18:30-19:30	***CLOSING LECTURE***

Marcelo Rubinstein

INGEBI-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

"On the molecular and functional genetics of food intake and body weight regulation"

Chairperson: José Luis Bocco

21:30	***CLOSING CEREMONY AND AWARDS*** ***CLOSING DINNER***

ADENDA
COMUNICACIONES ORALES

FRIDAY, November 14, 2014

Room A

Structural Biology (SB-C01 to SB-C05) and Microbiology (MI-C09 to MI-C12)

Chairpersons: Julia Cricco and Paula Vincent

18:00-18:15

SB-C05

A 2-HELIX COILED-COIL CONTROLS THE ACTIVITIES OF DESK, A BACTERIAL THERMOSENSOR

Saita E; Abriata L; Del Peraro M; De Mendoza D; Albanesi A
Instituto de Biología Molecular y Celular de Rosario - IBR E-mail: saita@ibr-conicet.gov.ar

Room B

Cell Biology (CB-C17 to CB-C21) and Neuroscience (NS-C01 to NS-C03)

Chairpersons: Marisa Colombo and Nora Calcaterra

17:30-17:45

CB-C20

BRADYKININ (BK) FAVOURS THE FORMATION OF MIGRATORY COLONIES OF NEPHROGENIC URETERIC BUD (UB) CELLS

Guaytima EV[1]; Brandán YR[1]; Megías FE[1]; Favale NO[2]; Sterin Speziale NB[2]; Marquez MG[1]
[1]Instituto de Investigaciones en Ciencias de la Salud Humana – UNLaR; [2]FFyB – UBA, IQUIFIB-CONICET
E-mail: edithguaytima@hotmail.com

17:45-18:00

CB-C21

DIFFERENT STRATEGIES FOR PROTEOMIC IDENTIFICATION OF SUMOYLATED PROTEINS IN *Trypanosoma brucei*

Iribarren PA[1]; Berazategui MA[1]; Bayona JC[1]; Tammsalu TT[2]; Hay RT[2]; Cazzulo JJ[1]; Alvarez VE[1]
[1]IIB-INTECH / UNSAM-CONICET [2]Centre for Gene Regulation and Expression, University of Dundee
E-mail: piribarren@iibintech.com.ar

Room C

Biotechnology (BT-C01 to BT-C08)

Chairpersons: Verónica González Pardo and Juan Carlos Diaz Ricci

17:45-18:00

BT-C08

RELATIONSHIP BETWEEN MUSCLE CELL PROLIFERATION AND GROWTH FACTORS IN PEJERREY DURING STARVATION

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LECTURES

L-01
PERSONAL RECOLLECTIONS OF A LIFE-LONG AFFAIR WITH CELL BIOLOGY

Sabatini DD.
New York University School of Medicine

I entered the field of cell biology during its formative phase and contributed technical advances in electron microscopy that gave new insights into the physiological roles of subcellular compartments. I concentrated for many years on the structure and function of the rough endoplasmic reticulum where I aimed at explaining mechanistically how the functional specialization of ribosomes bound to the endoplasmic reticulum membranes is achieved.This work eventually led to the formulation of the signal hypothesis with Gunter Blobel in 1971. Subsequently, my laboratory contributed to the birth of the field of protein traffic with the demonstration that membrane-bound ribosomes in the ER are also responsible for the synthesis of membrane and luminal proteins destined to other subcellular compartments, pointing to the existence of sorting signals in the newly synthesized polypeptides and corresponding discriminating trafficking mechanisms. I have derived great satisfaction from the fact that some of the work I will present, including the introduction with M. Cerejido of the MDCK cell line to study the development and properties of polarized epithelia and the discovery with Rodriguez-Boulan of the polarized budding of enveloped viruses, has helped many laboratories to continue to explore the fascinating mechanisms that contribute to generate the complex organization of eukaryotic cells.

L-02
CELLULAR ION HOMEOSTASIS: EMERGING ROLES OF INTRACELLULAR NHX-TYPE Na^+/H^+ ANTIPORTERS IN PLANT GROWTH AND DEVELOPMENT

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The homeostatic control of ions and pH in intracellular compartments are fundamental to basic cellular processes needed to maintain normal plant growth as well as the responses to stress.Intracellular NHX-type Na^+/H^+ exchangers are important for the regulation of pH and ion homeostasis and play significant roles in a variety of physiological processes. *Arabidopsis* contains six intracellular NHX isoforms; NHX1 to NHX4 reside on the vacuolar membrane (tonoplast) while NHX5 and NHX6, localize to Golgi and *trans*-Golgi network (TGN). We compareddouble, triple and tetra *nhx1 – nhx4* knockouts and showed the role of tonoplast NHXs on vacuolar K^+ uptake, osmotic regulation, cytoskeleton organization, the generation of vacuolar turgor needed to drive cell expansion as well as the cellular responses to high salinity.
Using *nhx5nhx6* double knockouts, we further investigated the role of intravesicular pH and ion homeostasis on vesicular trafficking and protein processing.Using a combination of approaches including *in vivo* protein-protein interactions, biochemical characterization of protein complexes, and *in vivo* endomembrane pH measurements, we provide evidence indicatingthat pH and/or ion homeostasis, controlled by NHX5 and NHX6, is a requisite for receptor mediated processing of storage proteins and trafficking of storage proteins to protein storage vacuoles.

L-03
VERY LONG CHAIN FATTY ACIDS: THEIR ROLE IN RETINA AND BRAIN FUNCTION

Anderson RE, *Hopiavuori B, Agbaga M-P.*
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In 1987, Marta Aveldaño identified very long chain polyunsaturated fatty acids (VLC-PUFA, ≥ C26) as significant components of retinal rod outer segment membranes. VLC-PUFA and VLC-SFA (saturated fatty acids ≥ C26) are synthesized by ELOngation of Very Long chain fatty acids-4 (ELOVL4). Four distinct mutations in *ELOVL4* are the cause of autosomal dominant Stargardt-like Macular Dystrophy-3 (STGD3), a juvenile form of macular degeneration. Studies from our lab have shown that the mutant ELOVL4 has no enzymatic activity for VLC-PUFA synthesis and that conditional deletion of *Elovl4* from mouse photoreceptors leads to rod cell death and synaptic dysfunction. Patients with STGD3 have no other systemic phenotypes. Recent studies by others have identified four additional mutations in *ELOVL4* that cause spinocerebellar ataxia (SCA, posture, gait, etc.) and erythrokeratodermia variabilis (EKV, skin problems), but without any retinal abnormalities. We found that deletion of *Elovl4* in mouse brain causes

early onset seizure/hyperactivity disorder. How is it possible that different mutations in the same gene can cause such tissue-specific phenotypes? ELOVL4 in retina and testis makes VLC-PUFA, while in brain and skin the major products are VLC-SFA. We hypothesize that the different mutations in *ELOVL4* affect the quality and the quantity of VLC-FA synthesized, either by altering substrate specificities for mutated ELOVL4 or by a dominant negative effect of the mutant protein on the products made by the wild type ELOVL4.

L-04
NOVEL LIPID MEDIATORS OF INNATE IMMUNITY AND INFLAMMATION

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Exposure of human peripheral blood monocytes to free arachidonic acid (AA) results in the rapid induction of lipid droplet (LD) formation by these cells. LD are formed by two different routes, namely (i) the direct entry of AA into triacylglycerol and (ii) activation of intracellular signaling leading to increased neutral lipid formation utilizing fatty acids coming from the de novo biosynthetic route. The latter predominates, accounting for 60-70% of total LD formation, and can be completely inhibited by selective inhibition of the group IVA cytosolic phospholipase A_2, pointing out this enzyme as a key regulator of AA-induced signaling. In other work, we applied mass spectrometry-based lipid profiling to study the levels of AA-containing phospholipids in macrophages. We identified an unusual inositol phospholipid molecule, PI(20:4/20:4), the levels of which do not decrease but actually increase by 300% after activation of the cells. Elevating the intracellular concentration of PI(20:4/20:4) by introducing the lipid into the cells results in enhancement of the microbicidal capacity of macrophages but does not change gene expression in response to inflammatory stimuli, highlighting the selectivity of action of PI(20:4/20:4). These findings suggest that PI(20:4/20:4) is a novel bioactive inositol phospholipid molecule that regulates innate immune responses in macrophages

L-05
PROTEIN LIPIDATION: ROLE IN MEMBRANE ASSOCIATION, INTRACELLULAR TRAFFICKING AND SIGNALING

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A wide variety of proteins are modified by covalently-linked fatty acids and/or prenyl groups, which may confer reversible association of the lipid-modified protein with membranes. Moreover, each distinct fatty acid or prenyl moiety provides particular information to assist proteins in finding their correct spatial organization and biological function. We have focused on understanding the consequences of proteins S-acylation on intracellular trafficking using as model protein members of the Ras family GTPases. S-acylation, unlike other lipid modification such as N-myristoylation and prenylation, is the only reversible fatty-acid modification. Despite the progress that has been made in identifying and characterizing protein acyltransferases involved in S-acylation much less is known about the thioesterases involved in protein deacylation. The recent identification in our laboratory of the second bona fide protein thioesterase (APT2), allow us to better characterize the deacylation process and its role in the spatial distribution of peripheral proteins. Overall, our findings illustrate how lipid modification of proteins plays an important role in dictating precise intracellular movements within the cells by regulating membrane-cytosol exchange and/or by modifying the flux of the proteins through vesicular transport systems

L-06
STUDY OF RESILIENCE, A SECOND DIMENSION OF HOST DEFENSE, IN INTESTINAL INFECTIONS OF THE GENETIC MODEL ORGANISM *Drosophila melanogaster*

Lee KZ[1], Lestradet M[1], Liegeois S[1], Limmer S[1], Yamba WM[1], Keime C[2], Schwab Y[2], Ferrandon D.[1]
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Host defense is not limited to immunity and encompasses the ability to endure and repair damages and to handle toxins and toxicants. After a general introduction on the *Drosophila* model and the notion of resilience to infections, I will describe the existence in the flyintestine of a common response to *Serratia marcescens* hemolysin, a pore-forming toxin, and to xenobiotics such as caffeine, soft or heavy metals, or a strong oxidant. Exposure of enterocytes

to hemolysin leads to the rapid formation of megamitochondria and a subsequent controlled extrusion of the cytoplasm along with damaged organelles, which may constitute a novel repair mechanism. This results in a thin intestinal epithelium that recovers its original shape in a few hours. The recovery process requires *CyclinJ*, an evolutionary conserved cyclin of hitherto unknown function. Finally, a primary exposure to toxin or xenobiotics induces cross-protection against a further hemolysin challenge, thus evoking a hormetic response, which can be elicited by ectopically expressing *what else,* a *CyclinJ*-dependent gene. Thus, *CyclinJ* plays a central role in this novel resilience mechanism that defends the intestine against infections or intoxications.

L-07
SHEDDING LIGHT ON ALTERNATIVE SPLICING

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Regulation of alternative splicing (AS) by transcription elongation can occur via changes in the RNA polymerase II (pol II) molecule itself or in chromatin structure. The first mode is illustrated by the effects of DNA damage caused by UV irradiation on AS. The UV effect does not imply damage of the DNA template in cis, and is caused by inhibition of pol II elongation due to hyperphosphorylation of its carboxy terminal domain. We have found that two different types of DNA lesions caused by UV irradiation [CPDs and (6-4)PPs] trigger different signaling effects on specific alternative splicing events. White light is a source of energy and also a regulator of plant physiological adaptations. In a second study we have shown that light/dark conditions affect alternative splicing of a subset of Arabidopsis genes preferentially encoding proteins involved in RNA processing. The effect requires functional chloroplasts and is also observed in roots when the communication with the photosynthetic tissues is not interrupted, suggesting that a signaling molecule travels through the plant. Using photosynthetic electron transfer inhibitors with different mechanisms of action, we deduce that the reduced pool of plastoquinones initiates a chloroplast retrograde signaling that regulates nuclear alternative splicing and is necessary for proper plant responses to varying light conditions

L-08
'SWEETENING' IMMUNE AND VASCULAR SIGNALING PROGRAMS IN CANCER

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The function of deciphering the biological information encoded by the glycome, which is the entire repertoire of complex carbohydrate structures expressed by cells and tissues, is assigned in part to endogenous glycan-binding proteins or lectins. Galectins, a family of animal lectins that bind N-acetyllactosamine-containing glycans, have many roles in diverse tumor-associated processes, including those relevant to tumor-immune escape and angiogenesis. How do galectins translate glycan-encoded information into immune and vascular signaling programs? We will discuss the essential roles of galectin-1 in promoting tumor-immune escape, regulating chronic inflammation and mediating angiogenesis rescue programs. Particular emphasis will be given to recent findings demonstrating that interactions between tumor-derived galectin-1 and complex N-glycans on endothelial cells can link tumor hypoxia, endothelial cell signaling, vascularization and tumor-associated inflammation. The therapeutic implications of these findings will be highlighted.

L-09
STRESS, MUTAGENESIS AND ADAPTATION IN BACTERIAL POPULATIONS

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Evolutionary success of bacteria relies greatly on the constant fine-tuning of their mutation rates, which optimizes their adaptability to constantly changing environmental conditions. When adaptation is limited by the mutation supply rate, natural selection favors increased mutation rates by acting on allelic variation of the genetic systems that control fidelity of DNA replication and repair. Mutator alleles are carried to high frequency through hitchhiking with the adaptive mutations they generate. Butwhen fitness gain no longer counterbalances the fitness loss due to continuous generation of deleterious mutations, natural selection favors reduction of mutation rates. However, bacteria also posses capacity to adapt mutation rates to theenvironmental conditions by modulating gene expression. This is interesting from an evolutionary point of view because, by reducing the increase in the mutation rate to the

phases of adaptation, it should reduce the overall cost of high mutation rates. However, the nature of selective pressure acting on the molecular mechanisms controlling stress-induced mutagenesisis hotly debated.For the first time,we provide, evidence that stress-induced mutagenesis is not only an unavoidable by-product of mechanisms involved in survival under stress, but that it could be selected for its capacity to increase evolvability of bacterial populations.

L-10
ON THE MOLECULAR AND FUNCTIONAL GENETICS OF FOOD INTAKE AND BODY WEIGHT REGULATION

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Energy balance in vertebrate animals is regulated by specialized brain areas that integrate central, peripheral and environmental signals that promote food intake or satiety. A group of neurons present in the arcuate nucleus of the hypothalamus mediate satiety by releasing the anorectic neuropeptides α- and γ-MSH encoded by the proopiomelanocortin gene (*POMC*). The physiological relevance of central melanocortins can be readily appreciated in mice lacking central *Pomc* expression which are hyperphagic and display early onset severe obesity, as humans carrying *POMC* null alleles. In this SAIB lecture I will present our studies on the transcriptional regulation of *Pomc* in the brain at the molecular, evolutionary and behavioral level. Neuronal *Pomc* expression is controlled by two upstream distal enhancers, nPE1 and nPE2, which are highly conserved in mammals. During mammalian evolution, nPE1 and nPE2 were co-opted as neuronal enhancers into the *POMC locus* after the sequential insertion of two unrelated retroposons. Since both enhancers drive identical spatio-temporal expression patterns to all POMC neurons, nPE1 and nPE2 are functional analogs that represent an authentic example of convergent molecular evolution of cell-specific transcriptional enhancers. I will discuss the adaptive value of having two apparently redundant enhancers, instead of just one, driving neuronal *Pomc* expression.

SYMPOSIA

Microbiology

MI-S01
ENZYMATIC DIVERSITY IN LIPOIC ACID MODIFICATION OF PROTEINS

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Lipoic acid (LA) is a covalently bound cofactor essential for the function of several key enzymes involved in oxidative and single carbon metabolism. The genes for LA synthesis were initially identified in *Escherichia coli*, and the current model for protein lipoylation is based in the mechanism employed by this bacterium. It involves two pathways: one in which exogenous LA is transferred to apoproteins in a process mediated by LA ligase (LplA), and an endogenous one, that involves LipB, which transfers octanoate to target proteins. These octanoylated domains are converted into lipoylated derivatives by lipoyl synthase (LipA).
We have previously shown that *B. subtilis* is able to synthesize LA, and if exogenously provided, ligate it to apoproteins. We have also demonstrated that LipL and LipM are essential for the attachment of octanoic acid to the apoenzymes. However, the role of each of these proteins was not clear. In this work we performed physiological and biochemical characterization of different LA auxotrophic mutants and used *in vitro* biochemical assays that allowed us to conclude that *B. subtilis* protein lipoylation is carried out through a novel mechanism. This pathway involves the sequential action of LipM and LipL and a third protein, the glycine cleavage system H protein, which acts as a novel lipoyl/octanoyl carrier. In *B. subtilis* four proteins, LipM, GcvH, LipL, and LipA, are essential for the endogenous protein lipoylation pathway, instead of the two-protein model of *E. coli.* Notably, LipL homologues can be found in pathogenic bacteria closely related to *B. subtilis* such as *Staphylococcus aureus* and *B. anthracis*.

MI-S02
EHEC SINGS: POUR SOME SUGAR ON ME

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Gastrointestinal (GI) bacteria sense diverse environmental signals, including host hormones and nutrients, as cues for differential gene regulation and niche adaptation. Although the impact of carbon nutrition on the colonization of the gut by the microbiota has been extensively studied, the extent to which carbon sources affect the regulation of virulence factors by invading pathogens has not been fully defined. The enteric pathogen enterohemorrhagic*Escherichia coli* (EHEC) gages sugar sources as an important cue to regulate expression of its virulence genes. Specifically, this sugar dependent regulation fine tunes the expression of the locus of enterocyte effacement (LEE) pathogenicity island, which encodes for a type three secretion system, effectors, and an adhesin necessary for the formation of attaching and effacing (AE) lesions on enterocytes. Glycolytic environments inhibit the expression of the LEE genes. Conversely, growth within a gluconeogenic environment activates expression of these genes. Part of this sugar-dependent regulation is achieved through two transcription factors: KdpE and Cra. Cra and KdpE interact to optimally directly activate expression of the LEE genes in a metabolite dependent fashion. This sugar dependent regulation is key during infection of the mammalian host, given that a *kdpE* mutant is attenuated *in vivo*. Additionally, a novel two component signal transduction system, named FusKR (where FusK is a membrane bound histidine sensor kinase, and FusR a response regulator) that senses fucose, controls expression of the LEE genes. This fucose-sensing system is required for robust EHEC intestinal colonization. During growth in mucus, the glycophagicprominent member of the GI microbiota, *Bacteroidesthetaiotaomicron,* supplies fucose to EHEC, modulating its virulence gene expression. Our findings suggest that EHEC uses fucose, a host-derived signal made available by the microbiota, to modulate EHEC virulence and metabolism, and suggest a new layer of complexity in the inter kingdom signaling that underlies EHEC pathogenicity

MI-S03
THE ROLES OF HEAVY METAL TRANSPORT ATPases IN BACTERIAL VIRULENCE

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Transition metals (copper, zinc, iron, manganese, etc.) are essential micronutrients for all living organisms. However, at high concentrations they are toxic. TransportP-ATPases are membrane proteins that couple the hydrolysis of ATP to the efflux of cytoplasmic metals. These have two overarching physiological functions: to maintain cytoplasmic metal levels and to provide metals for the periplasmic assembly of metalloproteinssecreted out of the cell. Our studies have shown that both roles are critical for bacterial virulence. Cu^{+}-ATPases appear key to overcome high phagosomalCu^{+} levels and are required for the assembly of cytochrome oxidases,essential for survival in extreme oxidant environments. In a distinct example, *M. tuberculosis* CtpC, a Mn^{2+}-ATPases, is required for the synthesis of superoxide dismutase, a relevant enzyme for *M. tuberculosis* growth in the human host.

MI-S04
INFLAMMASOME ACTIVATION IN RESPONSE TO INFECTION WITH INTRACELLULAR PATHOGENS

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Activation of the inflammasome occurs in response to a notably high number of pathogenic microbes and is a broad innate immune response that effectively contributes to restriction of pathogen replication and generation of adaptive immunity. Activation of these platforms leads to caspase-1- and/or caspase-11-dependent secretion of proteins, including cytokines, and induction of a specific form of cell death called pyroptosis, which directly or indirectly contribute for restriction of pathogen replication. Not surprisingly, bona fide intracellular pathogens developed strategies for manipulation of cell death to guarantee intracellular replication. In this sense, the remarkable advances in the knowledge of the inflammasome field have been accompanied by several reports characterizing the inhibition of this platform by several pathogenic microbes. Data to be presented illustrate the activation of the inflammasome in response to pathogenic microbes and the microbial strategies to bypass inflammasome activation.

Cell Biology

CB-S01
THE SECRETORY PATHWAY AND ITS ADAPTATION TO A HIGH SECRETORY DEMAND

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Membrane transport from the ER to the Golgi complex requires multiple molecules located in at least three distinct membrane entities: at specialized subdomains of the Endoplasmic reticulum called ERES (ER exit sites), at VTCs (Vesicular tubular clusters) and at *cis*-Golgi network. The mammalian Rab1 GTPase is essential for ER to Golgi transport and, through its interactions with diverse effector proteins, regulates the formation, tethering and fusion of transport carriers derived from the ER. It is clear that Rab1b is acting in sequential stages at the ER-Golgi transport and we propose a model that postulates that Rab1b is a coordinator of this step of transport.
Furthermore, since Rab1 isoforms (Rab1a and Rab1b) are ubiquitous and different tissues express dissimilar mRNA levels of Rab1 isoforms, we also analyzed cellular effects induced by changes of Rab1b levels. We report that an increase in Rab1b levels induces changes in Golgi structure and gene expression. Such gene expression changes require the activation of p38 mitogen-activated protein kinase (MAPK) and the cAMP-responsive element-binding protein (CREB) binding consensus site. The data strongly suggest that a Rab1b increase is required to elicit a secretory response.

CB-S02
INTRACELLULAR TRAFFICKING OF PHAGOSOMES: LESSONS FROM MYCOBACTERIA

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The dynamic and complex process of phagosome maturation in phagocytes is the result of multiple interactions between the phagosome and various intracellular compartments.Critical events occurring in the lumen and membrane of phagosomes have a profound impact in the development of an appropriate innate and adaptive immune response and intracellular pathogens, such as *Mycobacterium tuberculosis*, have learnt to subvert the normal phagosome maturation pathway for their own benefit. Using a combination of biochemical and advanced light microscopy techniques, we have recently contributed to the characterization of two small GTPases involved in the trafficking and function of phagosomes in macrophages.We have identified Rab34 and its effector Munc13-2 as important regulators of both a Rab7-independent pathway of phagolysosome biogenesis and intracellular killing of mycobacteria. Moreover, we identified Rab20 and its effector Rabex-5 as key players in a novel Rab cascade by which IFN-γ induces a delay in phagosome maturation in macrophages. Altogether, our studies uncover novel players in phagosome maturation and highlight the importance of uncharacterised Rab GTPases in regulating phagosome biology and killing of intracellular mycobacteria

CB-S03
THE MOLECULAR BASIS FOR PLATELET SECRETION

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Activated platelets release 100's of molecules needed for hemostasis and the *sequelae* of thrombosis. Platelets have 3 granule types: dense, alpha, and lysosomes. Cargo release requires SNAREs (Soluble NSF Attachment Protein Receptors). v-SNAREs from the granules and heterodimeric t-SNAREs from the plasma membrane, form a trimer that spans the fusing membranes. Using knockout mice, we defined the granule secretion machinery. Platelets contain 4 v-SNAREs (VAMP 2, 3, 7, and 8). Deletion of VAMP 8 attenuates cargo release; however deletion of VAMP 2, 3, and 8 is required for defective hemostasis. Platelets contain 6 t-SNAREs (Syntaxin 2, 4, 7, 11, 13, and SNAP-23). Patients lacking Syntaxin 11 show a loss of secretory function. Deletion of Syntaxin 2/4 results in an endocytic defect. SNARE regulation is also important. Phosphorylation of SNAP-23 by IkappaB Kinase (IKK) is important for SNARE complex formation and secretion. Acylation of SNAP-23 and Syntaxin 11 is also important for secretion. Multiple regulators have been identified. Munc18b is a Syntaxin 11 chaperone. Tomosyn-1 regulates the t-SNARE complexes. Munc13-4 is important for granule release. Analysis shows that Munc13-4 is required to increase the efficacy of membrane fusion. This greater understanding of platelet exocytosis will undoubtedly lead to the development of better anti-thrombotic therapeutics. Supported by HL56652.

CB-S04
MELANOSOME BIOGENESIS AND MECHANISMS OF INTERCELLULAR COMMUNICATION IN THE SKIN

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Melanosomes, the lysosome-related organelles (LROs) of pigment cells synthesize and store melanins fated for transfer to keratinocytes. Melanosome biogenesis requires trafficking of melanosomal proteins within distinct endosomal subdomains. During early melanogenesis, sorting of the protein PMEL to intraluminal vesicles of multivesicular bodies (MVBs) is concomitant with its cleavage and formation of PMEL-driven amyloid fibrils. Sorting of PMEL and fibril formation requires its interaction with the Tetraspanin CD63 and the protease BACE-2 processes PMEL to form the physiological amyloid. Late melanogenesis involves the transfer of melanogenic enzymes from early recycling endosomes to melanosomes. Proteins mutated in the Hermansky Pudlak syndrom regulate endosomal trafficking and late stages of melanogenesis and together with motors and Rabs are involved in endosome dynamics. The kinesin motor KIF13A interacts with the adaptor AP-1 to coordinate sorting and positioning of peripheral endosomal domains close to melanosomes and KIF13A via its interaction with Rab11 is essential for endosome tubulogenesis. Our current studies focus on how endosome dynamics, melanosome biogenesis and consequently melanosome transfer to keratinocytes is regulated by direct interactions with keratinocytes and whether secreted vesicles called exosomes modulate the pigmentation status of the melanocytes.

Lipids

LI-S01
CONTROL OF THE LEVELS OF PIP3: STRUCTURE AND FUNCTION OF THE LIPID KINASE PI3KALPHA

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Phosphatidylinositide-3-kinase-□ (PI3K□) catalyzes the phosphorylation of PIP2 to produce PIP3 in response to phosphorylated receptor tyrosine kinases (RTK) or their substrates. The increased level of PIP3 initiates a number of signaling pathways by recruiting other kinases, such as Akt, to the plasma membrane. PI3K□□(subunit composition: p110 and p85), is an oncogene frequently mutated in many cancer types. These mutations increase PI3K kinase activity leading to increased tumor cell survival, cell motility, cell metabolism, and cell cycle progression. Several structures of the enzyme, determined by X-ray diffraction□ reveal that the enzyme has a complex architecture in which each domain interacts with several domains of the same or the other subunit. The structural data show that physiological activation, as well as activation by some oncogenic mutations, involves relief of autoinhibition by dislodging the inhibitory nSH2 domain of the regulatory subunit p85 from its inhibitory position. Computational studies show that most of these effects involve, in addition to structural changes, modifications of the dynamics of the protein that alter the relative stabilities of the different states accessible to the enzyme. In contrast, another of the most common oncogenic mutation, H1047R in p110, activates the enzyme by different mechanism: it increases the interaction of the enzyme with the cellular membrane expanding its access to the substrate, a membrane component.

LI-S02
NEW INSIGHTS INTO THE ROLE OF STEAROYLCOA DESATURASE-1 IN THE REGULATION OF METABOLIC AND SIGNALING PATHWAYS IN CANCER

Igal RA.
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The constitutive activation of lipid biosynthetic enzymes is a metabolic hallmark of cancer cells and tumors. Initial studies from our lab and others showed that the levels of StearoylCoA Desaturase-1 (SCD1), the main enzyme that converts saturated (SFA) into monounsaturated fatty acids (MUFA), are markedly elevated in a wide range of human cancer cells and tumors. These observations suggested that SCD1 may play a crucial role in the mechanisms of cancer cell replication, survival and tumorigenesis. Indeed, by using specific genetic ablation or small molecule inhibitors of SCD1, we demonstrated that the desaturase is a central regulator of cell cycle, programmed cell death and invasiveness in cancer cells, as well as tumor formation in mice. We also found evidence that SCD1 controls the phenotype of cancer cells and tumors by modulating a series of interconnected metabolic and signaling pathways, including fatty acid biosynthesis and oxidation, glycerolipid production, SFA-mediated cell stress response, and key survival signaling cascades, such as the PI3K/Akt pathway. In this regard, recent studies from our lab revealed that the rate of activation of EGFR, a prototypical EGF receptor that is a main activator of Akt signals, is highly dependent on the levels of SCD1. In addition, we observed that SCD1 activity determines the distribution of molecular species of membrane phospholipids and the mobility of plasma membrane lipid domains, suggesting that SCD1 may concurrently modulate lipogenic and mitogenic pathways by affecting plasma membrane-resident signaling platforms, such as EGFR→PI3K/Akt.

LI-S03
LIPID METABOLISM IN MATERNAL DIABETES. ROLE OF PPARs ACTIVATION

Jawerbaum A.
Laboratorio de Reproducción y Metabolismo. CEFYBO-CONICET-UBA.

Maternal diabetes impairs feto-placental development and induces intrauterine programming of metabolic and cardiovascular diseases. Peroxisome proliferator activated receptors (PPARs) are ligand activated transcription factors that regulate metabolic, anti-inflammatory and developmental processes. In maternal diabetes, theinduced metabolic impairments, intrauterine pro-inflammatory environment and developmental defects make PPARsa relevant focus of investigation. The three PPAR isotypes(PPARα, PPARγ and PPARδ)are expressed and relevant in

the placenta, and the expression of PPARα and PPARγis altered in the placentaof experimental models of diabetes and diabetic patients. PPARs activation in the placenta has relevant function in the regulation of lipid metabolism and lipoperoxidation.In fetuses from diabetic rats, lipid metabolism, lipoperoxidation and several pro-inflammatory markers are regulated by the activation of the three PPAR isotypes. Impaired formation of arachidonic acid derivatives that activate PPARs is observed in diabetic intrauterine tissues. Maternal treatments enriched in unsaturated fatty acids are capable of activating PPARs in fetuses and placentas. Our results show that the intrauterine activation of PPARs leads to the regulation of impairments in lipid homeostasis, pro-inflammatory pathways and growth in placentas and fetuses from experimental models of diabetes in pregnancy. Moreover, their regulatory effects are evident also in the offspring of diabetic animals.

LI-S04
PROTEIN AND LIPID REGULATORS OF LIPID TRANSPORT TO THE LOCUS OF PHOSPHATIDYLSERINE DECARBOXYLASE 2

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A convenient method for following interorganelle movement of phosphatidylserine (PtdSer) uses decarboxyation of the lipid by PtdSer decarboxylases (Psd1p and Psd2p) to produce phosphatidyletnanolamine (PtdEtn). Previous work has established that PtdSer transport to Psd2p requires the Sec14p homolog PstB2p, a phosphatidylinositol 4-kinase, Stt4p, and a C2 lipid-binding domain within Psd2p. Using protein-protein interaction studies and lipid binding studies we probed the network of interactions involved in lipid transport to Psd2p. Protein interaction studies revealed that PstB2p binds to C2 domains within Psd2, and to a previously unknown protein, which we now designate as Pbi1p. Pbi1p also interacts with a tethering protein of the endoplasmic reticulum, Scs2p, which in turn binds to Stt4p. Thus a continuum of protein interactions can physically couple membranes harboring Psd2p to the endoplasmic reticulum, which is the site of PtdSer synthesis. In addition to protein interactions we tested lipid binding by all of the above described proteins. Psd2p, PstB2p and Pbi1p all bind to phosphatidic acid (PtdOH) with high affinity. This latter interaction raises the possibility that localized domains of PtdOH may regulate the assembly of the interacting proteins into a mature transport complex. Our emerging model predicts that tethering complexes between donor and acceptor membranes function to create zones of apposition where protein machinery assembles to transport phospholipid.
Supported by NIH Grant 5R37-GM32453

Signal Transduction

ST-S01
POST-TRANSLATIONAL MODIFICATION INFLUENCE THE SPLICING MACHINERY AND VICE VERSA

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Ourlaboratoryhas focused on the regulation of fibronectinpre-mRNA alternative splicing by signal transduction pathways triggered by cell-cell and cell-ECM interactions. We reported that the activation of a Pi3K-Akt signaling axisregulates the activity of two splicing factors of the SR protein family, SRSF1 and SRSF7, simultaneously altering alternative splicing and translation. We also revealed Akt as an SR protein kinase capable of phosphorylating these two factors. More recently, we described that SRSF1, apart from its multiple mRNA-related tasks,enhances SUMO conjugation to a variety of proteins,among them we uncovered Akt as a SUMO substrate.Phosphorylation and SUMOylation of Akt appear as independent events. However, several functions of this kinase are impaired by its lack of SUMOylation. In agreement with the pro-survival role of Akt, we found that its SUMOylation is relevant for G1/S transition along cell cycle progression and for the regulation of alternative splicing leading to the production of mRNA isoforms associated with cell proliferation and survival.These findings reveal SUMO conjugation as a novel level of regulation for Akt, a crucialsignaling molecule in human health and disease. We propose that this posttranslational modification does not regulate Akt activation, but it might regulate its subcellular localization andinteraction with downstream targets.

ST-S02
WHICH, WHEN AND WHERE: PROBING SPATIOTEMPORAL PROFILES OF EPIDERMAL GROWTH FACTOR PHOSPHORYLATION

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The intrinsic properties of Epidermal growth factor receptor (EGFR) leading to dimerization and trans-phosphorylation play an important role to regulate tyrosine phosphorylation. But is the interaction with other proteins, in particular with protein tyrosine phosphatases (PTP), what determines the systemic response. Signals, in the form of spatiotemporal tyrosine phosphorylation patterns, propagate inside the cell due to a local change in the balance of opposing kinase and phosphatase enzymatic reactions. To determine which PTPs regulate the phosphorylation of EGFR in space and time, we used quantitative imaging on single cells in combination with genetic perturbations. The phosphorylation of tyrosine residues of EGFR was imaged by high throughput Förster Resonance Energy Transfer measured by Fluorescence Lifetime Imaging Microscopy (FRET-FLIM) on cell arrays (CA-FLIM). In a set of experiments with opposing perturbations, individual PTPs were down-modulated or up-regulated in breast-tumor derived human cells. The resulting spatiotemporal profiles of EGF-induced EGFR phosphorylation were used to cluster PTPs according to when (early vs. late) and where (plasma membrane vs endosomes) they act. In this way, we have identified how each PTPs contributes to regulate initiation, maintenance and termination in space and time of the EGF-induced EGFR phosphorylation.

ST-S03
REGULATORY NETWORKS CONTROLLING DAILY AND SEASONAL RHYTHMS IN PLANTS

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Circadian clocks allow organisms to time biological processes to the most appropriate phases of the day and year. We are interested in deciphering the regulatory networks that control clock function in plants, since this knowledge could be used to manipulate flowering time, a key factor influencing crop productivity. Combining functional genomics and a reverse genetics approach we have identified a novel family of light induced genes that control the pace of the clock. We found that most light regulated genes in plants respond more strongly to light pulses given during subjective day-time, but clock associated genes respond more strongly to light in the middle of the night, a treatment that simulates extreme changes in photoperiodic conditions. Among a small subset of genes that are more strongly induced by light in the middle of the night, we identified a novel gene family that we named LNK, which controls the pace of the clock and the floral transition regulating the expression of genes with peak expression in the afternoon. In turn, LNK genes are directly regulated by members of the PRR family of core clock genes that act as transcriptional repressors, defining a novel loop withing the core of the plant oscillator. Thus, LNK genes constitute novel components that act linking light signals to the control of clock progression by modulating the plant transcriptome.

ST-S04
LIPIN-1 INTEGRATES LIPID SYNTHESIS WITH PROINFLAMMATORY RESPONSES DURING TLR ACTIVATION IN MACROPHAGES

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Lipin-1 is a Mg^{2+}-dependent phosphatidic acid phosphatase involved in the *de novo* synthesis of phospholipids and triglycerides. Using macrophages from lipin-1-deficient animals and human macrophages deficient in the enzyme, we have learned that after TLR4 stimulation those cells exhibit a decreased production of diacylglycerol, activation of MAPKs and the AP-1 transcription factor. Consequently, the generation of proinflammatory cytokines like IL-6, IL-12, IL-23, or enzymes like iNOS and COX-2 is reduced. Also, animals lacking lipin-1 have a faster recovery from endotoxin administration concomitant with a reduced production of harmful molecules in spleen and liver. In human macrophages lipin-1a colocalizes with lipid droplet (LD)-specific proteins such as adipophilin or TIP47 in the same cellular structures. Mass spectrometry determinations demonstrate that the fatty acid composition of triacylglycerol in isolated LDs from lipin-1–deficieent cells differs from that of control cells. Moreover, activation of cytosolic group IVA phospholipase A_2a, a proinflammatory enzyme that is also involved in LD biogenesis, is compromised in lipin-1–deficient cells. Collectively, these findings demonstrate an unanticipated role for lipin-1 as a mediator of macrophage proinflammatory activation and support a critical link between lipid biosynthesis and systemic inflammatory responses.

Translational Science

TS-S01
TAMING THE P53 NETWORK FOR THERAPEUTIC PURPOSES

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p53 is the most commonly inactivated tumor suppressor gene in human cancer. The p53 gene network is composed of functionally distinct gene modules mediating diverse cellular responses to stress including cell cycle arrest, senescence, apoptosis and autophagy. The molecular mechanisms defining how cells adopt a specific response upon p53 activation are poorly understood, which hampers the development of therapies harnessing the apoptotic potential of p53 for selective elimination of cancer cells. Why do some cell types survive whereas others die upon p53 activation?
Several projects in our lab investigate how pleiotropy is generated within the p53 transcriptional program and how the network can be manipulated to produce specific cellular responses upon p53 activation. We performed mechanistic studies using global measurements of nascent RNA synthesis (GRO-seq), steady state RNA levels (microarray gene profiling) and p53 occupancy (ChIP-seq) to demonstrate how the p53 transcriptional program is qualified at the transcriptional and post-transcriptional levels. We have also performed genome wide shRNA screens to identify signaling pathways that control the cellular response to p53 activation. Finally, we employed this knowledge to improve the therapeutic efficacy of p53-based targeted therapies currently being tested in clinical trials for the treatment of various cancers.

TS-S02
HOX GENE REGULATION DURING NEURAL DEVELOPMENT IN HEALTH AND DISEASE

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The Hox genes encode a family of evolutionarily conserved transcription factors that elicit distinct developmental programs along the head-to-tail axis of animals including humans. The specific regional functions of individual Hox genes largely reflect their complex expression patterns, the disruption of which can lead to serious developmental defects and disease including various neurological disorders. Here, I focus on the post-transcriptional regulation of Hox genes by small RNAs (microRNAs) showing that microRNA-dependent regulation of Hox genes within the nervous system can lead to significant changes in neural development and function in Drosophila. The projections of this work into the clinic including the possible use of microRNA diagnostics will be examined in the context of human disease by considering the case of Huntington Disease.

TS-S03
THE SODIUM/IODIDE SYMPORTER (NIS): AN UNENDING SOURCE OF SURPRISES

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The Na^+/I^- symporter (NIS) is a key plasma membrane protein that mediates active I^- transport in the thyroid. The ability of the thyroid to take up I^- has been known since the end of the 19th century, and used clinically for over 65 years in the diagnosis and treatment of thyroid disease, including cancer, with radioiodide. However, NIS was identified at the molecular level only in 1996, when our group isolated the cDNA encoding the protein. Against expectations, we have shown that NIS is not a thyroid-specific protein. Instead, it is expressed in other I^--transporting tissues, such as lactating breast and, significantly, breast cancer and its metastases. NIS mediates the inward movement of Na^+ and I^- with a 2:1 stoichiometry, resulting in electrogenic transport. NIS transports different anion substrates with different stoichiometries, as Na^+/perchlorate transport is electroneutral-a property unprecedented in any transporter. That NIS actively transports the pollutant perchlorate is relevant for public health, as high perchlorate levels have been found in the drinking water in many areas of the US. Valuable mechanistic information on NIS, which may prove useful in gene transfer studies intended to render cancer cells susceptible to destruction with radioiodide, has been obtained by the molecular characterization of NIS mutations that cause congenital I^- transport defect in patients.

TS-S04
DRUG TARGET SELECTION AND DRUG DISCOVERY FOR TRYPANOSOMES; THE PROMISE OF GLYCOSOMAL ENZYMES

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Trypanosomatid parasites are unique in compartmentalizing glycolysis inside peroxisome-related organelles called glycosomes. This compartmentalization has resulted a pathway of enzymes with a network organization, and structural and kinetic properties importantly different from those of other organisms including the human host of these parasites. Comparative control analysis studies allowed to identify in bloodstream-form *Trypanosoma brucei*, the glycolytic steps with the highest differential susceptibility for glycolytic flux inhibition compared to host cells. Moreover, determination of enzyme structures has shown the feasibility to select or design compounds that are expected to inhibit selectively the trypanosomatid glycolytic enzymes. These combined studies have then allowed the discovery and further optimization of inhibitors of several enzymes (phosphofructokinase (PFK), aldolase, glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase) that kill in vitro cultured parasites with no or little effect on human cells. Further structure-based optimization of PFK inhibitors has lead to chemotypes that are active at sub-micromolar concentrations, and following intraperitoneal dosing, have high plasma levels and, most importantly for human African trypanosomiasis treatment, are highly brain penetrant. They also appear benign in acute high dose mouse toxicity studies.

Plant Biochemistry and Molecular Biology (Plants)

PL-S01
ROLE OF CHROMATIN REMODELING PROTEINS IN UV-B RESPONSES IN PLANTS

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Terrestrial life evolved only after the stratospheric ozone layer formed and could absorb most damaging UV-B (280-315 nm) in solar radiation. The strong absorption of UV-B by biological molecules, particularly DNA, makes this radiation extremely dangerous. Because plants must absorb photons to power photosynthesis, they are inevitably exposed to damaging UV-B. As a result, they have evolved diverse responses to this type of radiation. Chromatin remodeling in response to UV-B has been implicated in plants. Chromatin conformation on DNA can have a role in the regulation of the expression of different genes: proteins that function in chromatin remodeling can activate or repress transcription recruiting co-regulators, or changing the structure of promoter regions, and thus affecting the interaction with the transcripcional machinery. On the other hand, the package of DNA in the chromatin affects the structure and accessibility of DNA, and therefore the velocity of formation and repair of damage in DNA molecules. Thus, we explored the role of chromatin proteins in UV-B stress. In particular, we investigated the role of histone acetylation in UV-B responses and the function of chromatin proteins in cell cycle regulation. Our results indicate that chromatin remodeling is a key process in acclimation to UV-B treatment and that lines deficient in this process are more sensitive to UV-B.

PL-S02
THE WORLD OF RNA EDITING IN MITOCHONDRIA AND CHLOROPLASTS IN PLANTS

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In mitochondria and chloroplasts of nearly all land plants, RNA editing alters individual nucleotide identities from C to U. The numerous different sites are distinguished by E class PPR (pentatricopeptide repeat) proteins. These recognize and address the target RNA sequence by specific interactions between the PPR elements and the nucleotide in the mooring sequence. However, the precise determinants of how PPR proteins recognize their target RNA editing sites still need to be worked out in detail. The enzymatic activity catalyzing the deamination or transamination step of the C to U editing may reside in the C-terminal extensions found in some of the E class PPR proteins or may be provided by an as yet unknown enzyme. Connecting the PPR protein attached to the RNA with either enzyme

structure may be mediated by the small group of MORF proteins. The MORF proteins are required for the RNA editing process in plant organelles in addition to the E-class PPR proteins. Interaction studies show that MORF proteins can interact with each other and in addition can bind to the PPR proteins. We will look at the progress made in recent years towards a better understanding of this post-transcriptional RNA editingprocess altering the primary RNA derived from the genetic information to produce a protein different from the one predicted from the genomic DNA.

PL-S03
PATTERNING AND FERTILIZATION OF THE *Arabidopsis* EMBRYO SAC IN *Arabidopsis thaliana*

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Gamete formation in plants takes place in special haploid structures called gametophytes. The male gametophyte is the pollen grain, while the female gametophyte is the embryo sac, which develops enclosed by the maternal tissues of the ovule. In *Arabidopsis*, the embryo sac is a polarized structure composed by seven cells: the egg cell, the central cell, two synergids and three antipodal cells. In our lab, we are interested in the molecular mechanisms underlying cell specification during embryo sac development. Particularly, we are focusing our studies on mitochondrial proteins. By studying insertional mutants in genes encoding ROS detoxifying enzymes, we showed a crucial role for ROS in cell fate and fertilization. Additionally, we have identified and studied *Arabidopsis* mutants with insertions in genes that encode for members of the mitochondrial electron shuttle Adrenodoxin-Adrenodoxinreductase(ADX-ADXR).These mutants show cell fate abnormalities and aberrant pollen tube attraction, suggesting a role for this shuttle in cell specification and communication between the female and malegametophytes. In vertebrates and insects, ADX-ADXRmediates electron transfer to a cytochrome P450, which constitutes the first step in steroid hormonessynthesis. We are currently analyzing the possible occurrence of a similar pathwayin *Arabidopsis* and the female gametophyte steroid profile.

PL-S04
FUNCTION OF BBX PROTEINS IN PLANT LIGHT SIGNALING

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Plants have evolved sophisticated photosensory systems to respond and convert the light information into biological signals. After germination, etiolated seedlings perceive light and promote de-etiolation that includes hypocotyl inhibition and development of photosynthetic cotyledons. After seedling establishment, plants grow and become mutually shaded. To increase the chances to compete for light, sun plants are capable to induce the shade avoidance syndrome, a group of physiological responses being the most prominent the elongation of stem and petioles. These developmental processes promote a massive transcriptional reprogramming finely modulated by the activity of transcription factors. Here in this talk, I will describe the functional characterization of a group of zinc-finger transcription factors that contain a tandem repeated B-boxes (BBX) involved in seedling de-etiolation and shade avoidance response. In particular, I will demonstrate the action of BBX24 and BBX25 in both processes. They are negative regulators of photomorphogenesis and act additively in the control of de-etiolation through the inhibition of HY5 activity. Furthermore, BBX24 and BBX25 promote additively cell elongation under shade light. This talk will present some evidence of BBX protein functions in the shade signaling pathway.

ORAL COMUNICATIONS

Biotechnology

BT-C01
RECOMBINANT GLYCOPROTEINS AS NOVEL ANTIGENIC TARGETS FOR DIAGNOSIS OF HEMOLYTIC UREMIC SYNDROME

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Human infection with Shiga toxin-producing *Escherichia coli* (STEC) is a major cause of postdiarrheal hemolytic uremic syndrome (HUS), a life-threatening condition characterized by hemolytic anemia, thrombocytopenia and acute renal failure. E. coli O157:H7 is the dominant STEC serotype associated with HUS worldwide although non-O157 STEC serogroups can cause a similar disease. Detection of anti-O157 LPS antibodies in combination with bacteriological procedures considerably improves diagnosis of STEC infections. In this work, we have exploited bacterial glycoengineering technology for the development of recombinant glycoproteins consisting of the O157, O145 or O121-polysaccharide attached to a carrier protein as serogroup-specific antigens for the serological diagnosis of STEC-associated HUS. We demonstrate that using these antigens it is possible to clearly discriminate between STEC O157, O145 and O121 infected patients and healthy children, even at early stages of the disease, as well as to confirm the diagnosis in HUS patients in which the classical diagnostic procedures failed. Additionally, in all the culture-positive HUS patients, the identified serotype was in accordance with the serotype of the isolated strain indicating that these antigens are not only valuable for the diagnosis of HUS but also as a tool for serotyping and guiding the following steps to confirm diagnosis.

BT-C02
PHOSPHOLIPASE C FOR SOYBEAN OIL DEGUMMING

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Crude vegetable oils must be refined prior to use for human consumption or biodiesel production. Oil “degumming” consists of phospholipids or “gums” removal, and is the process that causes the major lost in oil refining industry. Enzymatic degumming emerged as a valuable alternative due to its increase in oil yield. In this process, phospholipases are used to hydrolyze phospholipids, generating products with increased water solubility making easier their separation from oil. The aim of our study is to produce a phospholipase C with high efficiency in industrial soybean oil degumming. We designed an expression strategy to obtain *B. cereus* phospholipase C and three individual mutants in *Pichia pastoris*. We determined kinetic parameters of the three mutant proteins in aqueous media and oil and compared them to the *wt* enzyme. We selected one enzyme that shows superior catalytic properties in oil. In addition, we optimized reaction parameters such as temperature, pH and enzyme dosage to completely remove major phospholipids from crude oil, setting up reaction conditions that can be scaled up to meet industrial needs.

BT-C03
HIGHLY EFFICIENT PHOSPHOLIPASE C PRODUCTION FOR INDUSTRIAL OIL DEGUMMING USING *Pichia pastoris*

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Crude soybean oil is refined to remove impurities that adversely impact in oil stability, color and flavor. Phospholipids are a major class of impurities removed in conventional processes by oil degumming at significant cost to oil yield. In recent years, enzymatic degumming methods using phospholipase C (PLC) were introduced and are already available on industrial scale with further reduction in yield loss. In this study, *Pichia pastoris* was used as the expression system for the production of a recombinant PLC enzyme derived from *Bacillus cereus*. Secreted expression of PLC was driven by the methanol inducible promoter AOX1. In order to optimize the yield of expression, we performed genetic engineering on this strain including generation of multi-copy chromosomal integrations clones, gene codon optimization and the co-expression of several helper factors supporting protein

folding, processing and secretion processes. Based on screening activity tests, the most productive clone was selected. Different approaches were used in order to maximize the yield obtained from fed-batch fermentations reaching secreted protein levels up to 7 g/l. In conclusion, this work set the basis for the development of a scalable model for PLC production. Cost reduction of enzyme production is a basic principle for industrial applications.

BT-C04
TRANSGENIC *Arabidopsis* PLANTS BEARING *PROMOTERHAHB11::HAHB11* EXHIBIT SIGNIFICANTLY ENHANCED BIOMASS

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HD-Zips are plant specific transcription factors (TFs) combining a Homeobox Domain (HD) and a Leucine Zipper (Zip). HaHB11 is a sunflower divergent HD-Zip I TF. It was previously reported that the ectopic and overexpression of this gene in Arabidopsis driven by the 35S constitutive promoter conferred to transgenic plants relevant traits: tolerance to both drought and flooding stresses, larger rosettes and improved yield compared with controls. However, this constitutive expression also caused a developmental delay when the expression levels are very high. In order to avoid this penalty, the HaHB11 promoter was isolated from a BAC genomic library. Transgenic plants in which this promoter drives either the HaHB11 cDNA or the reporter GUS expression were generated and analyzed. GUS expression was clearly detected in roots, hypocotyls and petioles of plants grown in standard conditions. *ProH11::HAHB11* plants exhibited larger rosettes than controls either in control and mild stress conditions, significantly improved seed yield, larger roots and did not display developmental delays or penalties. However, these plants did not show enhanced stress tolerance. In conclusion, the construct *ProH11::HAHB11* resulted in an interesting and relevant biotechnological tool to improve biomass and yield. Further studies with enhancers or other crops would give a complete comparative scenario.

BT-C05
SENSITIVE WHOLE-CELL BIOSENSORS FOR THE DETECTION OF A BROAD-SPECTRUM OF TOXIC METAL IONS

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Environment pollution with toxic heavy metals emerges mainly as a consequence of anthropogenic activities or improper waste disposal. These pollutants not only affect human health, but also biodiversity, being a matter of world-wide concern. In the last decade, bacterial biosensors emerged as simple and cost-effective alternatives to conventional procedures for detection of contaminants. Moreover, because of their nature, they can easily be used for the construction of portable instruments allowing the screening of environmental samples in the field. In this study, we developed a set of non-selective bacteria biosensors able to detect a broad-spectrum of toxic heavy metals, including lead (Pb), mercury (Hg) and cadmium (Cd), three of the top ten most toxic chemicals according to the World Health Organization. In these devises, the sensor/transduction module is provided by modified versions of the Salmonella Au sensor/transcriptional regulator GolS, while a GolS-controlled fluorescent reporter construction supplies the output signal. These biosensors detect the presence of any of these metals in aqueous solutions in a single measure and with high sensitivity under laboratory conditions, even when more than one metal is preset in the sample. These studies can be easily applied as a primary screening tool for the detection of metal pollution in drinking-water or aquatic ecosystems.

BT-C06
METABOLIC ENGINEERING FOR THE PRODUCTION OF NEUTRAL LIPIDS IN *Escherichia coli*

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Environmental concerns, in addition to cost and finite supply of crude oil, have expanded global interest in the development of bio-based chemicals from sustainable sources. In view of the lubricant industry, vegetable wax esters have good properties but their high cost limits their use to specialized areas such as pharmaceutical or cosmetic. In this context, microbial-cell-factories are an attractive model for the production of specific products as it provides the opportunity to convert sustainable biomass into high value chemicals. In this work we have engineered into *Escherichia coli* a mycocerosic polyketide synthase-based biosynthetic pathway from *Mycobacterium tuberculosis*

and redefined its biological role towards the production of multi-methyl-branched-esters (MBE) with novel chemical structures. Our minimal set of proteins for the production of MBE, composed of *M. tuberculosis* enzymes FadD28, Mas and PapA5, enabled the biosynthesis of multi-methyl-branched-FA and their further esterification to an alcohol. The high substrate tolerance of these enzymes resulted in the biosynthesis of a broad range of structurally diverse MBE. Further metabolic engineering of our MBE producer strain coupled this system to long-chain-alcohol biosynthetic pathways resulting in de novo production of branched wax esters following addition of only propionate.

BT-C07
VARIANT-SPECIFIC SURFACE PROTEINS FROM *Giardia lamblia* AS A NEW TOOL FOR ORAL DRUG DELIVERY

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Oral administration is an ideal mean for providing therapeutic agents due to its painless administration, patient compliance and low cost. However, it is also the most difficult approach because of the gastrointestinal tract properties, such as extremely low pH in the stomach and enzymatic degradation in the upper intestine. Here we show that Variant-specific Surface Proteins (VSPs) of the intestinal parasite *Giardia lamblia* can be used as carrier for oral delivery of drugs. VSPs cover and protect the parasite from the harsh conditions of the upper small intestine. The extracellular region (ExR) of VSPs is rich in cysteine, mostly as CXXC motifs. Recombinant VSP ExRs produced in insect cells, similarly to native VSPs, are resistant to acidic pH and proteolytic degradation and adhere for hours to the intestinal mucosa. We initially used insulin and glucagon in combination with VSP ExRs as prototype drugs. Results in mice showed that VSP´s ExRs protect these bioactive peptides from degradation and promote their systemic biological action, namely the regulation of blood glucose levels. VSP ExRs were also able to protect other peptides, such as growth hormone, IL-10 and IFNα, both *in vitro* and *in vivo*, significantly assisting their oral absorption. Results demonstrate that combination of the ExR of VSPs with bioactive peptides is a promising approach for oral administration of drugs.

Cell Biology

CB-C01
A HIGH-VOLUME EXOPLASMIC HEMI-TMD IS A NOVEL SIGNAL FOR ENDOCYTOSIS AND POLARITY IN YEAST

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In *Saccharomyces cerevisiae*, polarized distribution of membrane proteins is maintained kinetically due to polarized secretion followed by endocytic recycling to the plasma membrane. The classical model for endocytosis of transmembrane proteins involves the recognition of cytosolic signals by adaptor proteins that drive the active concentration of cargoes in endocytic vesicles. As part of a systematic analysis of the influence of Transmembrane Domain (TMD) length and volume in intracellular localization of type-two membrane proteins, we observed that increasing the volume of the residues that constitute the exoplasmic hemi-TMD of the yeast SNARE Sso1, which is distributed homogenously in the plasma membrane, results in its polarized distribution. Expression of this mutant protein in strains affected in either endocytosis or recycling revealed that this polarization is achieved by endocytic cycling. A bioinformatic search of the yeast proteome identified proteins with high-volume exoplasmic hemi-TMDs, some of which exhibit a polarized distribution. We showed that the TMDs of two of these proteins; Hkr1 and Kre6, are also able to confer a polarized distribution to the cytoplasmic domain of Sso1. Overall, our experiments indicate that the geometry of TMDs represent a novel determinant for endocytosis and polarity in vivo.

CB-C02
REGULATION OF SECRETORY CAPACITY IN SPECIALIZED SECRETORY CELLS

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Secretion occurs in all cells, with relatively low levels in most cells and extremely high levels in specialized secretory cells. Although the molecular machinery involved in the secretory pathway is well studied, how secretory capacity is selectively upregulated in specialized secretory cells is not clearly understood. It has been demonstrated that CREB3 proteins, which are ER-localized bZip transcription factors, upregulate genes involved in secretory capacity and increased secretion of specific cargos. However, how changes in secretory capacity are coordinated to allow an efficient transport process are not well known. Using a thyroid cell line, in which thyroid-stimulating hormone (TSH) stimulates the synthesis of thyroid specific proteins, we analyzed the mechanisms that are involved in the adaptation to a higher secretory demand. We showed that TSH stimulation enhances the level of proteins required for membrane transport and ER folding (chaperones), as well as the Golgi volume, in agreement with an increase in specific cargo levels. Furthermore, we showed that one member of the CREB3 family is regulated by TSH. Our data indicate that, to maintain cellular homeostasis after stimulation, a global cell response is induced to cope with cargo increase, suggesting that common signaling pathways are able to modulate specific secretory production and traffic-related genes.

CB-C03
RAB27A CONTROLS HIV-1 ASSEMBLY BY REGULATING PLASMA MEMBRANE LEVELS OF PHOSPHATIDYLINOSITOL 4,5-BISP

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During the late stages of the HIV-1 replication cycle, the viral polyprotein Pr55Gag is recruited to the plasma membrane (PM), where it binds phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] and directs HIV-1 assembly. We demonstrate that the small GTPase Rab27a regulates Pr55Gag membrane association, both in primary macrophages and CD4+T lymphocytes, thus controlling viral assembly and replication. We show that Rab27a controls the trafficking of multivesicular endosomes (MVEs) carrying phosphatidylinositol 4-kinase type 2 alpha (PI4KIIalpha) towards the PM of CD4+ T lymphocytes. Hence, Rab27a promotes high levels of PM phosphatidylinositol 4-phosphate and the localized production of PI(4,5)P2 , controlling the formation of the HIV-1 assembly platform. Rab27a also controls PI(4,5)P2 levels at the Virus-Containing Compartments of macrophages. By screening Rab27a effectors, we identified that Slp2a, Slp3 and Slac2b are required for the association of Pr55Gag with the PM. We conclude that by directing the trafficking of PI4KIIalpha-positive endosomes towards the PM, Rab27a controls PI(4,5)P2 levels and HIV-1 replication.

CB-C04
HEMIN INDUCES MITOPHAGY IN K562 CELLS

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Autophagy is a lysosomal catabolic event which is implicated in the turnover and clearance of cytoplasmic components in healthy and damaged tissue via a lysosomal pathway. This pathway plays a significant role in the erythropoiesis process, contributing to the clearance of some organelles that are not necessary in the mature cells.
The main objective of this study was to determine whether hemin, a strong inducer of hemoglobin synthesis, induces erythroid maturation in K562 cells using autophagy. We demonstrated, in K562 cells, that hemin increased the number and size of autophagic vacuoles. These structures were labeled with lysosomal markers such as lysotracker or DQ-BSA. In addition, we have determined by Western blot a rise in the lipidated form of the autophagic protein LC3 (i.e. LC3-II) upon hemin incubation. Likewise, we have also demonstrated an increased expression level of autophagy related genes by semi-quantitative real-time RT-PCR (ie Map1a1b, Beclin1, ATG5). Moreover, we provide evidences that hemin induces mitochondrial membrane depolarization and that mitochondria sequestration by autophagy and requires the active form of NIX protein. Summarizing, we have found that hemin stimulates mitophagy in K562 cells, likely to allow a more efficient and faster erythroid maturation.

CB-C05
THE GIARDIAL EPSIN-LIKE PROTEIN POSSESS A DUAL EPSIN-EPSINR ROLE IN CLATHRIN-MEDIATED TRAFFICKING

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The epsin N-terminal homology (ENTH) domain is an evolutionarily conserved protein module that defines monomeric adaptor proteins of the epsin family. It is present in the epsin or epsin-related (epsinR) proteins, which are implicated in the endocytosis and Golgi-to-endosomes protein trafficking, respectively, in other eukaryotic cells. In Giardia we found a single gene encoding a protein containing an ENTH domain (GlENTHp for G. lamblia ENTH protein), which localized in the cytosol and, like epsin, was associated with αAP-2, clathrin, ubiquitin, interacted with PI3,4,5P3, and was involved in receptor-mediated endocytosis. This protein also bonded γAP-1, PI4P, and was implicated in ER-to-PV trafficking, like epsinR proteins. Alteration of the GlENTHp function affected trophozoite growth showing an accumulation of dense material in the lysosome-like peripheral vacuoles (PVs), indicating that GlENTHp might be implicated in the maintenance of the PV homeostasis. When the ENTH protein family was analyzed in an evolutionary context, it was suggested that the subfamily of epsin proteins was acquired more recently probably by duplication of the epsinR. In this study, we showed evidence that places GlENTHp at the beginning of the whole family, summarizing the function of epsin and epsinR and suggesting that GlENTHp might be the epsinR adaptor from which all the family evolves.

CB-C06
ROLE OF THE GTPASE RAB22A DURING ANTIGEN CROSS PRESENTATION BY DENDRITIC CELLS

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Cross presentation is the process by which exogenous antigens are processed and presented to the class I molecules of histocompatibility complex (MHC) to activate a CD8+ T cell response. Dendritic cells (DCs) have been shown to be the most adapted antigen presenting cells to perform efficiently cross presentation. To do this, DCs have developed a strong specialization of their internalization pathway and many of the intracellular features used for cross presentation have been elucidated. Nevertheless several open questions remain unclear, for instance to determine how the peptides derived from exogenous antigens are loaded into MHC-I molecules. We consider that recycling compartments may have an important role in this step of cross presentation by modulating the trafficking of MHC-I molecules. So, we focus our study on Rab22a, a GTPase that participates in the recycling of MHC-I molecules. We found that this protein localized to recycling compartments in DCs and was recruited to phagosomes very early after latex beads internalization. After silencing the expression of Rab22a, DCs failed to cross present antigens efficiently and this defect could not be explained by a lack of MHC-I molecules expression. These results evidence a critical role for Rab22a in antigen cross presentation and point it out as a potential target for immunotherapy.

CB-C7

RAB24 AND ITS CONNECTION WITH THE ENDOCYTIC/LYSOSOMAL PATHWAY

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The investigation of new therapy for neurodegenerative disorders and myopathies lead research efforts to an intensive study of intracellular protein degradation processes. One of the major intracellular protein degradation systems is the autophagy pathway, which is a catabolic process that involves delivery of cellular components to the lysosome for proteolysis. Lysosomes also receive material from the extracellular media via the endosome traffic, which represents the endocytic/lysosomal pathway. Results from our laboratory have demonstrated that Rab24, an atypical Rab protein whose function is currently unknown, is implicated in the autophagy pathway. This protein changes its distribution colocalizing with the autophagosomal markers LC3 and monodansylcadaverine upon autophagy induction. In the present report we have studied the role of Rab24 in the endocytic/lysosomal pathway in K562 cells. We demonstrate that Rab24 colocalizes with markers of the late endocytic/lysosomal compartments (i.e. Rab7 and LAMP1). In addition, the expression of a Rab24 mutant or the silencing of the protein by siRNA, block the lysosomal degradation process. According to our data about Rab24 and Rab7 colocalization and the specific and reciprocal effect in their intracellular distribution, we postulate that these Rabs proteins share regulatory factors, being both Rabs components of the same trafficking pathway.

CB-C08
IDENTIFICATION AND CHARACTERIZATION OF INHIBITORS OF HECT E3 LIGASES BY HIGH THROUGHPUT SCREENING

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Mutations, altered expression, or viral hijacking of different members of the E3 family of ubiquitin (Ub) ligases have been reported to contribute to the development of a wide variety of pathologies. Therefore, selective interference of this enzyme family might represent a powerful therapeutic tool. ITCH is a HECT domain-containing E3 Ub ligase that promotes the ubiquitylation and degradation of several proteins controlling cell growth and apoptotic processes. In agreement, ITCH depletion potentiates the effect of chemotherapeutic drugs, revealing ITCH as a potential pharmacological target in cancer therapy. Using high throughput screening, we identified several putative ITCH inhibitors one of which is Clomipramine, a drug that is used in the clinic to treat psychiatric disorders. We found that Clomipramine specifically blocks the HECT catalytic activity of ITCH. To obtain qualitative insight on how Clomipramine derivatives might obstruct ITCH ubiquitylation, we performed modelling studies and identified two potential binding sites of Clomipramine within the HECT domain of ITCH. Together, our study demonstrates the feasibility of using high throughput screening to identify E3 Ub ligase inhibitors and provide insight into how Clomipramine might interfere with ITCH and other HECT E3 Ub ligases catalytic activity.

CB-C09
GUANINE QUADRUPLEXES AND EMBRYO DEVELOPMENT

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Guanine quadruplexes (G4) are stable secondary structures of single-stranded DNA or RNA associated with gene expression control. The goal of this work was to assess the role of G4 as transcriptional regulatory elements present in genes required for embryonic development. An *in silico* search to find out sequences with the potential to form G4 was performed on promoter regions of human, mouse and zebrafish genes. The analysis yielded 23 genes present in the three species containing potential G4 within their promoters and displaying development gene ontology associated terms. Among them, *col2a1a*, *fzd5*, and *nog3* were selected for further studies. The G4 formation was determined *in vitro* both by ThT fluorescence and circular dichroism assays. The G4 regulatory role was assessed *in cellulo* by cloning the sequences upstream of a constitutive promoter that governs the expression of luciferase. G4 located in template (-) strand reduced the expression of luciferase while those located in coding (+) strand had no effect. Furthermore, microinjection of zebrafish embryos with DNA oligonucleotides complementary to the selected G4s resulted in transcriptional reduction, as measured by RT-qPCR and revealed by morphological changes accordingly. The results suggest the existence of conserved G4 structures displaying transcriptional regulatory roles during the embryonic development of vertebrates.

CB-C10
TREACLE DEPLETION IN *Danio rerio* INDUCES OXIDATIVE STRESS AND APOPTOSIS

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Treacher-Collins Syndrome (TCS) is a disorder of craniofacial development due to mutations in *TCOF1*, which encodes the protein Treacle. TCS patients show variable expressivity in phenotype. Treacle deficit seems to increase cranial neural crest cells apoptosis. Recently, it has been informed that Treacle has antioxidant activity in pneumocytes. We developed a TCS-like model injecting in zebrafish embryos morpholinos against the *tcof1* ortholog (TcofMO). The aims here were to assess in TCS-like zebrafish embryos: I) the apoptosis occurrence (pattern and gene markers) and, II) the oxidative stress induction. We studied *noxa, cyclinG1, trp53inp,* and *hsp70* expression by RT-qPCR; the levels of reactive oxygen species (ROS) by incubation with DA-DCFH; and apoptosis events by Acridine Orange stain. The expression of apoptotic marker genes was induced in TCS-like embryos (noxa 3.0±0.4, cyclinG1: 3.5±1.0 and, trp53inp1: 1.4±0.1 times vs. control morpholinos (MisMO) embryos). Besides, TCS-like embryos showed increased hsp70 expression (2.0±0.6 times) and ROS levels (MisMO=46.6±1.2 and TcofMO=69.8±2.5 UA, $p<0.001$). We also observed a rise on apoptotic cell number, mainly located on cephalic

structures. The results show that zebrafish TCS-like embryos have enhanced apoptosis and increased ROS production suggesting oxidative stress as a factor involved in TCS variable expressivity.

CB-C11
OXALATE INDUCES BREAST CANCER TUMOR

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Microcalcifications are routinely used to detect breast cancer in its early stages. Mammary microcalcifications are mainly composed of calcium oxalate (OX) or calcium phosphate. In order to study the biological significance and implication of OX within the breast tumor microenvironment, we measured Ox concentration in Human Breast tumor tissue and in adjacent non-pathological breast tissue. We found that all breast tumor tissues tested contained a higher concentration of Ox than their counterpart non-pathological breast tissue. Moreover, Ox induced cell proliferation and over-expression of the pro-tumorigenic gene c-fos in cultured MCF-7 and MDA-MB231 human breast cancer cells. Finally, when female mice received an injection of OX periodically in the mammary fat pad or in the back, only the mice injected with OX solution at the mammary fat pad area generated tumors indicating oxalate's tissue specificity. Since no tumor formation was observed when oxalate was injected into mice back. Cell line specificity was also observed in vitro; non-breast cell lines did not proliferate nor expressed c-Fos when oxalate was added to the culture. Our results support the hypothesis that the chronic exposure of breast epithelial cells to OX promotes the transformation of breast cells from normal to tumor cells.

CB-C12
ACYL-COA SYNTHETASE 4: A NOVEL REGULATOR OF THE MTOR PATHWAY IN BREAST CANCER CELLS

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Breast cancer comprises a heterogeneous group of diseases that vary in morphology, biology, behavior and response to therapy. Previous studies have identified an acyl-CoA syntethase 4 (ACSL4) expression which correlated with tumor progression in breast cancer. We have proven that cell transfection solely with ACSL4 cDNA renders a highly aggressive phenotype *in vitro* and *in vivo*. Although the role of ACSL4 in mediating an aggressive phenotype is well accepted, there is little evidence as to the early steps through which ACSL4 increases tumor growth. Therefore, we have performed a massive in-depth mRNA sequencing approach and functional proteomic to identify gene expression and functional proteomic signatures specific to ACSL4 overexpression. ACSL4 displays a distinctive transcriptome and functional proteomic profile, and results show that the most significantly up-regulated gene networks include genes associated to the regulation of embryonic and tissue development, cellular movement and DNA replication and repair. Specific pathways such as AKT-mTOR-SP6 kinase and Wnt are functionally required for ACSL4 action. Finally, by means of a combination of ACSL4 and mTOR inhibitors, we demonstrate a synergistic effect in the inhibition of cell growth. ACSL4 is an upstream regulator of tumorigenic pathways. Our data provide novel insights into a combined pharmacological approach.

CB-C13
C-FOS AS ACTIVATOR OF PHOSPHOLIPIDS SYNTHESIS: NEW TARGET IN BRAIN CANCER

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Glioblastomas multiforme constitute one of the most aggressive types of brain cancer. Patients diagnosed with this class of tumors have a survival period ~1 year. The conventional treatment for these malignancies includes surgery followed by radio and chemotherapy that, as evidenced by the short survival time post-treatment, results very ineffective. Consequently, the search for novel strategies and new targets for treatment of these tumors is highly important. We have described that c-Fos, in addition to its AP-1 transcription factor is able to activate the phospholipid synthesis by an AP-1 independent mechanism. Furthermore, we found c-Fos highly expressed in all tumors of the central nervous system examined (>150) contrasting with the low or absent expression of this protein in normal tissue. The aim of this study is to test deletion mutants of c-Fos as negative dominants to block its action as

activator of lipids synthesis. The overexpression of NA (aa 1-138), an deletion mutant of c-Fos, interferes in the interaction between c-Fos and the enzyme of phospholipid metabolism PI4KIIα thus inhibiting proliferation of human glioblastoma cells (T98G) in vitro. Truncated mutants of NA that contain the first 90 amioacids of this domain are sufficient to inhibit proliferation of T98G cells. At present we are testing the effect of injecting these peptides on tumor growth in vivo.

CB-C14
INVOLVEMENT OF PKCα INHIBITION AND RETINOID SYSTEM INDUCTION IN THE MAMMARY CANCER STEM CELL BIOLOGY

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It has been established that retinoids exert some of their effects on cell differentiation and malignant phenotype reversion through the interaction with different members of the PKC family. Objective: to study the modulation of the expression of PKCα and retinoic acid receptors (RAR) by Retinoic Acid (RA) and to analyze the effect of RA+PKCα inhibitor on proliferation, self-renewal and differentiation of cancer stem cells (CSC) from triple negative mammary cell line LM38-LP. By RT-PCR we found that RA decreased PKCα and increased RARβ and RARγ in CSC. RARγ increase was abrogated by the addition of PKCα inhibitor. RA and PKCα inhibitor synergized to reduce CSC growth (number and diameter of mammospheres). RA treatment showed an increment in self-renewal (secondary mammospheres formation) and clonogenic capacity of LM38-LP CSC, via RARγ. This effect was reversed when PKCα activity was inhibited. In a matrigel 3D culture assay, CSC generated large undifferentiated structures while PKCα activity inhibition together with RA treatment led to the formation of small and differentiated structures with evidence of cell death. Our data suggest that the CSC differentiation, reduced self-renewal and clonogenic capacity, induced by the pharmacological inhibition of PKCα together with the activation of retinoic system could be possibly through the modulation of RARγ.

CB-C15
SPHINGOMYELIN METABOLISM IS INVOLVED IN THE MAINTENANCE OF TISSUE ORGANIZATION OF COLLECTING DUCTS

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Adherens junctions (AJ) and focal adhesions (FA) are structures that mediate tissue organization at two different levels. Vinculin is a cytoskeletal protein associated with both structures, interacting with a-catenin through specific domains recruits F-actin to the AJs. Taking into account that we have demonstrated that both adhesion structures are located in sphingomyelin (SM) enriched membrane microdomain, we investigated the influence of SM synthesis in AJ and FA integrity. To this end, primary culture of differentiated collecting duct cells that preserve the fully epithelial phenotype reflected by the presence of primary cilium was incubated for 24 hs with increasing concentration of D609, a SM synthase1 inhibitor. Cell adhesion structures were analyzed by confocal immunofluorescence with anti vinculin and anti a-catenin. We observed a population of cells located in the middle of the colony without FA but with their borders delineated by both proteins, and other more peripheral containing FA. D609 treatment showed cells with irregular shape and a progressive alteration in the distribution of both proteins, denoting AJ impairment. At higher D609 concentration, cell-cell contact was lost but retained the spread morphology by increasing the amount of FA. These results suggest that the activity of SM synthase1 is essential for the maintenance of collecting duct tissue organization.

CB-C16
ALTERNATIVE LENGTHENING OF TELOMERES IN HUMAN GLIOMA STEM CELLS

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Glioblastoma multiforme (GBM) is the most aggressive primary tumor of the central nervous system. Glioma stem cells (GSCs), a small population of tumor cells with stem-like properties, are supposedly responsible for GBM relapse. In approximately thirty percent of GBMs, telomeres are not maintained by telomerase but through an alternative mechanism, termed alternative lengthening of telomere (ALT), which represent and interesting target for

the treatment of this subtype of brain tumors. We isolated and characterized for the first time GSCs elongating their telomeres trough ALT, named TG20 cells. Both in vitro and in vivo TG20 cells were significantly more resistant to ionizing radiation than GSCs with telomerase activity. Radiation resistance was related to interference of ALT pathway with DNA damage response. Therefore, our data show for the first time that the ALT pathway can confer to GSCs the capacity to sustain long-term proliferation and may also affect treatment efficiency. Furthermore, we developed the first mouse model of ALT glioma by performing intracerebral grafts of TG20 cells into NSG and nude mice. Using this model we demonstrate that 360B, a G-quadruplex ligand, prevents the development of TG20 tumors. TG20 cells are thus the first cellular model of GSCs displaying ALT and should prove to be useful for the development of specific treatment strategies.

CB-C17
HOW IS CHOLINE KINASE ALPHA REGULATED DURING RA-INDUCED NEURONAL DIFFERENTIATION?

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Neuronal differentiation involves several steps, being one of the most characteristic the neurites sprouting and elongation. As in this process there is high demand for membrane components, and phosphatidylcholine (PtdCho) is the major component of mammalian membranes, PtdCho biosynthesis plays a key role during neuronal differentiation. We have previously reported that the increased in PtdCho biosynthesis after retinoic acid (RA)-induced differentiation is supported, at least in part, by an enhanced expression of Chka gene. Moreover, we have demonstrated that the stimulation of CKa expression during RA-induced differentiation depend on the promoter region that contains two boxes, which are highly conserved in different species. The Box1 includes a CCAAT/Enhancer-binding Protein-b (C/EBPb) sites, and the Box2 is an inverted repeat sequence GGGGCCTTGGCCCC. We have demonstrated that RA induces Chka to the Box1.β expression by a mechanism that involves the binding of C/EBP However, the mechanism by which Box2 regulates Chka expression remains unresolved. We have determined that the Box2, preferentially under basal condition, recruits a putative unknown repressor protein. Our present efforts are focused in the elucidation of the nature of this protein.

CB-C18
E2F1 AND E2F2 INDUCTION IN RESPONSE TO DNA DAMAGE PRESERVES GENOMIC STABILITY IN NEURONAL CELLS

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E2F transcription factors regulate a wide range of biological processes, including the cellular response to DNA damage. We have previously reported that E2F1 and E2F2, the latter specifically in neuronal cells, are transcriptionally induced after DNA damage. This upregulation, which relies on ATM/ATR kinases activity and is conserved among different species, leads to increased E2F1 and E2F2 protein levels as a consequence of de novo protein synthesis. In the present study, we evaluated the significance of E2F1 and E2F2 induction in the maintenance of genome integrity in neuronal cells. Ectopic expression of these E2Fs reduces the level of DNA damage following genotoxic treatment, while ablation of E2F1 and E2F2 leads to the accumulation of DNA lesions and increased apoptotic response. Cell viability and DNA repair capability in response to DNA damage induction are also reduced by the E2F1 and E2F2 deficiencies. In addition, E2F1 and E2F2 accumulate at sites of oxidative and UV-induced DNA damage, and interact with γH2AX DNA repair factor. Finally, as it was previously established for E2F1, we show that E2F2 downregulation impairs Rad51 foci formation upon genotoxic insult. The results presented here unveil a new mechanism involving E2F1 and E2F2 in the maintenance of genomic stability in response to DNA damage in neuronal cells.

CB-C19
LYSOPHOSPHATIDYLCHOLINE: A SIGNAL FOR NEURONAL DIFFERENTIATION IN NEURO-2A CELLS

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Phosphatidylcholine (PC) is the most abundant phospholipid in mammalian membranes, and its biosynthesis is required in several processes like neuronal differentiation. We use Neuro-2a cells that can be induced to differentiate

by treatment with Retinoic Acid (RA). We previously demonstrated that stable cell lines that overexpressed enzymes involved in the biosynthesis of PC undergo neuronal differentiation in the absence of RA, suggesting a regulatory role of PC metabolism. We demonstrated that addition of PC liposomes promotes neuronal differentiation, and that this process depend on cytoplasmatic phospholipase A2 (cPLA2) activity. As a consequence, we observed that lysophosphatidylcholine (LPC) but not fatty acids induce neuronal differentiation. LPC promote the process by activating ERK cascade. Experiments of supplementation with lysophosphatidic acid showed that this lysophospholipid is unable to induce differentiation. However, the addition of 1-O-palmityl-sn-glycero-3-phosphocholine (LPAF, which has an ether bond in the 1-position that prevents its hydrolysis) exert the same effect that LPC. The pharmacological inhibition of LPC acyltransferase (a condition that increases the level of endogenous LPC) showed an induction of neuronal differentiation even in the absence of RA. The results suggest that LPC is a neurotrophin-like factor able to promote neuronal differentiation.

Lipids

LI-C01
LIPIDS AS SIGNALS IN STEM CELL DIFFERENTIATION

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Neural stem cells (NSCs) are a cell type able to divide asymmetrically: one daughter cell maintains the same characteristics, while the other differentiates into a neuron or glia cell. In an adult mammal, exist niches of NSCs which are activated after cellular damages. However, as there activation is not complete, the identification of molecules that can act as signal and promote this process is relevant. We study if the stem cell differentiation could be induced by phospholipids. NSCs were incubated with lipids for three days. Cell identity was analyzed by immunofluorescence with β-III tubulin (neurons) and GFAP (glia). We observed that Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) have a specific effect on neural differentiation; while Phosphatidyletanholamine (PE) has a specific effect on glia stem cell differentiation. We also analyzed the effect of the inhibition of the Kennedy pathway for PC biosynthesis. The results suggest that although the neuronal differentiation program is turned on, cells are unable to generate and elongate neurites.In order to study how PC biosynthesis affects stem cell differentiation, NSCs cultures were followed by time-lapse microscopy. Single cells were followed for several days to analyze the fates of individual cells and their progeny. In this way, we could study alterations in proliferation and the mode of cell division.

LI-C02
MOLECULAR MECHANISM OF THE PHOSPHOLIPID SYNTHESIS ACTIVATION BY C-FOS

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The oncoprotein c-Fos activates phospholipid synthesis through a mechanism independent of its genomic AP-1 activity. To accomplish an overall activation of this synthesis, only key metabolic steps are positively affected. c-Fos is capable of increasing the activity of PhosphatidatePhosphohydrolase, CDP-diacylglycerol synthase and Phosphatidylinositol 4-Kinase II. The kinetic constant Vmaxis increased in all the enzymes activated by c-Fos that were analyzed while no effect was seen on the Km (a measure of the substrate enzyme affinity). Furthermore, we have proved the activation in a purified in vitro system that contains only c-Fos, PAP1 enzyme and the substrate.In order to understand the mechanism of enzyme activation, we performed analysis of protein-protein interactions by Forster Resonance Energy Transfer (FRET). We have established a direct association specifically between c-Fos and the enzymes it activates; no association is observed between c-Fos and the enzymes it does not activate.Results point to a shared mechanism of phospholipid synthesis enzyme activation: c-Fos directly interacts with those whose metabolic steps it activates to satisfy cell phospholipid requirements such as membrane biogenesis. It also reinforces the concept of a protein capable of increasing pivotal enzymatic activities per se.

LI-C03
ENDOCANNABINOIDS IN *Caenorhabditis elegans* DAUER DIAPAUSE REGULATION

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The most studiedendocannabinoids (eCBs) in mammals, anandamide and 2-arachidonoylglycerol (2-AG), are produced from membrane phospholipids after cell stimulation and immediately released to target their canonical receptors. These metabolites have been detected in the nematode *Caenorhabditiselegans*, as well as some of the enzymes responsible for their synthesis and degradation. However, no eCB receptors have been identified in this organism. In addition, one of *C. elegans* most striking characteristics is its ability to carry out an alternative life cycle in hostile environments, where development into an extremely resistant and long-lived larva, known as dauer larva, takes place. We have determined that lipid starvation produced by reduction of de novo synthesis of fatty acids (FAs) induces the entry of *C. elegans daf-7(e1372)* mutant strain into the dauer state. Importantly, administration of exogenous eCBs can bring the nematode out of this phase, presumably because these molecules provide a signal that reverses the adaptation to reduced FA synthesis that leads to dauer formation. The finding that eCBs could be reproductive growth promoters provides a unique tool for understanding the molecular logic of eCB signaling in this animal model organism, where eCBs appears to be linking lipid metabolism with regulatory mechanisms concerning postembryonic development and longevity.

LI-C04
SPHINGOSINE 1 PHOSPHATE CONTROLS SPHINGOLIPIDS SYNTHESIS DURING MDCK CELL DIFFERENTIATION

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Sphingosine 1-Phophate (S1P) is an important sphingolipid mediator in cell fate, synthetized by Sphingosine Kinase (SK). We have demonstrated that hypertonic media induce the establishment of differentiated phenotype of MDCK cells. In this situation the sphingolipid metabolism change and increase in the salvatage pathway. We study the involvement of SK activity in this process. For this end, confluent MDCK cells were subjected to hypertonic medium with the concomitant knock down of SK or not (control). Additionally we use D,L-threo-dihydrosphingosine (DHS) as an SK inhibitor. After 48 h of incubation, the cell phenotype was visualized by fluorescence microscopy, evaluating actin cytoskeleton and Adherens Junction (AJ) formation. SK knock down induces Adherens Junction protein redistribution to intracellular localization resulting in disassembling of AJ, nuclear accumulation of β-catenin and actin cytoskeleton reorganization. SK inhibition also induces an increase in de novo sphingolipid synthesis with Ceramide (Cer) accumulation. In order to evaluate whether AJ disassembly is due to Cer accumulation, Fumonicin B1 (FB1) was used, an inhibitor of Ceramide Synthase. FB1 treatment recovers MDCK phenotype, suggesting that the disassembly of AJ due to inhibition of SK activity is an indirect effect produced by Cer accumulation.

LI-C05
IMPORTANCE OF THE SPHINGOLIPIDS IN THE CILIUM FORMATION OF MDCK CELLS

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The final stage in the differentiation of epithelial cells is the outgrowth of a primary cilium from the apical surface. The mechanisms underlying cilia formation are poorly understood. We have demonstrated that glycosphingolipid synthesis is essential in MDCK cell differentiation induced by hypertonicity. The aim of this study was to evaluate the role of different sphingolipids in primary cilium formation. Confluent MDCK cells were cultured under isotonicity or subjected to hypertonic media for 72h in the presence or absence of D-PDMP, t-DHS and D609, inhibitors of glucosylceramide synthase, sphingosine 1-phosphate and sphingomyelin synthase, respectively. The primary cilium formation was analyzed by immunofluorescence for acetylated tubulin to reveal the cilium, and for Zo1, a tight junction marker. Cells cultured under isotonicity failed in developing primary cilium, whereas cells subjected to hypertonicity showed cilium formation and this process was impaired by D-PDMP. D609 induced a fibroblastic-like phenotype, with no cilium development. On the other hand, t-DHS did not affect the cilium formation, but induced a dissipation of Zo1 in specific areas. These results demonstrate that, even under hypertonicity, MDCK cells cannot fully differentiate in the presence of D-PDMP and D609, indicating that glucosylceramide and sphingomyelin are essential for primary cilium formation.

LI-C06
Haematococcus pluvialis AS A SOURCE OF TRIACYLGLYCEROL FOR BIODIESEL AND VALUABLE CO-PRODUCTS

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Haematococcus pluvialis is an oleaginous microalga which has been previously described as an important source of astaxanthin and, recently, of triacylglycerol (TAG) for biodiesel production. In this work, our purpose was to describe the effects of light-induced stress on *H. pluvialis* lipid metabolism. For this end, native strain from Bahía Blanca (Arg.) was grown in appropriate culture medium and maintained under control conditions or light-induced stress (300 μmol photons m-2 s-1) for 3, 6 and 12 days. After that, pigment content and lipid profile were analyzed. Under light-induced stress conditions, microalga cultures showed increased levels of astaxanthin and phytosterols, and diminished chlorophyll content. After 12 days of light-induced stress, *H. pluvialis* cultures showed the maximal TAG accumulation (568, 8%) with respect to control conditions. TAG accumulation was accompanied by a strong diminution in the protein content and in cell density. Fatty acid profile analyzed by GC was similar in all the assayed conditions and met biodiesel standards. Our results suggest that light-induced stress in *H. pluvialis* triggers TAG accumulation suitable for biodiesel production and also the accretion of valuable co-products (astaxanthin and phytosterols).

LI-C07
NLPR CONTROLS GENES INVOLVED IN NITROGEN AND LIPID METABOLISM IN OLEAGINOUS *Rhodococcus* STRAINS

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Oleaginous *Rhodococcus* strains are able to accumulate large amounts of triacylglycerols (TAG) under nitrogen-limiting conditions from a broad range of carbon sources. Studies to establish physiological and molecular mechanisms for TAG biosynthesis/accumulation have been reported, but so far the regulatory aspects of these processes are unknown in such bacteria. In this work, we described NlpR (Nitrogen-lipid Regulator) as a putative transcriptional regulator which controls the expression of genes involved in nitrogen and lipid metabolism in *R. jostii* RHA1 and *R. opacus* PD630. Transcriptome analyses showed a higher expression of *nlpR* under nitrogen-limiting conditions, but it was repressed when an inhibitor (cerulenin) of *the novo* fatty acid biosynthesis was added. *NlpR* disruption resulted in a significant decrease of TAG content besides to a delayed growth with NO_3^- or NO_2^-; whereas its overexpression led to an improved TAG accumulation under nitrogen-rich conditions. Preliminary analyses by EMSA assays, showed a positive binding of purified NlpR to the upstream region of genes involved in NO_2^- assimilation (*nasK-nirB*) and TAG biosynthesis (*atf1*) among others lipid metabolism genes of RHA1 strain. The results of this work suggest that NlpR acts as a global regulator linking the nitrogen and lipid metabolism in response to nitrogen starvation conditions in oleaginous *rhodococci.*

Microbiology

MI-C01
IDENTIFICATION OF A METACASPASE PROTEIN SUBSTRATE CONSERVED AMONG EARLY DIVERGENT EUKARYOTIC CELLS

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Metacaspases, distant relatives of metazoan caspases, have been shown to participate in programmed cell death, progression of the cell cycle and removal of protein aggregates in unicellular eukaryotes. However, since natural proteolytic substrates have scarcely been identified to date, their roles in these processes remain unclear. To find metacaspase's putative substrates in *Trypanosoma cruzi* we tested 14 enzyme interactors previously identified by co-purification and mass spectrometry. Each one was co-expressed with metacaspase 5 (*Tc*MCA5) using a dual-vector *E. coli* system. By this method we identified for the first time a *Tc*MCA5 protein substrate. The cleavage site, determined by N-terminal Edman sequencing of fragments produced *in vitro* with both recombinant purified proteins, presented an Arg residue upstream the hydrolyzed peptide bond matching perfectly the known metacaspase

specificity. Moreover, replacement of this residue by Ala completely prevented cleavage. Similar results were obtained for *T. brucei* and budding yeast metacaspase orthologs on their respective substrates. Interestingly, in each case cleavage occurs at a linker region that connects different domains. The *in vivo* proteolytic event is currently being studied in *T. brucei*, and wild type or metacaspase null mutant yeasts.

MI-C02
EXPOSING THE SECRETS OF TWO WELL KNOWN DAIRY PHAGES: GENOMIC AND STRUCTURAL ANALYSIS OF J-1 AND PL-1

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Bacteriophages infecting lactic acid bacteria constitute a threat for dairy fermentations. Knowledge of phage-host interactions is a valuable tool for the design of rational strategies against infection. We have sequenced and annotated the genomes of phages J-1 (isolated from an abnormal fermentation of Yakult) and PL-1 (isolated from a J-1 derived resistant strain). Both genomes are almost identical but PL-1 has a deletion of 1.9 kbp in the immunity region and also differs in gene 16 that code for a tail component. All the structural proteins were identified by mass spectrometry analysis. The structure of gp16 was modeled based on distal tail proteins. However, a region that accounts for the differences between J-1 and PL-1 gp16 could not be fitted in the model and showed sequence similarity to carbohydrate-binding modules. J-1 and PL-1 gfp-gp16 fusions specifically bind to *Lactobacillus casei/paracasei*cells and addition of L-rhamnose inhibits binding of the recombinant protein and entire phage particles as well. Phage adsorption inhibition assays revealed a higher affinity of J-1 gp16 for cell walls of *L. casei* ATCC 27139 in agreement with differential adsorption kinetics observed for both phages in this strain. Altogether, this is the first report of an isolated Dit-like protein interacting with the cell surface and probably involved in determination of the host range.

MI-C03
BROMODOMAIN FACTOR 3 IS ESSENTIAL FOR *Trypanosoma cruzi* GROWTH AND DIFFERENTIATION

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Bromodomains (BrDs) are conserved protein modules capable of binding acetylated lysines (Kac) and are found in proteins associated with chromatin and in nearly every nuclear histone acetyltransferase. *Trypanosoma cruzi* Bromodomain Factor 3 (*Tc*BDF3) is the first exclusively non-nuclear bromodomain-containing protein reported so far. *Tc*BDF3 is expressed in all life cycle stages and interacts with acetylated α-tubulin, the major component of the flagellar and subpellicular microtubules. This ability to interact with acetylated α-tubulin was impaired *in vitro* when we mutated essential aminoacids of the bromodomain binding-pocket. Do to the impossibility to perform RNA interference in this organism we over-expressed the mutated protein in an inducible manner, which acted as a dominant negative mutant. When the mutated protein is over-expressed in *Trypanosoma cruzi* epimatigotes (non-infective stage) the parasites do not grow normally and the metacyclogenesis (differentiation to the infective stage) rates are diminished. Also, we quantified the infection in Vero cells by the parasites over-expressing the wild type and the mutated protein and the localization of the exogenous proteins was determined in all life cycle stages by fluorescence microscopy. These results allowed as concluding that *Tc*BDF3 is essential for *Trypanosoma cruzi* growth and differentiation.

MI-C04
OLIGOTYPING ANALYSIS OF A WASTEWATER TREATMENT PLANT MICROBIOME

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The assessment of bacterial diversity using high-throughput sequencing of 16S rRNA gene amplicons generally relies on clustering approaches that establish operational taxonomic units (OTUs). Clearly, these approaches are not capable of resolving ecologically distinct organisms having overall similar 16S rRNA sequences. This limitation is overcome by oligotyping, a recently developed computational approach, based on Shannon entropy, which identifies highly variable nucleotide sites in the 16S rRNA gene as markers for genomic variation between closely related

microorganisms. We have applied this method to compare the distribution of closely related organisms across a time series of high-throughput amplicon sequencing data of municipal activated sludge. We observed large time-dependent changes in oligotype distribution within several OTUs. Accordingly, relevant process variables (BOD, SVI, T) correlated with the abundance of some of the oligotypes, but not with the abundance of the corresponding OTU, implying differences in ecological niches between closely related oligotypes. Significant negative correlations, suggesting competition between related oligotypes, was also observed. Overall these results show that oligotyping can reveal aspects of ecological and functional diversity in activated sludge that are hidden in similarity-based analyses.

MI-C05
OUTER MEMBRANE VESICLES IN *Serratia*: PROTEOMIC ANALYSIS AND DELETERIOUS EFFECT IN AN INSECT MODEL

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Gram negative bacteria naturally release outer membrane vesicles (OMVs) into their environment. These OMVs are composed of lipopolysaccharide, phospholipids, outer membrane proteins and periplasmic components. Several studies have suggested a number of roles for vesiculization including the selective delivery of virulence factors in pathogenic species. *Serratia marcescens* is a member of Enterobacteriaceae that acts in humans as an opportunistic pathogen. Despite its clinical prevalence, mechanisms of *Serratia* pathogenesis remain unclear. In this work we applied an optimized protocol for purifying flagellin-free OMVs. Subsequent in-solution trypsin digestion coupled to MS allowed us to identify 26 proteins present when *S. marcescens* was grown to stationary phase, at 30°C. Parallel proteomic analysis of outer membrane fraction (OM) revealed differential components between both fractions under study, reinforcing the concept of OMVs as vehicles of selected cargo. Furthermore, we carried out OMVs and OM microinjections assays in *Drosophila melanogaster* and analyzed survival rates. Results showed that, in this infection model, OMVs have a deleterious effect. This effect was strongly dependent on the carriage of the PrtA protease, as revealed when *prtA*$^{-}$strain-derived OMVs were compared. These results reveal that the production of OMVs is a component of *S. marcescens* pathogenic arsenal.

MI-C06
IDENTIFICATION OF A COMPOUND FROM SOYBEAN HULL PYROLYSATE WITH ACTIVITY AGAINST *Salmonella enterica*

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Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. In *S. typhimurium*, the PhoP/PhoQ two-component system (TCS) controls the virulence, the adaptation to limited Mg^{2+} conditions, the resistance to antimicrobial peptides and the modifications of the LPS. This system is also involved in the bacterial entry mechanism into the host cell. Once inside the cell, PhoP-modulated genes contribute to define the intracellular survival of *Salmonella*. As signal transduction in mammals occurs by a different mechanism, the inhibition of these TCS may be a target for new antimicrobial agents. In Argentina, soybean hull constitutes a main agroindustrial subproduct, which is susceptible of being recycled by pyrolysis with the aim of obtaining economically useful compounds. By using a bio-guided strategy, we have discovered that soybean hull pyrolysates affect viability of both Gram(-) and Gram(+) bacteria, and have identified the compound responsible for this effect. Also, we have synthesized chemically modified derivatives of this compound that allowed us to determine the chemical functional groups that are required for the action over bacterial viability. In conclusion, our results highlight the importance of biomass pyrolysis to recycle low commercial value products into compounds with antimicrobial activity against pathogenic bacteria of sanitary relevance.

MI-C07
CO-DEPENDENCE ON COPPER EXCESS AND PERIPLASMIC STRESS TO CONTROL *Salmonella CUEP* EXPRESSION

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Copper (Cu) is an essential metal required as cofactor in numerous biochemical reactions, and at the same time is highly toxic. In most Gram(-) bacteria Cu toxicity is handled by the *cue* regulon, that includes *copA* and *cueO*, coding for a Cu-efflux pump and a periplasmic multicopper oxidase, controlled by the cytoplasmic Cu-sensor/regulator CueR. Most species also rely on the Cus system to cope with bacterial envelope Cu excess. This includes a two-component system that senses periplasmic Cu and induces the expression of a periplasmic Cu-efflux system CusCFBA. *Salmonella* does not harbor the *cus* locus. Instead, a CueR-regulated gene coding for a periplasmic protein, CueP, substitutes the function of the Cus system. We observed a differential expression within the *Salmonella cue* regulon that particularly affects *cueP* expression. By site-directed mutagenesis we detected a cis-acting sequence within *cueP* promoter responsible for this differential expression. *In vivo* as well as *in vitro* experiments allowed us to confirm this regulatory element, sensitive to the periplasmic stress caused by Cu excess. Phenotypic analysis allowed us to demonstrate that this periplasmic-stress sensory element acts in concert with CueR to ensure the correct supply of this component of the *cue* regulon when the environment becomes unfavorable for survival.

MI-C08
IDENTIFICATION OF A *Brucella* PERIPLASMIC PROTEIN CENTRAL FOR THE ADAPTATION TO ENVIRONMENTAL CHANGES

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All organisms, including bacteria, face constant environmental changes and must adapt to them in order to survive. In particular, many pathogens that alternate between free living conditions and their hosts, encounter different environments which vary in temperature, nutrient availability and presence of an immune system among other stimuli. In order to adapt to each of these scenarios, pathogenic bacteria must be able to sense the cues present in each of them and modify their biology appropriately. Here, we present the identification in *Brucella* of a gene (*cgpA*) that codes for a small protein with periplasmic localization. A null mutant in *cgpA* is unable to replicate at 23°C in solid media and, in liquid media, is incapable of transitioning from lag to log phase, although at 37°C the strain grows as the wild type one in both conditions, indicating that the mutant is unable to sense and respond to the temperature shift. Lipid composition analysis of both strains at 37°C and 23°C indicated that the mutant is also unable to modify its membrane content in response to temperature. Moreover, the mutant strain is severely attenuated in macrophages but overreplicates in mice, inducing a heightened inflammatory response. Overall, our results indicate that *cgpA* codes for a protein that is key for the capacity of the bacteria to sense and respond to a plethora of extracellular cues.

MI-C09
THE MISMATCH REPAIR PROTEIN MutS CONTROLS THE ACCESS TO REPLICATION OF THE DNA POLYMERASE IV

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We report that MutS regulates the access of the low fidelity DNA Polymerase (Pol) IV to replication through its interaction with the processivity factor β clamp in *Pseudomonas aeruginosa*. Based on *in vitro* assays, we found that MutS inhibits the association of Pol IV with β clamp, which is absolutely required for the activity of this Pol. Conversely, $MutS^{\beta}$, a mutant version that fails to bind β clamp, was not able to prevent Pol IV-β clamp interaction. These data suggested that MutS and Pol IV may compete for binding to β clamp. To analyze the relevance of this competition *in vivo*, we evaluated the effect of Pol IV overexpression on mutagenesis of diverse chromosomal genes in a mutant strain harboring a chromosomal *mutS*$^{\beta}$ allele compared to the parental strain. Pol IV overexpression increased ~10-fold mutation rates at all Pol IV expression levels tested in the parental strain, indicating that the Pol IV mutagenic activity is controlled in these cells. This control was not evidenced in the *mutS*$^{\beta}$ strain since it showed a concomitant increase of mutation rates with Pol IV levels reaching a maximum of ~45-fold. Finally, analysis of mutation spectra revealed that Pol IV strongly contributes to spontaneous mutations in the *mutS*$^{\beta}$ strain whereas it has a lesser impact on mutagenesis in the parental strain. This work reveals a novel mechanism by which MutS maintain replication fidelity.

MI-C10
DIVALENT METAL IS REQUIRED FOR LIGAND BINDING TO THE *Enterococcus faecalis* CITRATE METABOLISM REGULATOR

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In *Enterococcus faecalis*, the citrate metabolism regulator (CitO) belongs to the GntR superfamily. Within the FadR family CitO and its homologs constitute a subgroup associated in Gram+ organisms to citrate utilization. In presence of citrate, CitO binds to *cis*-acting sequences near the *cit* promoters inducing citrate metabolism. CitO binds specifically citrate with high affinity (KD=1.2 μM) as determined by isothermal titration calorimetry and differential scanning fluorometry. Structure-based site directed mutagenesis was used to identify residues involved in citrate binding. Mutations in CitO Q64A, H74A and L214A did not affect citrate binding. In contrast, changes to Ala in R97, F143 and H191 modified the binding affinities for citrate indicating that these residues mediate ligand binding. These results were confirmed *in vivo* using fusions to *lac*Z in *E. faecalis*. In CitO structure model, residues N147, H191 and H213 that may be involved in metal binding were found. Accordingly, it was observed that binding of citrate in CitO was dependent on the presence of metal evidenced as decreased DNA binding upon preincubation with EDTA. The effect could be reverted by addition of Ca^{2+}, Zn^{2+} or Mn^{2+}. The metal-binding site is buried within CitO structure, which suggests that citrate carboxylic group could interact directly with the metal at the bottom of the ligand-binding cavity.

MI-C11
CHARACTERIZATION OF ESSENTIAL ACYL- COENZYME A CARBOXYLASES OF *Mycobacterium tuberculosis*

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The two Fatty Acid Synthase (FAS) systems of the human pathogen M. tuberculosis work in concert to synthesize mycolic acids. FAS I builds a long-chain acyl-CoA (C24-CoA) and FAS II generate a very long-chain fatty acid (meromycolic acid). A long-chain acyl-CoA carboxylase activates the C24 acyl-CoA, and the acyl-AMP ligase FadD32 activates the meromycolic acid chain. These two chains are later condensed by Pks13 to yield the final mycolic acid. Besides its importance in mycolic acid biosynthesis, there are no conclusive results of the subunit composition of the acyl-CoA carboxylase responsible to generate the long-chain carboxyacyl-CoA. We found that an accD5-accE5 conditional mutant in Mycobacterium smegmatis have reduced levels of long-chain acyl-CoA carboxylase activity. Also, 14C-acetate labeling and TLC analysis of FAMEs and MAMEs of this mutant showed accumulation of previously uncharacterized compounds. LC-MS analysis of these compounds showed that they were meromycolic acids methyl esters. These results allow us to speculate that the subunits AccD5 and AccE5, that are part a of well-characterized propionyl-CoA carboxylase, are also involved in the long-chain acyl-CoA carboxylation. To solve this issue, we performed in vitro assays and we found evidences that the subunits AccD5 and AccE5 are part of the active long-chain acyl-CoA carboxylase.

MI-C12
APPLICATION OF FLUOROPHAGES FOR TB-DETECTION AND WHOLE CELL SCREENING OF ANTITUBERCULAR DRUGS

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Tuberculosis (TB) is a major cause of human mortality with 9 million new cases and nearly two million deaths annually; approximately two billion people are infected with the causative agent, *Mycobacterium tuberculosis*. We have described the development of Fluoromycobacteriophages containing a fluorescent reporter gene that provide a simple means of revealing the metabolic state of mycobacterial cells, and therefore their response to antibiotics. We have constructed an optimized version of these mycobacteriophages carrying an*mCherrybomb* gene with an enhanced expression to increase the signal and shorten the time-to-detection. Fluorescence can be detected easily by fluorescent microscopy or by flow cytometry and is maintained for at least two weeks after fixation, increasing biosafety and facilitating storage or transportation of samples. We used this improved version to test specific protocols for sputum processing to achieve efficient phage infection of mycobacterial cells directly in clinical samples. Together, these characteristics result in a simple, rapid, and specific diagnostic test for TB. Finally, we set up the conditions for infection of mycobacterial cells in a convenient automated multiwell format using a fluorimeter. As a result, we

describe a novel application of fluorophages for rapid drug susceptibility testing and whole cell screening of new antitubercular drugs.

Neuroscience

NS-C01
ROLE OF C-FOS DURING NEUROGENESIS.

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c-Fos is a proto-oncogene involved in diverse cellular functions. Its dysregulation has been associated to abnormal development and oncogenic progression. c-fos-/- mice are viable but present a clear reduction in the body weight and size their brain reaches. Herein we examine the importance of c-Fos during development of the neocortex. We studied the cerebral cortex at 14.5 days of gestation (E14.5) (peak of neurogenesis), analyzing neocortex thickness, apoptosis, mitosis and expression of markers along the different stages of Neural Stem Progenitor Cells (NSPCs) differentiation in c-fos-/- and wild-type mice. A ~15% reduction in the neocortex thickness of c-fos-/- embryos was observed which correlates with an increase both in the number of non-differentiated cells (Sox2-positive cells) and the content of apoptotic cells in the ventricular zone. No difference in the rate of mitosis was observed, although the mitotic angle was predominantly vertical in c-fos-/- embryos, suggesting a reduced trend of NSPCs to differentiate. More AP-1/DNA complexes were detected in nuclear extracts of cerebral cortex from c-fos-/- embryos with no differences in the lipid synthesis activity between both groups of embryos. Taken together, these results indicate that c-Fos is involved in the normal development of NSPCs by means of its AP-1 activity.

NS-C02
C-FOS REGULATES BRANCHING FORMATION IN NEURONS.

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Neuronal differentiation is a complex event where lipid synthesis for membrane biogenesis plays a key role. We have previously shown that c-Fos associates to membranes of the endoplasmic reticulum (ER) and activates phospholipid synthesis. This activation mechanism might be associated with the molecular events that allow the higher rate of membrane genesis required for neuronal differentiation. When c-Fos expression is blocked in primary cultures of rat hippocampal neurons either using a lentiviral vector that expresses a specific sequence against c-Fos or a specific antibody, differentiation is impaired and no development of axonal processes can be observed. Immunofluorescence analysis of c-Fos shows it co-localizing with ER markers in the neuronal soma and mainly forming structures at the branching sites of the neuronal processes. We examined if CTP:phosphocholine cytidylyltransferase-β2 (CCTβ2), an integral enzyme of the ER membranes that plays an important role in the formation of axon branches, is activated by c-Fos. Both co-immunoprecipitation assays and FRET microscopy consistently showed a physical interaction between CCTβ2 and c-Fos. Taken together, our results point to a possible mechanism for axonal branching regulation and support our hypothesis of the importance of c-Fos dependent activation of phospholipid synthesis for neuronal differentiation.

NS-C03
PEA3 TRANSCRIPTION FACTORS: MEDIATORS OF BDNF-MEDIATED HIPPOCAMPAL DENDRITE GROWTH DURING DEVELOPMENT.

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The precise development of complex and specific dendrite morphology is essential in the development of the functional properties of neural circuits and many neurodevelopmental disorders are due to structural abnormalities of dendrites and their connections. In the present work, we show that two members of the Pea3 family of transcriptional factors, Etv4 and Etv5, are key regulators of growth and elaboration of pyramidal cell dendrites in the developing hippocampus. We show that hippocampal neurons co-express Etv4 and Etv5 during development. Downregulation of Etv4 or Etv5 in pyramidal neurons, which express endogenous levels of both transcription factors, resulted in reduced complexity of dendritic arborization and a prominent reduction of spine density. Overexpression of Etv4 and Etv5

resulted in an enhanced dendritic growth and spine development. We also provide evidence indicating that the induction of Etv4 and Etv5 by the Brain Derived Neurotrophic Factor (BDNF) is essential to control dendrite patterning. Our experimental data support the idea that BDNF induce transcriptional programs, which involve Etv4 and Etv5, which play a crucial role in the establishment of hippocampal connectivity.

Plant Biochemistry and Molecular Biology (*Plants*)

PL-C01
ATHB1, AN ARABIDOPSIS HD-ZIP I TRANSCRIPTION FACTOR, IS INVOLVED IN HYPOCOTYL ELONGATION

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Homeodomain-leucine zipper (HD-Zip) transcription factors are only present in the plant kingdom and have been involved in developmental processes associated to environmental conditions. AtHB1, an Arabidopsis thaliana HD-Zip I member, has been previously described as a transcriptional activator, probably involved in leaf development. However, up to date its function is still largely unknown. Here we show that AtHB1 is mainly expressed in hypocotyls of seedlings grown under a short photoperiod regime. *AtHB1* knock-down mutants showed longer hypocotyls than WT while AtHB1 overexpressors exhibited the opposite phenotype, especially in short-day-conditions. By measuring *AtHB1* transcript levels in *pif1* mutants and GUS activity in tobacco leaves co-transformed with *PromAtHB 1::GUS* and *35S::PIF1*, it has been observed that PIF1 induces *AtHB1* expression. Additionally, by analyzing *athb1*, *pif1* and *athb1/pif1* mutants and PIF1 or AtHB1 overexpressors in WT, *athb1* or *pif1* backgrounds, we were able to determine that PIF1 regulates *AtHB 1* expression to promote hypocotyls growth. Transcriptomic approaches revealed that AtHB1 regulates several genes involved in cell wall composition and elongation. The results suggested that AtHB1 acts downstream PIF1 to regulate growth-related proteins expression to promote hypocotyl cell elongation in response to short photoperiod conditions.

PL-C02
CARACTERIZATION OF NEW GENES REGULATED BY GRF TRANSCRIPTION FACTORS

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The GRFs (GROWTH-REGULATING FACTORs) are a family of transcriptional factors composed by nine members in Arabidopsis thaliana. Seven GRFs are negatively regulated by a microARN miR396 at the post-transcriptional level. This regulation is conserved at least in angiosperms and gymnosperms. There is evidence showing that the GRFs are important regulators of organ size in plants. Furthermore, we have recently shown that they also regulate plant longevity, suggesting that the GRFs have functions that go beyond the control of cell proliferation. Although there is plenty information describing the roles of the GRFs in plant development, little is known about the genes directly regulated by these transcription factors. In other to identify these target-genes, we carried out a metanalysis of microarray experiments from plants with altered GRF levels. We complemented this approach with ChIP-seq experiments from transgenic plants carrying a GRF3-GFP transgene. We found that the GRFs directly regulate another transcription factor of the ZF-HD (Zinc Finger-HomeoDomain) family. We focused in this target to perform a detailed biochemical characterization of the system. These results will provide new insights into the gene-networks controlled by the miR396-GRF system.

PL-C03
SEARCHING FOR THE N-END RULE PATHWAY IN CHLOROPLASTS AND ITS SUBSTRATE SELECTOR

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The caseinolytic protease (Clp) is crucial for chloroplast biogenesis and proteostasis. In *Arabidopsis thaliana*, Clp complexes are composed of the proteolytic chamber ClpPR and the chaperones ClpC 1/2 and ClpD, which select and unfold the substrates. The N-end rule degradation pathway states that the half-life of a protein is determined by the nature of its amino-terminal residue. *Escherichia coli ClpS* (ecClpS) recognizes N-end rule substrates and presents them to the ClpAP protease. Since ClpS is found in plants, we postulate the existence of the N-rule in chloroplasts. Gel filtration chromatography showed that the *A. thaliana* recombinant ClpS (atClpS) exists as dimers and trimers, in contrast with ecClpS, which is a monomer. These results were confirmed by chemical crosslinking. Also, atClpS was found to associate with Hsp 100 chaperones. We previously demonstrated that atClpS is able to bind substrates recognized by ecClpS. We designed mutant proteins in which the amino acids involved in substrate recognition were altered. We found that one amino acid of the recognition site is essential for peptide binding and that the surface charges at this site may define substrate specificity, which is different in plants and bacteria. Our results indicate that ClpS functions as a substrate selector of the Clp system and by the N-end pathway, discriminates a different spectrum of substrates.

PL-C04
EVIDENCE OF *IN VIVO* PYRUVATE CARBOXYLASE ACTIVITY FOR AN ARABIDOPSIS NADP-MALIC ENZYME

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NADP-malic enzyme (NADP-ME) catalyzes the reversible oxidative decarboxylation of malate to pyruvate, CO_2 and NADPH and is present as a multigene family in *A. thaliana*. The reverse reaction catalyzed by recombinant AtNADP-MEs was faster than reported for other animal or plant isoforms. Particularly, AtNADP-ME2, the cytosolic isoform that mostly contributes to the ME activity in all organs of the plant, presented the higher catalytic efficiency of the family. In this work, AtNADP-ME2 as well as its N-truncated version NADP-MEdel2 (unable to catalyze the carboxylation of pyruvate *in vitro*) were functionally expressed in pyruvate carboxylase-negative (Pyc^-) *Saccharomyces cerevisiae* strains, which are incapable of growing on glucose as the sole carbon source. Experiments conducted on agar plates as well as in batch cultivation under ambient and thermodynamically favorable conditions showed that heterologous expression of both NADP-ME2 and NADP-MEdel2 enabled growth of Pyc^- *S. cerevisiae* on solely glucose. This capacity of rescuing Pyc^- strains from C_4 auxotrophy was dependent on the provision of the reaction substrates, particularly CO_2. These results indicate that AtNADP-ME2 is able to play an anaplerotic role *in vivo* and provide a basis for the study of the carboxylase activity of malic enzyme, which may contribute to the synthesis of C_4 compounds in plant cells.

PL-C05
ATHSCB AND ITS ROLE IN PLANT IRON HOMEOSTASIS

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Iron is an essential nutrition element in all organisms. Since Fe is a substrate of the [Fe-S] cluster biogenesis, it has been suggested an intimate connection between Fe homeostasis and Fe-S cluster formation. In this work we evaluate the effect of AtHscB (co-chaperone, involved in the Fe-S cluster biogenesis) on iron transport in A. thaliana. We characterized two AtHscB over-expressing and AtHscB knock-down mutant lines. The activities of [Fe-S] enzymes ACO and SDH were reduced in both leaves and shoots knock-down lines. Conversely, these activities were increased in over-expressing roots, but notoriously decreased in their leaves. Under Fe sufficient conditions, over-expressing and knock-down lines present altered Fe levels distribution between roots and shoots. Furthermore, these lines are less sensitive to growth under Fe deficiency. Studies after 48 h of metal absence showed that all lines decrease iron levels in root. In addition, Fe content in wt, knock-down mutant leaves decreases in comparison to their control conditions, while a clear increment in over-expressing leaves was shown. Moreover, changes in foliar iron content were correlated with variations in [Fe-S] enzymes activities. Our results indicate that AtHscB might produce a deregulation of the iron transport root to shoot, suggesting that Fe-S clusters could regulate the Fe transport in plants.

PL-C06
DIFFERENT CONTRIBUTION OF MITOCHONDRIAL METABOLISM TO SYNTHESIS OF LIPID IN SOYBEAN AND CASTOR SEEDS

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Soybean and castor bean seeds have important roles providing feedstocks for human and animal diet and industrial processing. In these oilseeds the synthesis *de novo* of the fatty acids occurs in the plastids and requires precursors generated in the cytosol mainly by glycolisis. Malate is a carbon transporter molecule abundant in plant tissues that supplies carbon skeletons and reductive power for fatty acids synthesis through the action of a plastidic malic enzyme (ME). In this work we studied the ME families of soybean and castor bean. The comparison of the family members, expression patterns, enzymatic activities and organic acids contents reveal differences in the roles of the MEs in these oilseeds. Most significantly, mitochondrial NAD-ME showed an important role in the maturation of soybean seeds driving the relocation of carbon skeletons from mitochondria to plastids to support the fatty acids synthesis in the last stages of seed filling.

PL-C07
A GENOME WIDE EXPRESSION PROFILE OF YERBA MATE (*Ilex paraguariensis* A. ST.-HIL.)

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Yerba mate is cultivated on 326,000 ha in Argentina, Brazil and Paraguay, with a total yield production of more than 1,000,000 t. Yerba mate gene sequence information is scarce. In order to elucidate the yerba mate gene landscape by means of NGS, we explored and discovered a vast collection of Ilex paraguariensis transcripts. Total RNA was sequenced by Illumina HiSeqTM-2000 obtaining 72,031,388 pair-end 100 bp sequences. High quality reads were de novo assembled into 44,907 transcripts encompassing 40 million bases and ~180X coverage. Multiple sequence analysis allowed us to predict that yerba mate contains ~32,355 genes and 12,551 gene variants. Based in sequence conservation we were able to annotate 77% of the transcripts. We identified and categorized members of more than 150 metabolic pathways. Overall, we have identified ~1,000 putative transcription factors, and hundreds of genes related to biotic and abiotic stress. We have pinpointed several members of the gene silencing pathway, and characterized the silencing effector AGO1. We predicted a diverse supply of putative microRNA precursors involved in developmental processes. We present here the first draft of the transcribed genomes of the yerba mate chloroplast and mitochondrion. This contribution broadly expands the knowledge of yerba mate genes, and is presented as the first genomic resource of this important crop.

PL-C08
THE COX ASSEMBLY PROTEIN ATCOX 10 IS INVOLVED IN PLANT EMBRYOGENESIS AND SENESCENCE

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Cytochrome c oxidase (COX) biogenesis requires more than 20 accessory proteins implicated, among other processes, in copper and heme a insertion. COX10 catalyzes the conversion of heme b to heme o, and is essential for the insertion the heme a in the COX1 subunit in yeast. By complementation analysis of a yeast Δcox 10 mutant, we observed that the Arabidopsis homologous protein AtCOX10 was able to restore growth on a non-fermentable carbon source. We also studied Arabidopsis mutant plants with a T-DNA insertion in the coding region of the AtCOX 10 gene. Segregation analysis demonstrated that it was not possible to obtain knockout plants, suggesting that AtCOX10 is essential during plant embryogenesis. In addition, low levels of AtCOX10 impact directly on the onset and progression of both natural and induced senescence. Heterozygous mutant plants reached senescence before wild type, showing less chlorophyll content, lower Fv/Fm and higher transcript levels for Senescence Associated Genes (SAGs). Our results suggest that AtCOX 10 function is important during plant senescence, probably due to its role in COX assembly. We hypothesize that this is due to a main role of COX in nutrient remobilization and energy supply for cellular processes during senescence.

PL-C09
CHLOROPLAST-GENERATED REACTIVE OXYGEN SPECIES PLAY A ROLE IN LEAF DEVELOPMENT AND SENESCENCE

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Leaf development can be divided in several processes: i) initiation of primordia, ii) cell proliferation, iii) cell expansion, and iv) senescence. Reactive oxygen species (ROS) are involved in both the early steps of leaf development, and leaf senescence. Noteworthy, the onsets of cell expansion and senescence represent extreme stages of leaf development with a feature in common, an imperfectly assembled photosynthetic electron transport (PET) chain, which is in the process of full assembly in the first case and of collapse in the second. Non-functional PET leads to runaway ROS production. A plastid-targeted flavodoxin (Fld) can increase tolerance to multiple stresses in plants by acting as a general antioxidant specific for chloroplasts preventing ROS accumulation in these organelles. Therefore Fld-expressing lines can be used as tools to probe the role played by chloroplast-generated ROS in different processes undergone by plants. In plants grown under controlled conditions, leaves of the Fld transformants showed a decrease in leaf size caused by a repression of cell expansion. Likewise, Fld expression significantly delayed senescence. The "stay-green" phenotype was reflected by extended preservation of leaf pigments and photosynthetic activity. Taken together, the results indicate that chloroplast-generated ROS are involved in leaf development and senescence.

PL-C10
AN IRON DEPENDENT DEATH PATHWAY INVOLVED IN THE RESPONSE OF PLANTS TO BIOTIC AND ABIOTIC STRESSES.

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In plants, regulated cell death plays critical roles during several developmental processes and plant-specific responses to external stimuli. However, the molecular mechanisms underlying plant cell death remain unclear. While some pathways appear to be conserved in animal and plant cells, others are specific. In this work, we examined whether an iron-dependent, oxidative processes similar to the recently described to occur during ferroptosis in animal cells, could be relevant to plant cell death during development or following abiotic or biotic stresses. Remarkably, although ferroptosis seems not involved in megasporogenesis, embryogenesis or root development, it appears to be implicated in the regulated cell death that follows heat shock stress (HS-RCD) and in the HR response. Thus, this oxidative iron-dependent type of cell death emerges as a new mechanism through which plants might respond to specific environmental signals. Our results show that a group of morphological, biochemical and genetic features that seem to be specific for this particular lethal pathway are present in Arabidopsis roots in response to HS and suggest a physiological role for ferroptosis in plants restricting pathogen infection. (Supported by CONICET, AGENCIA y HHMI)

Structural Biology

SB-C01
ENHANCED CRYSTALLIZATION OF *Leptospira interrogans* HEME OXYGENASE BY DIRECTED MUTAGENESIS

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LepHO is a heme oxygenase from *Leptospira interrogans*, the causative agent of leptospirosis. It catalyzes the conversion of heme to iron, biliverdin and carbon monoxide employing oxygen and reducing equivalents. Efforts to crystallize this enzyme have been unsuccessful. Therefore, various modifications were designed to obtain a more compact and stable protein abolishing dimer formation and removing the C-terminal 20 amino acids. By structural modeling we have inferred that this region is exposed. Cys-26 and Glu-205 were replaced with Ser and a stop codon respectively. All LepHO mutants were cloned and expressed in *Escherichia coli* cells and the recombinant products

were purified as soluble proteins. Their enzymatic properties were analyzed by UV-visible spectroscopy. We confirmed that all mutants were able to bind the substrate heme and complete its turnover while receiving electrons from its redox partner, the ferredoxin-$NADP^+$ reductase. Their activities were comparable with that of the wild-type LepHO. Crystals of heme complexes were obtained by the sitting drop vapor diffusion method using 24% (w/v) PEG 4000 and 0.2 M sodium acetate in 0.1 M Tris-HCl (pH 8.5) as precipitant. Only mutants containing a stop codon in position 205 were able to crystallize, suggesting that the C-terminal impedes the crystals formation. LepHO structure elucidation is in progress.

SB-C02
ANTIBIOTIC RESISTANCE IN *Staphylococcus aureus* BY ALLOSTERIC CONTROL OF PENICILLIN-BINDING PROTEIN 2A

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The expression of penicillin-binding protein 2a (PBP2a) is the basis for the broad clinical resistance to β-lactam antibiotics by methicillin resistant *Staphylococcus aureus* (MRSA). The high molecular mass PBPs of bacteria catalyze the transglycosylase and transpeptidase activities required for the biosynthesis of the peptidoglycan polymer, which comprises the major constituent of the bacterial cell wall. In bacteria susceptible to β-lactam antibiotics, the transpeptidase activity of their PBPs is lost as a result of irreversible acylation of an active site serine by the β-lactam antibiotics. In contrast, the PBP2a of MRSA is resistant to β-lactam acylation and successfully catalyzes the DD-transpeptidation reaction necessary to complete the cell wall. We report herein the identification of an allosteric domain of PBP2a - a remarkable 60 Å distant domain from the DD-transpeptidase active site - by X-ray crystallography and kinetic studies. When this allosteric site is occupied, a multiresidue conformational change culminates in the opening of the active site to permit the substrate entry. The ability of an anti-MRSA β-lactam antibiotic to stimulate allosteric opening of the active site, thus predisposing to PBP2a inactivation by a second β-lactam molecule, opens an unprecedented realm for β-lactam antibiotic structure-based design.

SB-C03
GAINING INSIGHT INTO THE ACTIVATION MECHANISM OF THE RESPONSE REGULATOR NTRX FROM *Brucella abortus*

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Brucella abortus is a pathogen that has to overcome oxygen deficiency during the course of infection. Our group identified the two-component system NtrY/X that is activated under reducing conditions and is important for the induction of genes involved in the adaptation to low oxygen tension. Given that NtrX has not been previously characterized, we decided to study the changes induced in its isolated receiver domain (REC) upon phosphorylation. We solved the structure of the REC domain in the absence and presence of the phosphoryl analog beryllofluoride by X-ray crystallography. We could determine that phosphorylation promotes dimerization of the REC domain, and analysis of the structures showed the assembly of an interface between helix α_4 and sheet β_5 from neighboring monomers. Also, major changes in the backbone of loop $\beta_4\alpha_4$ were observed, allowing interactions between residues W55-H85, as well as repositioning of helix α_5. To study the relevance of H85 we obtained the REC(H85A) mutant, which is not efficiently phosphorylated and is autodephosphorylated faster than the wild-type domain. These results present a first glimpse into the activation mechanism of NtrX, with changes in the interface between monomers and movement of helix α_5 as possible transducer events, and H85 as an important residue to determine phosphorylation efficiency and half-life of the activated response regulator.

SB-C04
PHOSPHORYLCHOLINE PHOSPHATASE OF *Pseudomonas aeruginosa*: STRUCTURAL INSIGHTS INTO INHIBITION SITE

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Phosphorylcholine phosphatase (PchP) has been involved in the pathogenesis of *Pseudomonas aeruginosa*, hydrolyzing the substrate phosphorylcholine (Pcho) producing choline and phosphate. PchP exhibits distinctive

inhibition behavior caused by high substrate concentrations that has never been reported for another Pcho hydrolyzing enzyme. We proposed the existence of two sites for Pcho: an active one and other for inhibition, based on the recently published crystallographic structure of PchP plus kinetic data. Docking and molecular dynamics simulations were employed to analyze the binding of Pcho to the protein and characterize the different binding sites. Free Energy Perturbation method (FEP) was used to evaluate the binding relative free energy (ΔGbind) of Pcho by native PchP and E42A mutant. The results confirmed the existence of a second binding pocket in PchP, which is adjacent to the catalytic site and acting as an inhibitory site. The simulations revealed the interactions which determine the binding of Pcho molecules within both sites. Finally, the energetic and structural analysis performed on the E42A mutant showed that E42 residue is key in the affinity of Pcho for the inhibition site, and provides relevant information to explain the access of this substrate to the enzyme.

Signal Transduction

ST-C01
SRC IS A CONNECTING PLAYER BETWEEN PKA ACTIVY AND HYPERPOLARIZATION DURING MOUSE SPERM CAPACITATION.

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Mammalian sperm must undergo capacitation before being able to fertilize, involving hyperactivation and the potential to suffer the acrosome reaction (AR). Capacitation entails ser/thr and tyr phosphorylation events and plasma membrane hyperpolarization (Em) among other processes. Previous studies showed that sperm from Src KO mouse undergo normal ser/thr and tyr phosphorylation, albeit they are infertile. The aim of this research is to gain insight into the role of Src tyr kinase in mouse sperm capacitation. Pharmacological inhibition of Src impaired the acrosome reaction (AR), without affecting sert/thr and tyr phosphorylation. Since sperm membrane hyperpolarization is both necessary and sufficient to prepare the sperm for the AR, we evaluated the role of Src in Em. Using the carbocyanine DiSC(3)5, we found a time-dependant hyperpolarization during capacitation becoming maximal and steady after 30 min. Moreover, inhibition of Src blocked hyperpolarization. It is known in other cell types that Src can be directly phosphorylated by PKA at Ser17, as well as auto-phosphorylated at tyr416. Our western blot analysis showed that whereas pSer17-Src is observed at 5 min after capacitation starts, pTyr416-Src begins after 15 min. These data are consistent with a role of Src upstream the cascade of events leading first to hyperpolarization and ultimately to the preparation to undergo the AR.

ST-C02
SPATIO-TEMPORAL CELL SIGNALING DYNAMICS: TOWARDS AN AKT SUBCELLULAR LOCALIZATION FINGERPRINT

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Cell-to-cell variation (CCV), also known as phenotypic heterogeneity, is a commonly observed phenomenon in normal and pathological conditions. Cancer is perhaps the most challenging situation in which to investigate what information is contained in CCV and how to use this information in therapy. Deregulation of Akt has been widely linked to cancer. This kinase orchestrates many biological functions including cell proliferation and survival. Our leading hypothesis is that the information contained in the expression level, posttranslational modifications, activity and specific subcellular localization of each Akt isoform can explain and predict variation in Akt target specificity, cell fate choices as well as in anti-tumor drug sensitivity displayed by individual cells. To test this hypothesis, we have developed immunofluorescence technics and designed fluorescent reporters to study CCV in the spatio-temporal dynamics of the Akt pathway. Here we describe a protocol to automatically image and quantitatively measure and analyze the pattern of CCV in what we call the Akt subcellular localization fingerprint. We found novel compartments were Akt can be recruited and are now analyzing the molecular mechanisms that govern relocalization to these compartments, which in turn regulate specific crosstalk with other signaling pathways.

ST-C03
ANALYSIS OF THE CELL CYCLE REENTRY DURING MATING PHEROMONE RESPONSE IN *S. cerevisiae*

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In haploid yeast, pheromones cause cells to arrest the cell cycle and initiate mating events. This “decision” depends on pheromone concentration. At low pheromone yeast ignore its presence and bud while at high concentrations arrest. At intermediate doses these two phenotypes coexist and an intermediate phenotype appears: some cells respond, but after a variable time, they reenter the cell cycle. Here, we studied the mechanism of this “switching” behavior. We hypothesized that the key was the mutual inhibition between the pheromone pathway and the cell cycle: Far1, a CKI activated by pheromone inhibits the G1 Cdk, and active G1 Cdks block the pheromone response. Therefore, we built an ordinary differential equations (ODE) mechanistic mathematical model in which the winner of this “tug of war” decides the cell fate. Simulations of this model capture the “switching” behavior. A core assumption of the model is that a positive feedback in the synthesis of the cyclins causes the observed behavior. We tested this idea experimentally and found that removal of this feedback did not diminish the number of “switching” cells. This key experiment allowed us to reject the model. We are now exploring another hypothesis that involves an incoherent feed-forward loop in the system. In general, our work illustrates the usefulness of models as tools for the understating of complex cellular behaviors.

ST-C04
ACTIVATION OF THE TERMOSENSOR DESK INVOLVES REVERSIBLE FORMATION OF A TRANSMEMBRANE SERINE ZIPPER

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DesK is a bacterial thermosensor involved in maintaining membrane fluidity in response to changes in environmental temperature. Here we aimed to elucidate the mode of action of DesK by studying a chimerical minimal sensor-DesK (MS-DesK), in which sensing and signaling are captured in a single transmembrane segment. This simplified version of the sensor allows investigating how membrane properties modulate protein activity by using synthetic peptides, corresponding to the membrane spanning parts of functional and non-functional mutants of MS-DesK incorporated in different lipid bilayers. The behavior of the peptides was investigated by circular dichroism, tryptophan fluorescence, in vitro activity and molecular modeling. Based on the results we constructed a working model for the mode of action of DesK, which was then tested in vivo with functional studies on MS-DesK mutants. The results suggest a new mechanism for sensing in which the protein responds to an increase in bilayer thickness by incorporation of a C-terminal hydrophilic motif at the intracellular water-lipid interface. Elongation of the transmembrane segment results in the exposure of three serines on the same side of the transmembrane helix to form a serine zipper motif. This switches the dimerization interface of the transmembrane segment of MS-DesK resulting in activation of the kinase state of DesK.

ST-C05
TUMOR SUPPRESSOR ACTIVITY OF KLF6 TRANSCRIPTION FACTOR IN ONCOGENESIS TRIGGERED BY ACTIVATED H-RAS

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KLF6 is a member of the Krüppel-like/Sp1 family of transcriptional regulators whose loss of function has been associated to cancer development. We have reported that endogenous KLF6 expression is induced by oncogenic Ras (H-Ras^{G12V}). However, KLF6 silencing did not modify the malignant phenotype triggered by H-Ras^{G12V} as determined by colony formation assays and tumor development in immunodeficient mice. This result indicates that KLF6 is dispensable for H-Ras^{G12V}-mediated tumorigenesis but instead is suggesting that KLF6 induction could be part of a failsafe mechanism of cells undergoing oncogenic activation, which is overriden by the dominant activity of H-Ras^{G12V}. Thus, silencing endogenous KLF6 levels in NIH3T3 cells allows cell growth in the absence of serum, formation of transformed foci and generation of spontaneous tumors upon xenotransplantation in nude mice. These results correlate with a significant reduction of p21 expression. Conversely, KLF6 overexpression produces a G1 cell cycle arrest, which can be resumed by siRNA-mediated decrease of p21. Finally, KLF6 overexpression significantly hinders tumorigenesis triggered by H-Ras^{G12V} through a mechanism involving JNK activity and p21

upregulation. Hence, our data provides evidence about a KLF6 function in a tumor surveillance system that is activated by oncogenic stress, playing an important role in tumor suppression.

ST-C06
NRF2 MEDIATES HO-1 EXPRESSION TRIGGERED BY KSHV-VGPCR - ROLE OF MAPK SIGNALING PATHWAY COMPONENTS

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Heme oxygenase-1 (HO-1) is an enzyme upregulated in cells infected with Kaposi´s sarcoma-associated herpesvirus (KSHV) and highly expressed in human Kaposi Sarcoma (KS) lesions. The oncogenic G protein-coupled receptor (KSHV-GPCR or vGPCR) is expressed by the viral genome in infected cells and is involved in HO-1 and vascular endothelial growth factor (VEGF) expression and consequent KS development. We have characterized that vGPCR induces HO-1 expression and HO-1 dependent transformation through the Ga13 subunit of heterotrimeric G proteins and the small GTPase RhoA. Narrowing down the molecular components that regulate vGPCR triggered HO-1 expression at the promoter level we found several lines of evidence that support a role for Nrf2 transcription factors and its associated family members as targets for vGPCR-Ga13-RhoA signaling. Cells expressing vGPCR show activation of different protein kinase pathways and our data indicates that the ERK2 and p38MAPK pathway act as intermediates in signaling from vGPCR to Nrf2. We found that the ERK2 MAPK pathway has an important role in Nrf2 translocation to the cell nucleus and in Nrf2 transactivation activity. We are focusing on Nrf2 family members to identify the target for MAPK dependent phosphorylation that leads to the increase observed in HO-1 expression in vGPCR expressing cells.

ADENDA
COMUNICACIONES ORALES

SB-C05
A 2-HELIX COILED-COIL CONTROLS THE ACTIVITIES OF DESK, A BACTERIAL THERMOSENSOR

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DesK, a histidine kinase from *Bacillus subtilis*, is a membrane-bound thermosensor suited to remodel membrane fluidity when the temperature drops below~30°C. This sensor protein behaves as a kinase at cold temperatures, promoting the phosphorylation of its cognate response regulator DesR, which in turns activates the transcription of *des*, coding for the acyl-lipid desaturase Δ5-Des. Promotion of membrane fluidity by Δ5-Des switches DesK from kinase to a phosphatase, shutting down the expression of the desaturase. Recently, our group reported the crystal structures of the cytoplasmic region of DesK in three different functional states. Analysis of these structures prompted us to construct a set of mutants and determine, through *in vivo* and *in vitro* experiments, the crucial role of the N-terminal 2-helix coiled coil (2-HCC) that links the sensing and catalytic domains, in controlling the phosphatase/kinase output activity. In addition, molecular dynamics simulations strongly support the experimental data, and remark hydration as a key player in the structural changes linked to activity modulation. Altogether, our results highlight the 2-HCC as an essential structural element in the control of the activity of the thermosensor DesK.

CB-C20
BRADYKININ (BK) FAVOURS THE FORMATION OF MIGRATORY COLONIES OF NEPHROGENIC URETERIC BUD (UB) CELLS

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In mammals, nephrogenesis is completed posnatally. We have shown that primary cultures of renal papillary UB cells of neonatal rats-which give rise to the collecting duct-, display a phenotype of migratory sheet of epithelial cells. Now we explore the implication of BK in these phenomena. We examined by immunofluorescence the cellular distribution of F-actin and vinculin (Vin). After 58h, cells formed low compacted colonies making contacts with neighboring cells through filopodium and interacting with the substratum through Vin positive focal adhesions. F-actin cell cortex and stress fibers were also observed. After BK treatment the colonies packed, typical of epithelial cells, and Vin-stained cell-cell adhesions appeared. A continuous F-actin belt along the outer contour of the colonies and ruffling-lamelipodium were observed, reflecting the formation of migratory colonies. Pre-treatment with a selective BK-B2 receptor (B2R) antagonist avoided the BK-induced cell-cell adhesion and F-actin colony delineation. Pre-treatment with a PI3K inhibitor impaired the BK–induced cell compactation and ruffle formation. Since B2R blockade results in abnormal tubular differentiation, we propose that in kidney development, BK favors cell compactation to form migratory colonies, and this behavior could explain the collective advance of the UB from papilla to renal cortex during nephrogenesis.

CB-C21
DIFFERENT STRATEGIES FOR PROTEOMIC IDENTIFICATION OF SUMOYLATED PROTEINS IN *Trypanosoma brucei*

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SUMOylation is a post-translational modification involving the covalent attachment of a small ubiquitin-like protein called SUMO to a variety of proteins. In T. brucei, SUMO resulted essential for procyclic (PC) and bloodstream (BS) forms. Moreover, there is an association between SUMO and the expression of the variable surface glycoprotein. The aim of this work is to compare different strategies for proteomic identification of SUMO conjugates. We generated different cell lines with SUMO chromosomal tagging that enable its expression at physiological levels and provide tags for tandem affinity purification of the conjugates. In this way, we achieved a significant improvement in the yield of the purification but the proteins identified by MS did not differ significantly from the control. To reduce the purification of contaminants we applied a complementary approach utilizing Lysine-deficient SUMO (Matic et al.,2010) with the introduction of an R residue prior to the GG motif in the C-terminus of SUMO to map its acceptor

K in substrates. Meanwhile, since this strategy creates a GG signature that can also derive from other Ubls, we started to work on a new scheme with a K residue prior to the GG motif. This enables to enrich for GG-K containing peptides following their digestion with Lys-C allowing the identification of the acceptor sites in an unambiguous manner (Tammsalu et al.,2014).

BT-C08
RELATIONSHIP BETWEEN MUSCLE CELL PROLIFERATION AND GROWTH FACTORS IN PEJERREY DURING STARVATION

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Insulin-like growth factors (IGFs) are key regulatory elements for growth and maintenance of skeletal muscle. Myostatin (MSTN) negatively regulates skeletal muscle growth, through the inhibition of myoblast cell cycle progression and the activation of p21 and MyoD for terminal differentiation. Our previous data indicate that IGF-1 and MSTN are down regulated in muscle of pejerrey (*Odontesthes bonariensis)* during starving conditions. In order to gain insight into the relationship between expression of growth factors and cell proliferation, 300± 20 mg juveniles pejerrey were starved for 2 weeks (starved group) or fed twice a day (control group). On day 7 and 10 of treatment fishes received an intra peritoneal injection of 5-ethynyl-2'-deoxyuridine (EdU) in order to track and label proliferative muscle cells. Fluorescent EdU labeled cells were analysed in transverse cryostat muscle sections. Our results indicate that proliferation of muscle progenitor cells, although significantly lower, continued during starved periods. Interestingly lower proliferation levels did not correlate with higher expression levels of MSTN. This suggests that MSTN could modulate muscle cell proliferation only under food availability.

POSTERS

Biotechnology

BT-P01
Lactococcus lactis PRODUCING ENT35-MCCV INHIBITS GROWTH OF FOODBORNE PATHOGENS *in situ*

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Bacteriocins are being used as food biopreservative agents. The main drawback is that they have a restricted spectrum of action against related bacteria to those that produce the bacteriocin. In order to obtain a broad spectrum bacteriocin-producing lactic acid bacterium, we carried out a transcriptional fusion between the sequence enconding the signal peptide of enterocin P (a bacteriocin secreted by SEC system) and Ent35-MccV (a broad spectrum chimerical bacteriocin). The hybrid gene *SPentP-munA-cvaC* was fused by PCR downstream to the constitutive P32 promoter. The genetic construction was cloned into a shuttle *Escherichia coli-Lactococcus* vector named pAK80 obtaining the pRUK. *L. lactis* NZ9000 (pRUK) was tested for the bacteriocin production and secretion by antimicrobial activity and colony MALDI-TOF. Moreover, *in-situ* inhibition of *L. lactis* NZ9000 (pRUK) on *Listeria monocytogenes* and *E. coli* food isolates mixed cultures in skim milk were assayed. The results showed that *L. lactis* NZ900 (pRUK) was able to produce Ent35-MccV and could inhibit the growth of both, the Gram (+) *L. monocytogenes* and the Gram (-) *E. coli* in skim milk. As far as we know, the results of this study indicate that Ent35-MccV is the first example of a lineal genetically-engineered bacteriocin produced *in situ* as an effective approach to control Gram (-) and Gram (+) foodborne pathogens in food products.

BT-P02
BIOLOGICAL FUNCTION COMPARISON BETWEEN A PROPOSED BIOSIMILAR AND ORIGINATOR RITUXIMAB

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Similar biotherapeutic products (SBPs) are reaching the market, earning acceptance and becoming a reality. A wide range of SBPs are under development or are already licensed in many countries. In this scenario, functional comparability assays are key elements in demonstrating biosimilarity, particularly when they assess the actual mechanisms of action of the molecule. In this work, we report a comparison of the binding capacity and the in vitro ADCC induction of a SBP and Mabthera©. By quantitative FACS we evaluated the binding of the SBP to CD20-positive cells. No differences in binding capacity were observed between the products. It has been described that FcγRIIIA-158 polymorphisms phenylalanine/valine (F/V) or valine/valine (V/V) are important for predicting rituximab ADCC response. We tested these polymorphisms in healthy donors by PCR and sequencing, finding that 4 out of 12 were positive. The induction of ADCC was evaluated with a lactate dehydrogenase release-based method using peripheral blood mononuclear cells of a positive donor in an Ab concentration-response manner. We observed equivalent efficacy between SBP and Mabthera©. Additionally, our results also confirmed that donors with F/F polymorphism induced a lower ADCC response as it is reported. In summary, similar binding and ADCC activity were shown for both Mabthera and the evaluated biosimilar.

BT-P03
DI- AND TRISIALOGANGLIOSIDE MICELLES LOAD FOUR TIMES MORE PTX THAN GM1 MONOSIALOGANGLIOSIDE

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Objetives: Characterize self- aggregate structures of ganglioside with two (GD) or three (GT) sialic acid for Ptx and Dox vehiculization and compare the results with those previously described with GM1 micelles. Methods: HPLC, UV-Vis spectrometry, DLS, EM were used to characterize water-solubility and structure. Hep-2 and HeLa cell cultures were used to assess cytotoxicity. Results: The incorporation of drugs at room temperature for both, GD1 and

GT1b, complexes achieves an optimum at a molar ratio of 5/1 and were stable in solution for at least 3 months. This stability is also observed upon freeze-thawing, lyophilization-solubilization cycles and high stress condition. GD1 and GT1 micelles showed a size and shape similar to those of GM1. However, contrary to what happens with GM1, the temperature does not change the effectiveness of load of di and tri sialo gangliosides. Finally, in vitro biological activity of the drug contained in GD1 and GT1 micelles was qualitatively equivalent to that of free drug. Conclusion: GD1 and GT1 load four times more Ptx than GM1 micelles. Complexes thus formed are stable and does not affect the biological antimytotic activity of Ptx. These self-assembled nanostructures are new options as drug vehiculization systems.

BT-P04
PHYTOTOXICITY AND ANTIPROLIFERATIVE ACTIVITY ON TUMOR CELLS *in vitro* OF PLANT EXTRACTS

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The interaction of plants with the environment and other organisms causes them to make compounds with diverse biological activities. These compounds are used as active ingredients for many applications. It is estimated that 85% of the world flora is still unexplored. Our goals are to identify plant extracts as source of active ingredients for agro and pharma industry. Therefore we evaluated the phytotoxic activity of plant extracts, as well as, their capacity to inhibit *in vitro* the proliferation of tumor cell lines. A total of 103 aqueous extracts were prepared from 62 plant species collected in Argentina. Germination assays were performed using all these extracts against two dicot and three monocot species. Four extracts were interesting because they significantly inhibited germination of these five species at 1 and 8 mg/mL. In addition, they showed pre- and post-emergence negative effect on plant growth at 1 mg/mL. On the other hand, four extracts showed anti-proliferative activity against the tumor cell line MDA-MB-231, from breast adenocarcinoma. Moreover, other four extracts were active against the H1299 line, from lung carcinoma. The proliferation inhibition was over than 50% when a dilution 1/2000 of each extract was applied. These results show promising possibilities for these samples and identification of the molecules responsible for the effects is in progress.

BT-P05
A NOVEL BAEYER-VILLIGER MONOOXYGENASE AS BIOCATALYST

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The Baeyer-Villiger oxidation of ketones to produce esters or lactones is a valuable synthetic reaction for the preparation of building blocks, pharmaceutical intermediates, bioactive compounds and natural products. The use of enzymes as catalysts takes advantage of the selectivity, efficiency and mild conditions of enzyme-mediated reactions. Type I Baeyer-Villiger monooxygenases (BVMOs) are FAD-dependent enzymes that catalyze the insertion of one atom from molecular oxygen into the substrate, whereas the other atom is reduced to water at the expense of NADPH. The aim of this work was to characterize a novel BVMO in order to expand the number of biocatalysts available for chemical applications. We identified a putative BVMO from *Leptospira biflexa* (Paris) proteome and found that this flavoprotein is distantly related to the main groups of previously characterized BVMOs according to phylogenetic analysis. Besides, its structure was predicted by homology modelling compared to published BVMO structures. We studied the substrate preference profile of this BVMO by biotransformations in recombinant whole-cell systems (growing and/or resting cells) with different ketones as substrates. Our results indicate that the BVMO from *L. biflexa* is able to oxidize linear short-chain ketones as well as some cyclic and aromatic compounds. Funded by ANPCyT, CONICET, UNR.

BT-P06
ANTIBIOTIC LOADED MODIFIED SILICA NANOPARTICLES

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In the present study, the antimicrobial activity of antibiotic loaded bare (SiNP) and modified silica nanoparticles was tested. The modifications were carried out with 3-Aminopropyltriethoxysilane (SiNP-NH2), 3-mercaptopropyltrimethoxysilane (SiNP-SH) and a third modification was achieved by oxidizing the last ones (SiNP-SO3-). As examples of antibiotic gentamicin sulfate and sodium rifamycin were used. The antimicrobial activity was tested performing the susceptibility disk diffusion test on LB agar. Pseudomonas aeruginosa, a gram negative, and

Staphylococcus aureus, a gram positive, were used as model microorganisms. It was observed that the loading capacity of gentamicin sulfate was higher in the sulfonate modified nanoparticles (SiNP-SO3-) and the unmodified nanoparticles (SiNP). This is indicative that electrostatic interactions are involved in the loading of this antibiotic; these NPs are negatively charged at the working pH while the drug has a positive charge. Regarding rifamycin, which is negatively charged, the highest loading capacity was found with the thiol (SiNP-SH) rather than with amino modified nanoparticles (SiNP-NH2). The loading of this drug does not seem to involve primarily electrostatic interactions but of a different nature, probably hydrophobic interactions.

BT-P07
SCREENING OF KERATINOLYTIC BACTERIA FROM PATAGONIAN MERINO WOOL WITH POTENTIAL FOR TEXTILE PROCESSES

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Keratinolytic enzymes attenuate wool fiber scales imparting shrink-resistance, softness and whiteness to the wool. Our aim was to isolate native bacteria from patagonian Merino wool and assess their wool keratinase activity regarding the potential for shrink-proofing process. For bacterial isolation, wool samples were divided in two sub-samples, one of them was suspended is saline solution and plated on Skim milk (SM) and selective actinomycete agar. The second sub-sample was incubated in mineral salt medium with wool as a sole source of carbon and nitrogen, and then plated on SM. To detect the best protease producers, spots of culture supernatants were plated on SM and the diameter of the digestion halos measured. Keratinase activity was assessed using milled wool as substrate. One unit of enzyme activity (UE) was defined as the amount of enzyme which liberates 1 µmol tyrosine/min. From 135 isolates, 66 showed proteolytic activity. Seventeen strong protease producers were selected to test keratinase activity. Nine strains hydrolyzed wool proteins producing about 0,05 to 0,01 UE/ml of supernatant. Wool keratin shows high recalcitrance to proteolytic degradation due to the tight packing of its protein chains. The capability of these isolates to produce wool keratinolytic enzymes makes them interesting candidates for the development of shrink-proofing treatments for the wool industry

BT-P08
MOLECULAR EPIDEMIOLOGY OF HPV IN HEALTHY SKIN FROM IMMUNOSUPPRESSED AND IMMUNOCOMPETENT INDIVIDUALS

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Papillomaviruses (PVs) are DNA tumour viruses infecting mucosal and cutaneous epithelia of vertebrates. More than 190 human PVs (HPVs) types have been completely sequenced. Although the clinical implications of the cutaneous HPV types are still unknown, it has been proposed that immunosuppression (UV + immunosuppressive treatment) may contribute to HPV infection. In order to explore the diversity of cutaneous HPVs on healthy skin from individuals with different immunological status, we designed a transversal descriptive study including 100 immunocompetent (IC) and 64 immunosuppressed (IS) individuals. Skin samples were collected from the forehead using a non-invasive procedure and analyzed by "hanging droplet" PCR with the FAP primer system. HPV DNA was more frequently found in the IC group (65% IC vs. 56% IS). In both populations, the majority of HPV types and putative types identified belonged to the β- and γ-PV genera, being HPV-20 (β-1) the most prevalent type. Time of sun exposure and age were significantly higher ($p \leq 0.05$) in IC HPV-infected subjects. Using "hanging droplet" long PCR assay, the full-length genomes of two γ-PV putative types were amplified. Novel putative types EP1 and FA91 showed 85% and 73% of sequence identity in L1 ORF to the HPV-173 (γ-1) and HPV-148 (γ-12), respectively. These findings expand our knowledge of the Papillomaviridae family.

BT-P09
GENERATING STRUCTURAL DIVERSITY IN MULTI-BRANCHED FATTY ACIDS AND WAXES DERIVATIVES

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Recent advances in the field of synthetic biology and metabolic engineering have renewed interest in "bio-production" of fuel precursors and chemicals from renewable raw materials. In this context, as an initial aim, we designed and constructed a recombinant *E. coli* strain capable of producing tetra-methyl branched fatty acids and

wax-esters derivatives (MBE). These new compounds have potential as oleochemical intermediates, especially as biolubricants. The heterologous production of this MBE was based on the mycoserosic polyketide synthase biosynthetic pathway from *Mycobacterium tuberculosis*; which uses Faal28, Mas, and PapA5 enzymes as minimal set of proteins. Here, we analyzed different strategies to generate structural diversity in these compounds in order to vary the range of their physicochemical properties. Firstly, we studied the capability of the Faal28-Mas-PapA5 system to utilize alternative substrates. Secondly, in a different approach with the aim of introducing additional methyl branching groups; we cloned, expressed and optimized the expression in the recombinant *E. coli* strain of *faal23-pks2-papA1* genes- paralogous of *faal28-mas-papA5*, respectively. Pks2 is responsible for synthesizing hepta and octa-methyl branched fatty acids that are constituents of sulfolipids of *M. tuberculosis*.

BT-P10
EVALUATION OF HAEMOSTATIC ACTIVITY OF A SERINE PROTEASE ISOLATED FROM *Solanum tuberosum* LEAVES

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Blood coagulation and fibrinolysis, two important processes associated with haemostasis and wound healing, involve a series of serine proteases. Fibrinogenolytic activity and anticoagulant ability of several plant serine proteases have been reported. Previously, we have reported the purification and partial characterization of a serine protease from *Solanum tuberosum* leaves, named as *St*SBTc-3. The aim of this study was to evaluate the fibrinogenolytic and anticoagulant capacity of *St*SBTc-3. The fibrinogenolytic activity of *St*SBTc-3 was analyzed by SDS-PAGE 10%, incubating different amounts of *St*SBTc-3 at several times (0-6 hours) with human fibrinogen isolated from human serum plasma. Thrombin time of pooled normal human plasma was determined to evaluate anticoagulant activity of *St*SBTc-3. The results obtained here show that *St*SBTc-3 is able to degrade human fibrinogen in a dose dependent manner. Contrary to the results obtained for other plant serine proteases, *St*SBTc-3 is able to cleave all chains of human fibrinogen. Thrombin time results show that *St*SBTc-3 has anticoagulant activity in a dose dependent manner, being the maximum effect at 14 µg/ml like others plant serine proteases reported. The results indicate that *St*SBTc-3 may have potential and therapeutic applications.

BT-P11
POTENTIAL APPLICATION OF MCCJ25(G12Y) IN FOOD BIOPRESERVATION

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The antimicrobial peptide Microcin J25(G12Y) has a potential use in food preservation since is susceptible to chymotrypsin and resistant to extreme pH and high temperatures. It has been demonstrated that MccJ25(G12Y) is inactivated by digestive enzymes from rat intestinal contents and its administration has not a negative effect on coliform intestinal population. The goal of this work was to evaluate the potential of MccJ25(G12Y) to preserve food. To this end, three objectives were raised: 1-Determine the antimicrobial activity of MccJ25(G12Y) against a collection of pathogen and food spoilage strains. 2-Evaluate the stability and antimicrobial activity of MccJ25(G12Y) in yogurt against *Escherichia coli* O157: H7, *Salmonella cholerae* and *Enterobacter cloacae*. 3-Test the activity of MccJ25 (G12Y) in meat and fish hamburgers against *E. coli* O157: H7 and *E. cloacae*, respectively. We note that MccJ25(G12Y) was active on 28 of 51 strains evaluated and was able to reduce significantly the amount of pathogens in all food analyzed. We also observed that these microcin was stable and active in yogurt during 30 days. The results show that MccJ25(G12Y) has a potential use in food preservation since, in addition to its promising features previously mentioned, it is active against a large number of pathogenic and food spoilage bacteria and preserves intact its antimicrobial activity in food.

BT-P12
BIOREMEDIATION OF HYDROCARBON-CONTAMINATED ANTARCTIC SOIL: OPTIMIZATION AND BIOPILE-SCALE FIELD ASSAY

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Introduction. Hydrocarbon contamination is a global problem, affecting even Antarctic regions. Bioremediation, particularly biostimulation, is an adequate tool for the recovery of contaminated soils. Nonetheless, the addition of an

excess of these nutrients may result in inhibition of biological activity. A 100:10:1 C:N:P ratio is considered as reference for biostimulation. However, each soil presents its own optimum ratio. Objective. Find the optimum C:N:P ratio for an antarctic gasoil-contaminated soil using Response Surface Methodology (RSM) and to test lab results in a field assay at higher scale. Methods. Experimental design for the RSM was a 23 factorial (N and P concentration). An optimum C:N:P ratio was obtained and used for the field assay. Hydrocarbon removal was evaluated as response. Experimental systems consisted in closed geomembranes biopiles (430kg of contaminated soil, 2180ppm). Results. Obtained model predicted a maximal removal for a 100:20:2. C:N:P ratio. Biostimulated biopile showed a removal of 75.9% after 40 days, while control system obtained a 49.5% removal. Conclusion. The best N and P levels for biostimulation of this Antarctic soil were different than those considered as reference, resulting in a 100:20:2 C:N:P ratio. An on-site biopile containing this C:N:P ratio was efficient for hydrocarbon removal from Antarctic soils (75% of removal in 40 days).

BT-P13
AN ENZYMATIC PROCESS FOR THE REMOVAL OF STERYL GLUCOSIDES, MAJOR CONTAMINANTS OF BIODIESEL

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Biodiesels are mostly produced from lipid transesterification of vegetable oils, including those from soybean, jatropha, palm and rapeseed. Unfortunately, transesterification of oil produces various unwanted side products, including steryl glucosides (SG), which precipitate and need to be removed to avoid clogging of filters and engine failures. So far, efficient and cost-effective methods to remove SGs from biodiesel are not available. Here we describe the identification, characterization and heterologous production of an enzyme capable of hydrolyzing SGs, and its implementation on a biodiesel treatment process. A steryl glucosidase (SGase) was selected from a family of thermophilic β-glucosidases. The synthetic gene was efficiently expressed and purified from *E. coli*; and SGase was used to treat soybean derived biodiesel containing 100 ppm of SGs. Several variables of the process were optimized, including temperature, pH, buffer, water content and salinity. Adjustments on the reaction conditions allowed a reduction of the SGs present in biodiesel below 5 ppm. Treatment was successfully scaled from 1 g to 1 ton of biodiesel. The resulting biodiesel was evaluated by the most stringent quality tests with excellent performances. These remarkable results suggest a path for the removal of SGs from biodiesel on industrial scale using an environmentally friendly enzymatic process.

BT-P14
DEVELOPMENT OF A FERMENTATION PROCESS OF EGASE1, AN ENZYME ABLE TO IMPROVE BIODIESEL QUALITY

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Biodiesels derived from vegetable oil suffer from some problems arising from their organic origin, mainly due to the formation of precipitates at various points in the production chain, during transport and in use. The main components of precipitates in biodiesel are steryl glucosides (SGs). Although some alternative methods for removing these precipitates have emerged, none of them has been entirely satisfactory for technical or economic reasons. Recently, we developed an enzymatic process that completely eliminates SGs from soybean biodiesel. The enzyme involved, named EGase1, hydrolyzes SGs to generate soluble sterols and glucose. The aim of this work is to produce EGase1 using a fermentation process at a cost that makes feasible its industrial implementation. The improvement of the expression of EGase1 was addressed by different approaches. These included the optimization of codon usage, the evaluation of different culture conditions, the use of promoters with different strengths, the coexpression of EGase1 with molecular chaperons and the use of alternative *E. coli* host strains. Such modifications had different impact on EGase expression levels and were combined into a final optimal production system. The resulting strain was used to produce the enzyme in a 1L fermentor using glycerol as carbon source and L-Ara as inductor reaching 10g/L of EGase 1.

BT-P15
ENHANCING VP6 ANTIGEN EXPRESSION IN *Lactococcus lactis*

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Group A rotaviruses are the major etiologic agents of acute gastroenteritis worldwide in children and young animals. Among its structural proteins, VP6 is the most immunogenic. The nisin-controlled expression system was used to display the VP6 protein at the cell surface of *Lactococcus lactis*, which is considered a promising candidate for the development of mucosal live vaccines. In line with this idea, this work focuses on enhancing antigen presentation on the bacterial surface by means of *L. lactis* codon usage optimization of the VP6 sequence (398 aminoacids). Moreover, expression of two different VP6 fragments (AB, 198 and BC, 206 aminoacids) was evaluated. The different constructs were transformed into *L. lactis* and protein expression was optimized. Additionally, cytoplasmic and cell-wall fractions were obtained and analyzed by Western blot using polyclonal anti-rotavirus antibodies. Results showed that codon usage optimization did not result in higher protein expression. This was not the case for the two assayed fragments which showed increased expression when compared to VP6 wild type protein, being AB fragment the more efficiently expressed (aproximately 50% more respect to wild type VP6). The immunogenicity and capacity to elicit a protective response of this optimized *L. lactis* expressing both fragments will be evaluated by oral and intranasal immunization of mice.

BT-P16
OPTIMIZATION OF RECOMBINANT Z (JUNIN VIRUS)-N (MEASLES)-GFP VLPS PRODUCTION

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In the last decades, VLPs have been widely studied for the development of new vaccines. It has been previously described that VLPs can be formed when a fusion protein Z (from Junín virus)-GFP is expressed in 293T cells. Moreover a C-terminal fragment of the measles nucleoprotein (N_{CT}) was cloned in frame with both Z and GFP in order to generate quimeric VLPs carrying the heterologous viral antigen. This work is mainly focused on the determination of optimal conditions for a laboratory scale VLPs production. pZ-N_{CT}-GFP plasmid was transfected into Cos-7 cells. Transfected cell percentage was followed through flow cytometry at different times post transfection and remaining transfected cells after cell spliting and recombinant protein expression levels were also determined. In order to optimize VLPs production effectively using the transfection reagent, cell culture conditions and ultracentrifugation protocols, the VLP production scaling up was evaluated using roller bottles or T180 tissue culture flasks and different transfection/splitting strategies. Results obtained so far indicate that 168 h after cell spliting the transiently transfected cell population is still maintained up to 60 %. The best VLPs recovery was obtained from cell supernatant derived from a transfected T75 flask splitted into two T180 collected at day 3 after trasfection and day 4 after cell splitting.

BT-P17
RECONSTITUTION IN YEAST OF THE PRO-VITAMIN D3 - SYNTHESIS COMPLEX FROM *Tetrahymena thermophila*

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Using a reverse genetic approach, we have previously identified a Rieske oxigenase as the sterol C7 desaturase of T. thermophila. It is involved in the biotechnologically relevant conversion of cholesterol into 7-dehydrocholesterol or pro-vitamin D3. In order to carry out its biochemical characterization, the oxigenase gene was fully synthesized with the codon usage of yeasts, and expressed in an appropriate Saccharomyces cerevisiae strain. C7-desaturase activity was assayed providing cholesterol to the yeast culture and analyzing the sterol profile by TLC and GC/MS. Reconstitution of an active system, with significant 7-dehydrocholesterol production, was possible only after coexpressing the human NADPH cytochrome P450 oxidoreductase, indicating that yeasts lack a compatible electron donor for the Rieske oxigenase. A BLASTp search revealed the presence of four putative oxidoreductase orthologues in T. thermophila. Applying the same reverse genetics technique, based on the RNA interference by feeding, one of the genes was identified as the natural electron donor in the enzymatic complex. It makes feasible the development of a sustainable system for the industrial production of provitamin D3.

BT-P18
PROTEOMIC ANALYSIS OF THE VLPS DERIVED FROM JUNÍN VIRUS Z PROTEIN

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Junín virus Z protein is capable of producing enveloped virus-like particles (VLPs), from its single expression in mammalian cells. Even when heterologous sequences are fused via its C-terminal region. This fact allows the production of chimeric VLPs derived from expression of the protein fused to Z. And these VLPs are capable of deliver antigens for the stimulation of the immune response.
Virus-like particles obtained from the Z protein or recombinant versions are produced from the plasma membrane of the host cell. Thus the protein content may contain cellular proteins, both as soluble and membrane anchored. In order to know the identity of these cellular proteins incorporated into the VLPs of Z, proteomic analysis by nano-HPLC-MS was performed. This study was conducted based on the VLPs derived from the Z protein and its recombinant version Z-eGFP, obtained in mammalian 293T cells. This study is of relevance in the context of the potential application of the VLPs derived from the Z protein of Junín virus, for use as a delivery of antigens for stimulating the immune response.

BT-P19
ISOLATION AND CHARACTERIZATION OF POTENTIAL PGPR BACTERIA FROM THE RHIZOSPHERE OF SUGAR CANE

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Sugar cane is one of the most important crop in Tucumán. Actually, Tucumán is the principal producer of sugar cane in Argentine. This crop is the main raw material in the production of ethanol and electricity generation, for this reason exist great interest in generate strategies to increase the biomass produced from sugar cane. The bacteria that inhabit the rhizosphere and have ability to cause a positive effect on plants are called plant growth promoting Rhizobacteria (PGPR). Our laboratory addressed a screening for rhizospheric bacteria from sugar cane featuring plant promoting traits and antibiotic action against plant pathogens. We studied bacterial biochemical markers of the isolates associated with plant growth promotion, i.e. siderophore production, synthesis of indole acetic acid and phosphate solubilization. We found that five isolates (R6, R10, R17, R22 and E35) presented characteristics related to a PGPR bacteria. Also, the strains R17, R22 and E35 showed antibiotic activity against the pathogen *Acidoborax avenae*, causal of the red stripe disease of sugar cane. Preliminary *in vivo* assays, using corn plants showed a positive effect of the R17, R22 isolates on plant growth. These results indicate a promising role of these strains in the formulation of an bioinoculant for plant growth promotion.

BT-P20
Pseudomonas putida LIKE BIOINOCULANT ON SOYBEAN AND CORN IRRIGATED WITH VINASSE

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The vinasse is the residue of ethanol production, which is the largest source of water pollution from the Sali-Dulce Basin. Actually it is used to irrigate crops (fetiriego). Recent work in *Pseudomonas putida* KT2440 shows that the role of matrix components LapA y LapF which are the two major secreted proteins are involved in the development of biofilms. This study showed that mutants defective in these proteins have increased production of exospolisacarido (compensatory mechanism) compared to the wild type, giving greater resistance to various stressors. In this work the influence of KT2440 and mutant were studied as bioinoculants in corn and soybean plants treated with vinasse. First, we tested the influence of irrigation with pure and diluted vinasse in the growth of these crops. Then we determine the MIC of vinasse and growth in mixed media where LB / M9-vinasse was obtained in both cases that shows a better growth of KT2440 and mutant LapF. We performed *in vitro* and *in vivo* with corn and soybeans irrigated with vinasse where bioinoculated with the above strains. A better germination and plant growth were shown in both cases. These results suggest that the use of *P. putida* as bioinoculant in soybean and corn generates the vinasse plant tolerance.

BT-P21
INHIBITION ACTION MECHANISM OF ENANTIO-PYOCHELIN ON *Xanthomonas citri,* CAUSAL AGENT OF CANKER DISEASE IN LEMON

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Bacteria able to inhibit *Xanthomonas citri* subsp *citri,* causal agent of canker disease in lemon, were isolated from citrus leaf surfaces. Our study was focused in one isolate which showed a remarkable inhibition activity. We studied biochemical markers associated with growth promotion i.e. siderophore production and synthesis of indole acetic acid (IAA). The strain was characterized as *Pseudomonas fluorescens* after sequencing its 16S rRNA. The active compound was purified and identified as enantio-pyochelin (E-Pch). In an attempt to identify the action mechanism of E-Pch, we evaluated the ability of E-Pch to generate reactive oxygen species (ROS) in *X. citri* subsp. *citri* and the correlation with its inhibitory activity. In fact, we observed increased ROS levels when E-Pch was added. In addition, the reducer agent ascorbic acid, lowered ROS levels and the antibiotic activity, implying that inhibition is probably caused by oxidative stress. We studied the biofilm formation of *P. fluorescens* on the surface of lemon leaves. Finally, we assayed the use of E-Pch in a model of canker disease in Limoneira 8A. We observed that E-Pch reduced canker formation while ascorbic acid addition reversed its effect. Our results showed E-Pch as a promising compound for citrus canker biocontrol and demonstrated that its mechanism of action *in vivo* and *in vitro* is by ROS increment.

BT-P22
HIGH YIELD TAG PRODUCTION BY ENGINEERING A *Streptomyces coelicolor* PATHWAY INTO *Escherichia coli*

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Microbial lipid production represents a potential alternative feedstock for biofuel and oleochemical industries. Since *Escherichia coli* exhibits many genetic, technical and biotechnological advantages over native oleaginous bacteria, we aimed to construct a metabolically engineered *E. coli* strain capable of producing high levels of triacylglycerol (TAG) and to evaluate its neutral lipid productivity during high cell density fed-batch fermentations. The *Streptomyces coelicolor* TAG biosynthesis pathway, defined by the acyl-CoA:diacylglycerol acyltransferase (DGAT) Sco0958 and the phosphatidic acid phosphatase (PAP) Lppβ, was successfully reconstructed in an *E. coli* diacylglycerol kinase (*dgkA*) mutant strain. Diacylglycerol, one of the substrate of the pathway, is present in high concentration in this strain. The expression levels of the *S. coelicolor* genes were found to be critical for the global process and were further optimized to reach high TAG content. Next, fed-batch fermentation in a 1-L stirred-tank bioreactor performed with the optimized strains resulted in cultures with an OD_{600nm} of 80 and a product titer of 722.1 mg TAG L^{-1} at the end of the process. This study describes a systematic approach for a heterologous metabolic pathway optimization and represents the highest-reported fed-batch productivity of TAG reached by a model non-oleaginous bacterium.

Cell Biology

CB-P01
TRANSCRIPTIONAL REGULATION OF ACYL-COA SYNTHETASE 4 (ACSL4) IN HUMAN BREAST CANCER CELLS

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ACSL4 over-expression correlates with tumor progression and we have demonstrated its functional role in human breast cancer cells, describing that promotes tumor aggressiveness. However the regulation of ACSL4 expression remains unclear. For that purpose we amplified the ACSL4 promoter in order to identify the regulatory mechanisms that are involved in the over-expression of this enzyme in breast cancer cells. Using human genomic DNA, we amplified a 1.7 kb fragment of the ACSL4 promoter containing most of exon 1 and it was subcloned in pNL1.1 vector. We also produced two other constructions by partial deletion of the 5 'or 3' ends of the cloned promoter. The constructions were transfected in MCF-7 and MDA-MB 231 cells. We show that there is an element in the 5´ region that negatively regulates the promoter activity in both cell lines. On the other hand the 3' deletion significantly

decreases the activity of the promoter only in MDA-MB-231 cells. We interpret that this region could be responsible for the over-expression of ACSL4 in the more aggressive breast cancer cell line. An inhibitor of histone deacetylase enhances the transcription of ACSL4 on both cell lines while an inhibitor of DNA methylation increases the ACSL4 expression only in MDA-MB-231. Furthermore we demonstrated that JNK and p38 are involved in the regulation of ACSL4 transcription in these cells.

CB-P02
S-ACYLATION OF GLYCOLIPID GLYCOSYLTRANSFERASES

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Glycosyltransferases (GTs) catalyze the glycosylation of proteins and lipids in the Golgi complex. These are type II transmembrane proteins, with a short N-terminal cytoplasmic domain, a transmembrane domain (TMD), and a catalytic region oriented to the lumen. Sequence analyses revealed the presence of conserved cysteine residues at the cytosolic border of- or within the- transmembrane domain. Cysteines in similar positions are known to be S-acylated (or palmitoylated) in SNAREs and viral fusion proteins but, to date, the lipid modification of mammalian GTs has not been described. Our experiments reveal that the N-Terminal regions of Ganglioside-GTs, SialT1, SialT2 and GalNAcT can be S-acylated in CHO-K1 and HEK293F cells. S-acylation in mammals is mediated by a family of Palmitoyltransferases (PATs), which localize mostly to the Golgi, however, two members of this family, DHHC4 and DHHC6, localize to the ER. Using a GalNacT ER-retention mutant we found that GTs palmitoylation takes place within this organelle, suggesting that these are candidate PATs for GTs. Results suggest that DHHC4 but not DHHC6 is involved in GalNacT and SialT2 S-acylation. Additionally, we found that GTs form DTT-sensitive multimers, mediated by disulfide links between the cysteines that could be target for S-acylation, indicating that GTs palmitoylation could be regulating GTs multimer formation.

CB-P03
CYTOTOXIC EFFECTS OF MEROCYANINE 540 (MC540) AND PHOTOACTIVATED PMC540 IN HUMAN CARCINOMA CELL LINES

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Photodynamic therapy (PDT), an alternative treatment for cancer, involves a photochemical reaction between a sensitizer, light and oxygen to generate cytotoxic reactive oxygen species (ROS) leading to cell death. Another strategy implies the pre-activation of the sensitizer prior to its use where photooxidation or breakdown into smaller substances biologically active could result by light irradiation. The present study was carried out to characterize pre-activated merocyanine 540 (pMC540) products by UV-vis, fluorescence and FT-IR spectroscopy and compare their properties and cytotoxicity in human carcinoma cell lines respect to MC540. Cytotoxicity was determined in human breast (MCF-7) and colorectal (Caco-2) carcinoma cells treated with different doses. Cell viability was determined by crystal violet assay. Our results suggest that photoactivation breaks down MC540 generating at least two pMC540 products which cytotoxicity is dependent on the cell line type. Either MCF-7 or Caco-2 cells were not susceptible to MC540, pMC-540 showed to be dose-dependent for MCF-7 cells. Caco-2 colorectal adenocarcinoma cells were not susceptible to pMC540 photoproducts. We hypothesize that these differences could be derived from the differential interaction of MC540 and/or pMC540 on different cell membranes constituents. Further assays and analysis are ongoing to strengthen our hypothesis.

CB-P04
CHAPERONING RNA AND DNA G-QUADRUPLEXES: PUTATIVE CNBP ROLE IN PROTO-ONCOGENES EXPRESSION CONTROL

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Cellular nucleic acid binding protein (CNBP) is a conserved nucleic acid chaperone associated to cell proliferation processes that participates in the complex transcriptional control of *c-MYC*. Recently, we have shown that CNBP binds *in vitro* to G-rich sequences present in the promoters of several human proto-oncogenes, including *c-MYC*, unfolding their guanine quadruplex (G4) structures. G4s are stable secondary structures of G-rich single-stranded DNA or RNA formed by the stacking of planar layers of four guanines. G4s have been found overrepresented not only in DNA transcriptional control regions of human proto-oncogenes, but also in their RNA translational control regions. We evaluated the effect of CNBP on RNA-G4s present in the translational control regions of *VEGF*, *NRAS*

and *BCL2* proto-oncogenes by Circular Dichroism (CD). We observed a clear decrease of the characteristic CD signals for *NRAS* and *BCL2* G4s, and a milder decrease for *VEGF*. These results may indicate that CNBP destabilizes structured RNA G4s. Moreover, Electrophoretic Mobility Shift Assys (EMSA) showed that CNBP preferentially binds to unfolded rather than folded RNA-G4 sequences. These results are in agreement with previous DNA-G4 results and with a model where CNBP chaperones G4 unfolding by stabilization of unpaired guanines and displacement of G4 equilibrium towards the unfolded state.

CB-P05
FUNCTIONAL CHARACTERIZATION OF GROWTH HORMONE RECEPTOR TYPE A (GHRA) IN ZEBRAFISH EMBRYOGENESIS

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Growth hormone receptor (GHR) mediates growth hormone activity in most animal cells. Teleosts have two growth hormone receptors genes (*ghra* and *ghrb),* which expression begins early during embryo development. The purpose of this work was to elucidate the role of GHRa during early fish development using zebrafish (*Danio rerio*) as model organism. One-cell embryos were injected with specific morpholinos that inhibited translation (tMO) or splicing (sMO) of *ghra* transcripts. GHRa depletion caused abnormal phenotypes (higher vitelline cell size, narrow yolk sac extension, smaller body size, and trunk deviation) 24 hour post fertilization (hpf). Abnormal phenotypes were observed in 77% of embryos injected with sMO (9% presented severe phenotype) and in 90% of tMO morphants (17% severe phenotype). The development of embryos with severe abnormal phenotype stopped, and intermediate and mild phenotypes returned to normal phenotype between 48 and 72 hpf. Defects in blood circulation (less number of blood cells and lower blood flow) were also observed in most of aberrant phenotypes between 48 hpf and 6 days post fertilization. Our results suggest the involvement of GHRa in the utilization of nutritional reserves, the development of the circulatory system and embryo growth. This work constitutes the first report on the role of GHRa during fish embryonic development.

CB-P06
CRITICAL ROLE OF PTP1B IN TRANSIENT INTEGRIN-MEDIATED REPRESSION OF MYOSIN ACTIVITY

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Integrin-mediated cell adhesion regulates the activity of protein tyrosine kinases and Rho GTPases in specific spatiotemporal patterns. In the current study, we show that protein tyrosine phosphatase PTP1B is critical for this regulation. PTP1B is required for an early and transient β3 integrin-mediated activation of the Src/FAK signaling pathway. During initial cell adhesion to fibronectin and vitronectin, aggregation of β3 integrin, paxillin, phospho-paxillin, and activated Src/FAK occurs at small puncta localized within the lamellipodium. All these structural and signaling hallmarks were significantly reduced in PTP1B null (KO) cells compared to PTP1B reconstituted (WT) cells. Inhibition of Src/FAK signaling pathway or expression of constitutive active RhoA in WT cells induced a KO cell phenotype. Conversely, expression in KO cells of constitutive active Src, or incubation with the myosin inhibitor blebbistatin, rescued the WT cell phenotype. We propose a model in which PTP1B cooperates with β3 integrins to activate Src/FAK signaling pathway, transiently repressing myosin-dependent contractility at the cell periphery. These events promote the stability of cell-matrix adhesions and the lamellipodium required for cell spreading.
Supported by CONICET and ANPCyT.

CB-P07
P120 CATENIN RECRUITS NSF TO THE N-CADHERIN PRECURSOR AND PROMOTES ITS PROCESSING AND TRAFFICKING

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N-cadherin is synthesized in endoplasmic reticulum (ER)-bound ribosomes as a precursor protein (pro-N-cad) and processed at the trans-Golgi network to generate a mature protein that carries its function at the cell surface. Binding of p120-catenin to N-cadherin occurs at the ER, and is critical for N-cadherin stability at the cell surface. Here we examined the role of p120 to promote the traffic of pro-N-cad through the biosynthetic pathway. We show that by preventing p120 binding to pro-N-cad, either by mutation of the p120-binding site or by p120 depletion, the precursor accumulates, while mature N-cadherin decreases. Confocal microscopy analysis reveals that pro-N-cad accumulation

occurs at the ER. Reconstitution experiments in p120-deficient SW48 cells show that expression of all three major isoforms of p120 (p120 1A, 3A and 4A) have similar capacity to promote the processing of pro-N-cad and its localization at cell-cell junctions. We found that N-ethylmaleimide sensitive factor (NSF), an essential regulator of vesicular trafficking, is recruited by p120 to the pro-N-cad complex. Functional NSF is required for N-cad trafficking, maturation and localization at cell-cell junctions. Our results suggest a novel role of p120 in the processing of pro-N-cad and its trafficking to the cell surface, likely through the recruitment of NSF to the complex. Supported by CONICET & ANPCyT.

CB-P08
EXPRESSION AND GLYCOSYLATION STATUS OF NA^{+}/CA^{2+} EXCHANGERS IN NORMAL AND CDG PATIENT PLATELETS

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Congenital Disorders of Glycosylation (CDG) are genetic diseases due to defects in the synthesis of glycoconjugates. The clinical features are often neurological impairments and thrombus-hemorrhagic events. A tight regulation of Ca^{+2} is necessary to prevent inappropriate thrombus formation in platelet activation. The Na^{+}/Ca^{2+} exchangers (NCX protein families) participate in Ca^{+2} cellular homeostasis. Recently, it has been described that human platelets expresses K^{+}-independent (NCX1 and NCX3) and K^{+}-dependent Na^{+}/Ca^{+2} exchangers (NCKX1). In microsomal fractions of human platelets we assay the Na^{+}-dependent $^{45}Ca^{+2}$ uptake. The immunopurification of both proteins and Western blot were done using lectins (ConA and WGA) and NCX antibodies. Our first results indicated NCX1 is an N-glycoprotein and NCKX1 an O-glycoprotein. Additionally, two PMM2-CDG patients were detected. The P1 (p.R141H;p.E139K) and P2 (p.R141H;V231M) presented a severe phenotype and abnormal secondary platelet aggregation (P2). We found that the expression of NCX1 as well as the Na^{+}-dependent $^{45}Ca^{+2}$ uptake was greatly diminished in platelets from PMM2-CDG patient with abnormal platelet aggregation. We report for the first time, experimental data about the glycosylation status of Na^{+}/Ca^{+2} platelet exchangers and a putative role of these proteins in the thrombus-hemorrhagic events associated to CDG.

CB-P09
EZRIN AND PROTEINS OF THE RHOA PATHWAY PARTICIPATE IN *Coxiella burnetii* INTERNALIZATION

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Coxiella burnetii (*Cb*) is an obligate intracellular bacterium that causes Q fever in humans. We have shown that internalization of *Cb* by HeLa cells is regulated by actin and Rho GTPases. It is known that Rho proteins are regulated by GEF, GAP and RhoGDI. Moreover, RhoA activates ROCK, a kinase that phosphorylates Ezrin, a protein that acts as a linker between the plasma membrane and the cortical F-actin. Our goal was to study the role of Ezrin and some RhoA-interacting proteins in *Cb* internalization. HeLa cells were transfected with pMyc-ROCK (DN). -ROCK (CAT), pVSVG Ezrin-Nter, pEGFP-Ezrin WT, -Ezrin T567A, Ezrin T567D, pEGFP-RhoGDIα WT, -RhoGDIα (D45A) or -RhoGDIα (D185A), and then infected with *Cb*. After 6 h, cells were processed for indirect immunofluorescence and analyzed by confocal microscopy. We observed that overexpression of the truncated N-terminal ezrin inhibited *Cb* uptake. We also found that overexpression of a non-phosphorylatable (T567A) mutant decreased *Cb* internalization while the phosphomimetic (T567D) mutant increased it. An increased uptake was observed in cell overexpressing a catalytic domain (CAT) of ROCK. Opposite result was observed with the dominant negative mutant (DN). Finally, the overexpresión of RhoGDIα WT reduced *Cb* uptake. These results suggest that ezrin and proteins associated to RhoA pathway participate in *Cb* internalization into HeLa cells.

CB-P10
CHLOROGENIC ACID BIOAVAILABILITY IN *Caenorhabditis elegans*

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Tomato is one of the most consumed vegetables in the world and an important source of nutraceutical compounds. Recently, we measured the ability of tomato extracts to rescue *C. elegans* worms from thermal stress death. This activity was associated with the polyphenol composition of theses extracts. We concluded that chlorogenic acid (CGA) is the most important bioactive compound in contributing to the tolerance to thermal stress. The CGA activity

might be explained by the effect of intact CGA or/and their biotransformed metabolites. However there is no information about the bioavailability of CGA in *C. elegans*, therefore, we developed a study to assess its bioavailability. To conduct this study, adult worms were exposed to active doses of CGA for 24 hours. Subsequently, CGA and its metabolites were extracted from the exposed worms and analyzed by HPLC-DAD-MS. CGA and at least four metabolites (caffeic acid, quinic acid, ferulic acid and 3-4 dihydroxyphenylacetic acid) were identified. These compounds were later assayed in *C. elegans* under thermal stress conditions, using equimolar biological active doses of CGA. All of them showed the ability to rescue the worms from death by thermal stress at different doses. These results reveal a CGA bioavailability mechanism in *C. elegans*similar to mammals and its biotransformed metabolites could mediate the CGA thermotolerance activity.

CB-P11
INTRACELLULAR TRAFFIC OF LRP1 AND GLUT4 IN ALPHA2-MACROGLOBULIN-STIMULATED MIO-M1 CELLS

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Low density lipoprotein receptor-related protein 1 (LRP1) is an endocytic and signaling receptor, which binds alpha 2-macroglobulin-protease complex (α2M*) and promotes cell migration of Muller glial-derived cell line, MIO-M1. The α2M* induces LRP1 translocation to plasma membrane (PM) by two possible pathways; i) Rab11-dependent recycling route, and ii) exocytosis from LRP1-stored vesicles and Rab11-independent pathway, which can be also stimulated by insulin. Glucose transporter type 4 (GLUT4) is a insulin-regulated glucose transporter found in adipose and muscle tissues. Specialized GLUT4 storage vesicles (GSV) can be translocated to PM by insulin through a Rab10-dependent pathway, increasing the glucose uptake. The failure of GLUT4 translocation is involved in insulin resistance and type 2 diabetes mellitus. LRP1 is the main protein component of GSV, although its function is not completely understood. To examine whether α2M* induces GSV translocation to PM, we have characterized the subcellular localization of LRP1 and GLUT4 in MIO-M1 cells. By confocal microscopy and biotin-labeling protein assay we determine that LRP1 and GLUT4 shown a high colocalization in intracellular vesicles, compatible with GSV, and LRP1 was translocated to PM by α2M*, which was unaffected by the presence of a dominant-negative Rab11. We conclude that α2M* induced LRP1 traffic to PM from GSV.

CB-P12
CHLOROGENIC ACID CONFERS THERMAL STRESS RESISTANCE IN *Caenorhabditis elegans* BY A MECHANISM THAT REQUIRES HIF-1

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In previous studies, we demonstrated that tomato extracts confer thermal stress resistance in *C. elegans*, where chlorogenic acid (CGA) resulted their main bioactive compound. CGA is one of the most abundant polyphenol in the human diet with biological activities such as antioxidant and anticarcinogenic. Nevertheless, its role in the stress resistance in *C. elegans* is not fully understood. The objective of this study was to investigate the molecular mechanism of CGA-induced thermal stress resistance in *C. elegans*. Synchronized adult were incubated with different doses of CGA (5000, 500 and 50 ng/mL) at 20°C for 18 h; and enhanced heat tolerance (increase in survival) was observed in wild-type (N2) worms subjected to heat stress (37°C for 6 h). In contrast, the HIF-1 (hypoxia inducible factor) loss of-function strain (ZG31) did not show significant increase in heat tolerance. The protein levels of HIF-1 in adult worms were evaluated after CGA (5000 ng/mL) incubation at 20°C for 18 h, and significant increase in the levels of HIF-1 was evidenced. In addition, the analysis of the expression of two HIF-1 target genes (F22B5.4 and K10H10.2) showed significant increase in mRNA levels after incubation. We conclude that HIF-1 is required for the thermal stress resistance conferred by CGA in *C. elegans*. Likewise, CGA produces an increment in HIF-1 protein levels and HIF-1 target genes.

CB-P13
Trypanosoma cruzi HIGH MOBILITY GROUP B PROTEIN: WHAT WE KNOW ABOUT ITS NUCLEAR AND EXTRACELLULAR FUNCTIONS

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High Mobility Group Box (HMGB) proteins are evolutionary-conserved components of chromatin. They act as architectural factors taking part of important nuclear processes like transcriptional regulation, DNA repair and replication. Beyond these intranuclear functions, some HMGBs can be secreted by certain cells and play important roles as inflammatory mediators. The HMGB protein from *Trypanosoma cruzi* (TcHMGB) has two HMG box domains and a 110 N-terminal domain unique of kinetoplastid HMGBs. It is a nuclear protein expressed in all life cycle stages and shows architectural properties. The specific N-terminal domain seems to have an additional DNA binding site and a putative nuclear localization signal. We found that TcHMGB can also be secreted, and protein acetylation seems to enhance this process. To test if TcHMGB can act as an immune regulator, we stimulated RAW 264.7 macrophages with recombinant TcHMGB for different time periods. Culture supernatants were collected and nitric oxide production was analyzed measuring nitrite release by Griess reaction. Also, total RNA was purified from treated macrophages and mRNAs for different cytokines were quantified by qRT-PCR. Recombinant TcHMGB showed to be able of inducing macrophage activation in vitro, suggesting a role in inflammation. These preliminary results indicate that TcHMGB may have both nuclear and extracellular functions.

CB-P14
Chlamydia trachomatis PHOSPHORYLATES AKT SUBSTRATE 160 TO CAPTURE OF HOST SPHINGOLIPIDS

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Chlamydia trachomatis, an obligate intracellular bacterium, replicates in a vacuole called inclusion, where intercepts certain Rab-mediated transport pathways to capture nutrients from host cells. Rab proteins, the master controllers of intracellular trafficking, are GTPases that switch between inactive GDP-bound and active GTP-bound forms. This cycling is modulated by GTP Activating Proteins (GAPs) and GDP Exchange Factors (GEFs) that respectively, promote the GTP hydrolysis, or the replacement of GDP by GTP. We demonstrated that Rab14 is specifically recruited to the inclusion and promotes the delivery of host Golgi-synthesized sphingolipids to the inclusion. Our findings suggest that *C. trachomatis*, through Akt phosphorylation, might inactivate AS160, a GAP of Rab14, to favour Rab14 GTP-bound state and thereby the arrival of sphingolipids to the inclusion. Our results reveal that both enzymes, Akt and AS160, present two waves of phosphorylation in infected cells. The first one is around 30 minute post-infection (pi) and the second one occurs at mid-stage of chlamydial lifecycle between 8-12 hours pi. Specific Akt inhibitors (iAkt) impair AS160 phosphorylation, and consequently, bacterial growth. *C. trachomatis* usurps Akt pathway to inactivate AS160, thus promoting Rab14-mediated sphingolipid transport to the inclusion.

CB-P15
PLASMA MEMBRANE-BOUND SIALIDASE NEU3 MODULATES CLATHRIN-MEDIATED ENDOCYTOSIS

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Gangliosides are sialic acid containing glycosphingolipids mainly expressed at the plasma membrane. Sialidase NEU3 is a key enzyme in the catabolism of gangliosides and its up-regulation has been observed in cancer cells. In the case of clathrin-mediated endocytosis (CME), although this has been widely studied, the role of NEU3 and gangliosides in this cellular process has not yet been well established. Here, we found an increased internalization of transferrin (Tf), the archetypical cargo for CME, in cells expressing complex gangliosides with high levels of sialylation. The ectopic expression of NEU3 led to a drastic decrease in Tf endocytosis, suggesting a participation of gangliosides in this process. However, the expression of NEU3 in ganglioside-depleted cells only slightly affected its inhibition on Tf endocytosis. Additionally, the internalization of LDL, another typical ligand in CME, was also decreased in NEU3 overexpressing cells. In contrast, cholera toxin β-subunit internalization, which occurs by both clathrin-dependent and clathrin-independent mechanisms, remained unaltered. NEU3 overexpressing cells showed an altered subcellular distribution of clathrin adaptor AP-2, but did not reveal any changes in the membrane distribution of clathrin, phosphatidylinositol 4, 5-bisphosphate or caveolin-1. Overall, these results suggest a specific and novel role of NEU3 in CME.

CB-P16
EFFECT OF ACYL-PROTEIN THIOESTERASES ON MEMBRANE ASSOCIATION AND SPATIAL ORGANIZATION OF H-RAS

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S-acylation is a reversible post-translational modification catalyzed by palmitoyltransferases and acyl-protein thioesterases (APTs). The acylation/deacylation cycle is necessary to maintain the subcellular distribution of S-acylated peripheral proteins, such as members of the Ras family. Due to the importance of a proper H-Ras localization to connect with downstream signaling molecules, we decided to investigate the acylation of this GTPase. H-Ras is dually acylated (Cys181,184) and arrives to plasma membrane (PM) using the secretory pathway. Mono- and non-acylated H-Ras mutants showed differences on their localization and the inhibition of H-Ras acylation dramatically reduced its PM association but maintained the Golgi location when expressed de novo. Then, we focused to investigate the deacylation process of H-Ras by analyzing substrate accessibility to APT1 and APT2. No significant deacylation over time was observed for H-Ras and H-Ras(C181S) associated to PM and Golgi, respectively. However, PM deacylation rate on H-Ras(C184S) mutant was enhanced in the presence of APT1 or APT2. Results support the fact that each fatty acid moiety provides particular information for spatial organization of H-Ras and suggest a differential accessibility of fatty acids to APTs. The insights gained here might be of particular value to investigate how APTs level modulate H-Ras function.

CB-P17
VARIATIONS IN NUTRITIONAL CONDITIONS PROMOTE METABOLIC ADAPTATION OF *Salmonella* WITHIN THE HOST

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Salmonella enterica serovar Typhimurium (*S. typhimurium*) is gram-negative pathogen that causes various host-specific diseases. During their life cycle, *Salmonella* must survive frequent exposures to a variety of environmental stresses, e.g. carbon-source (C) starvation. The virulence of this pathogen relies on its ability to establish a replicative niche, named *Salmonella*-containing vacuole (SCV), inside host cells. However, the microenvironment of the SCV and the bacteria and the metabolic pathways required during infection are largely undefined. The objective of this work is to develop different biological probes whose expression is modulated by the environment and the physiological state of the bacterium. We constructed transcriptional reporters by fusing promoter regions to the *gfpmut*3a gene, to monitor the expression profile of genes involved in the main metabolic pathways, focused in glucose utilization a lipid catabolism. The induction of these probes by a specific metabolic change was first tested *in vitro*, and then during different condition of infection in macrophages. We found sub-populations of bacteria expressing genes corresponding to differential pathway for utilization of source of C. These populations are altered in presence of different metabolizing substrate, suggesting the coexistence of *Salmonella* with diverse metabolic states during the infection.

CB-P18
DEVELOPMENT OF A MOLECULAR TOOL TO MEASURE *IN VIVO* PROTEIN HYPOGLYCOSYLATION IN FISSION YEASTS

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During *N*-glycosylation the endoplasmic reticulum (ER) membrane oligosaccharytransferase complex (OST) transfers $Glc_3Man_9GlcNAc_2$ from a dolichol-PP donor to the sequence Asn-X-Ser/Thr of proteins that are entering into the ER. The addition of a bulky hydrophilic glycan enhances protein folding efficiency as it reduces aggregation of folding intermediates. Defects in the glycan transfer reaction due either to OST mutations or to truncated glycan structures may result in protein hypoglycosylation (lack of glycosylation of sites normally occupied) thus causing ubiquitous defects and in human diseases known as congenital disorders of glycosylation type I. It has been recently shown that a GFP variant in which an *N*-glycosylation site was introduced (Gly-GFP) loses fluorescence when such site is occupied by a glycan. We expressed GFP and Gly-GFP variants fused to an N-terminal *S. pombe* signal peptide and a C-terminal ER retention signal (ER-GFP and ER-GlyGFP), both in a wild type and in a *Δalg6* mutant in which hypoglycosylation occurs. Our results showed that while expressed ER-GFP fluoresces in the ER of both strains, ER-GlyGFP only fluoresces in the ER of *Δalg6* mutant, indicating that the fluorescence intensity of this construction may be used to test the glycoprotein hypoglycosylation *in vivo* in *S. pombe*.

CB-P19
POTENTIAL ROLE OF RAB 21 AND VARP IN THE EXOCYTOSIS OF AUTOPHAGIC VESICLES

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Autophagy is a process by which long-live proteins and cellular components are recycled in a lysosomal-based degradation. LC3 is the main protein present in the membrane of autophagic vesicles (autophagosomes). Several signals can trigger autophagy in mammalian cells such as nutrient privation. Recent work has demonstrated that VAMP7 is necessary to deliver ATP-containing autophagosomes to the edge of the cell upon starvation, promoting the exocytosis of this nucleotide. VAMP7 is involved in the traffic from Golgi to plasma membrane, associated with the GTPase Rab21 and its GEF, Varp. The aim of this study was to evaluate the role of the Rab21 and Varp, in the autophagosomes transport to the cell periphery. HeLa cells were cotransfected with plasmids encoding RFP-LC3 and GFP-Rab21, YFP-Rab32 or GFP-VARP. Cells were incubated under starvation conditions or in complete medium in the presence of rapamycin or resveratrol for 4 hours. Our data show that autophagy induction generates an increased number of autophagic structures labeled with Rab21 or Varp localized at the cell periphery. In contrast, Rab32 positive autophagosomes were distributed at the perinuclear region, where colocalized with the lysosomal marker lysotracker. Our results suggest that Rab21 and Varp are vesicular transport proteins that participate in the redistribution of the autophagosomes toward the cell periphery.

CB-P20
VERO CELLS HARBOR A POLY-ADP-RIBOSE BELT PARTNERING THEIR EPITHELIAL ADHESION BELT

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Poly-ADP-ribose-polymerase (PARP) family members synthesize poly-ADP-ribose (PAR) both in cell nuclei and cytoplasm. While it is clear that nuclear PAR modulates chromatin compaction, affecting nuclear functions (gene expression, DNA repair), PAR cytoplasmic distributions and functions are still ill-defined. We have examined through immunofluorescence and confocal microscopy, the subcellular localization of PAR in an epithelial cell line (VERO). Actin filaments disruption with cytochalasin D was paralleled by PAR belt disruption. Conversely, PARP inhibitors 3-aminobenzamide, PJ34 or XAV 939, but not Olaparib, affected PAR belt synthesis, actin distribution, cell shape and adhesion. Extracellular calcium chelation displayed similar effects. Our results demonstrate the existence of PAR in a novel subcellular localization. An initial interpretation of all the available evidence points towards TNKS-1 as the most probable PAR belt architect. We propose for the first time that cytoplasmic PAR would be involved in adherens junction complexes that connect E-cadherin, α- and β-catenin, vinculin and actin microfilaments in polarized epithelial cells. The present work expands our perspectives regarding PAR functions, with deep implications in pathological situations, where PAR abundance or scarcity may affect the epithelial structure as well as transcendent cell signaling pathways.

CB-P21
HUMAN SPERM CHEMOREPULSION, A NOVEL REGULATION FOR FERTILIZATION?

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Progesterone (P) is secreted by the cumulus cells that surround the oocyte and diffuses generating a concentration gradient along the cumulus and beyond. Under in vitro conditions, sperm can be attracted by a gradient of picomolar dosses of P, mechanism that has been proposed to attract and retain spermatozoa at the fertilization site. Ulipistral acetate (UPA) is a selective progesterone receptor modulator mostly used as an emergency contraceptive which mainly acts as an antagonist of the P receptor. The aim was to evaluate if UPA may regulate human sperm chemotaxis toward P. Sperm chemotaxis was determined by a videomicroscopy-image analysis system and by the Sperm Selection Assay. Spermatozoa were previously incubated with nM doses of UPA (range of concentration determined in women serum after taking a UPA pill) and then exposed to the chemotaxis assay. As expected, UPA significantly inhibited sperm chemotaxis toward P. Surprisingly, UPA also stimulated sperm chemorepulsion from the P attracting source. Sperm repulsive behavior was observed only in the subpopulation of capacitated spermatozoa (those ready to fertilize the oocyte). This is the first observation of sperm chemorepulsion which may have both biological and medical implications, preventing either polyspermy under natural conditions or fertilization under pharmacological treatment with UPA.

CB-P22
TIME WINDOW WHERE MOST SPERM SAMPLES ARE AT OPTIMUM PHYSIOLOGICAL STATE

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At the beginning of in vitro incubation, sperm (S) are not capacitated (physiological state essential to fertilize the oocyte). Along time, consecutive sperm subpopulations become transiently capacitated, to later acquiring a non-functional "post-capacitated" state. The timing of this sequence of events is highly variable between S samples. The aim of this study was to define a time window where most of the S samples are in the best physiological state. Human S were separated from seminal plasma by migration-sedimentation and then incubated for 24 h. Every hour, the induced acrosome reaction (as a measure of capacitation) and the sperm selection assay (SSA; which selects S in the best physiological state by chemotaxis) were determined. The % of capacitated S gradually increased along time, showing a pattern of transient cycles with an amplitude of 2-4 h. The proportion of samples that failed to capacitate was higher at early times, significantly decreasing along the incubation time, while all samples were capacitated after 18 h of incubation. Similar kinetic were observed in the percentage of S selected by chemotaxis towards the progesterone in the SSA. In conclusion, the S incubation for at least 18 h guarantees that most sperm samples have a subpopulation of capacitated S, which are able to be chemotactically selected by progesterone, as a sign of a good S physiological state.

CB-P23
REGULATION OF SPERM PHYSIOLOGY BY EXTRACELLULAR MICROVESICLES FROM ENDOMETRIAL EPITHELIAL CELLS

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The uterus and oviduct synergistically induce sperm capacitation, essential process to fertilize the oocyte. Conditioned medium from an endometrial epithelial cell line (EEC) stimulates sperm capacitation, which might be regulated by extracellular microvesicles (EMV). The aims of this study were: to isolate and characterize EMV secreted by EEC; to investigate the sperm-EMV interaction; and to evaluate the effect of EMV on human sperm physiology. The EMV were obtained by ultracentrifugation and then characterized by electron microscopy and Western blot. The interaction of EMV with the cells was analyzed by incubating PKH26-stained EMV with spermatozoa, and the effect of EMV on sperm physiology, was determined by evaluating sperm induced acrosome reaction, viability and motility. The EMV had a size of 50-200 nm and showed the presence of MFGE8 protein as specific marker. After co-incubating sperm with PKH26-labeled EMV, a fluorescent staining was observed on the sperm surface, indicating membrane transference from EMV. A short time incubation of sperm with EMV-enriched medium significantly increased the induced acrosome reaction, whereas viability and motility were not affected. These results suggest that the brief transit through the uterus may contribute to the sperm acquisition of fertilizing capacity, being this process possibly mediated by the transfer of EMV to the spermatozoa.

CB-P24
CIRCADIAN REGULATION OF mRNA GRANULES

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Stress granules (SGs) and Processing bodies (PBs) are microscopically visible foci formed by mRNAs, translation factors, RNA-binding proteins, and other proteins involved in the processing of messengers in the cytoplasm. SGs and PBs are intimately related to mRNA translation, degradation and storage. Since a number of P-body and SG components display circadian rhythms, we hypothesized that these mRNA granules could be temporarily regulated. We analyzed PBs and SGs by ICC with anti-GE-1/hedls and anti-eIF3 antibodies respectively, in synchronized NIH3T3 cell cultures. We found that both SGs as PBs display circadian changes in number. We found no changes in phosphorylated eIF2alpha, a protein involved in SG assembly. Then we analyzed by RT-qPCR the temporal expression of the mRNAs of several RNA-binding proteins that are in RNA granules and could be involved in the temporal changes observed. Interesting, *Tia1, Brf1, hnRNPQ*, and *Lark* transcripts presented temporal changes in their levels. TIA1 protein has been implicated in SG assembly; we found that its levels also oscillate. LARK, which regulates the translation of the clock protein PER1, also presented temporal variations in its induction by stress. Our results show that PBs and SGs are temporarily regulated and that SGs may be involved in the circadian regulation of stress response.

CB-P25
DIFFERENT MECHANISMS REGULATES DISC LARGE 1 PROTEIN LEVELS IN DIFFERENT BIOLOGICAL PROCESSES

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Human Disc large (DLG1) has been demonstrated to be involved in the control of cell polarity and maintenance of tissue architecture. However, the mechanisms controlling DLG1 expression levels are poorly understood. This is relevant since DLG1 is lost in many tumours during the later stages of malignant progression. In this sense we initiated studies to analyse the mechanisms regulating DLG1 expression. We reported previously the discovery of an alternative splicing event in the 5' untranslated region (5´UTR) of DLG1 mRNA that generates two types of transcripts differing in their translation efficiency: the Short and Large 5´UTR variants. In this study, we further examined the impact of the DLG1 transcriptional rate and the role of the differential expression of the alternative 5´UTRs variants on DLG1 protein levels. We analyzed these mechanisms in cell processes like differentiation, cell cycle progression and cell-cell contacts, where the importance of DLG1 protein levels was previously established. Data presented here suggest that the transcriptional regulation of DLG1 strongly contributes to DLG1 abundance and show that differential expression of the alternative 5´UTRs variants also cooperates, depending on the cell type and cell situation. This study provides new evidence for understanding the mechanisms involved in DLG1 expression during different biological context.

CB-P26
CELL POLARITY DISRUPTION DURING CARCINOGENIC PROCESSES: ANALYSIS OF CELLULAR AND VIRAL CONTRIBUTIONS

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We are interested in the expression of polarity proteins during malignant progressions, and particularly, those associated to Human papillomavirus (HPV) infections. We are focused on the DLG1 and PAR3 PDZ polarity proteins, which expression is altered in tumors. By luciferase assays, we found that DLG1 promoter activity is increased in the presence of the HPV E6 and/or E7 proteins. In raft organotypic cultures, expressing HPV E6 and E7, DLG1 and PAR3 were found to be up-regulated and misdistributed, as ascertained by Western Blot and Immunohistochemistry (IHC). These data suggest that the viral proteins could contribute to the changes in the cell proteins expression observed in biopsies. Also, to study if epigenetic regulation mechanisms could contribute to DLG1 regulation in malignant processes in general, we analyzed the methylation pattern of DLG1 promoter in cultured cell lines, by treatment of DNA with sodium bisulfite and subsequent PCR amplification and sequencing. We designed primers suitable for amplification of bisulfite treated DNA and we found a demethylated state of the promoter in transformed cells. Also, we evaluated the methylation status using biopsies, and we found a difference between normal and tumoral colon samples, which could be related with the DLG1 expression, as we tested by IHC. Though, this mechanism could also be involved in regulating DLG1 abundance.

CB-P27
SUPPRESSION OF STARD7 DECREASES ABCG2 AND ENHANCES SENSITIVITY TO CHEMOTHERAPY DRUGS IN HUMAN CANCER

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Drug resistance during chemotherapy is the major obstacle to the successful treatment of many cancers. Several ABC transporters have been recognized as a source of drug resistance in the treatment of malignancies. StarD7 is a member of the START domain superfamily, which is involved in lipid transfer, metabolism, and modulation of signaling pathways. Previous results indicate that StarD7 silencing decreases ABCG2 multidrug transporter level, cell migration, proliferation, and phospholipid synthesis; whereas an increase in biochemical and morphological differentiation marker expression was detected in the choriocarcinoma JEG-3 cells. Here, we report that inhibition of StarD7 by siRNA led to a marked decrease of ABCG2 expression in HepG2 and A549 cell lines. Using flow cytometry and a microplate reader assay, we found that intracellular accumulation of the chemotherapeutic agent mitoxantrone was enhanced in cell lines transfected with StarD7 siRNA. In addition, StarD7 silencing led to increased ROS basal level as well as ROS-mediated cell toxicity induced by H2O2. More importantly, knocking down StarD7 resulted in enhanced sensitivity of cancer cells to chemotherapeutic agents. In summary, our results

suggest that targeted inhibition of StarD7 may be a novel therapeutic approach to overcome chemoresistance of epithelial cancer cells. Supported by FONCyT, CONICET, SECyT-UNC.

CB-P28
HORIZONTAL TRANSFERENCE OF GENETIC MATERIAL IN BACULOVIRUS DRIVEN BY TRANSPOSONS

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Baculoviridae constitutes an important viral family inside viruses which have large dsDNA's genomes. One interesting aspect of these entities is that they show evidence of evolutionary processes similar to the organisms because they achieve speciation processes due to the gain or lose of sequences caused by structural mutations, in which the transposition would be one of the main responsible factors. PiggyBac (2.5 kbp) is a Class II transposon isolated from insect cells that encodes a transposase. The insertion is in TTAA sequences, and once duplicated, remains attached to the transposon's ends once the process concludes. Using different variations of a PiggyBac isolated in our laboratory, with and without the flagging TTAAs, it has been proved that even those which didn't had the sequence have been able of move from donor molecule to another target, although with reduced activity using TTAAs sterically next. In this way, PiggyBac would be able to mobilize sequences between two loci (donor and target). Moreover, it has been evaluated in an insect cell line using RT-PCR that the transcript's level which encodes the transposase did not considerably oscillate both in presence or absence of a baculoviral infection, including different times of virus cycle. These evidences support the hypothesis that predicts a prominent role of transposons in the dsDNA genome evolution.

CB-P29
EVALUATION OF IN-VITRO ANTIOXIDANT ACTIVITY OF ANDEAN POTATO

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Potato contains vitamins, minerals, fibers and significant quantities of antioxidant substances, such as secondary (poly)phenol metabolites. This polyphenols are associated to beneficial effects on human health, including antioxidant, antimicrobial and antitumoral. The aim of this work was to evaluate in vitro antioxidant activity of Andean potatoes. Methanolic extracts of four varieties of potatoes were obtained and total phenolic acids, anthocyanins and flavan-3-ols were quantified. We also evaluated the antioxidant properties of these extracts by DPPH scavenging, superoxide radical scavenging, ferric reducing power and ferrous chelating capacity. Therefore, we characterized the profile of phenolic compounds in the extracts by HPLC. Currently, we are investigating the antioxidant effect of polyphenolic extract on hydrogen peroxide (H2O2)-induced oxidative stress in human neuroblastoma cells (SH-SY5Y). Our results show that peel has more polyphenols and antioxidant capacity than flesh in all four varieties. The CL658 variety was the one with major levels of polyphenols and the HPCL profile show chlorogenic acid (phenolic acid) and pelargonidin (anthocyanin) as the most abundant compounds. Finally, preliminary results indicate that CL658 extracts would decrease (H2O2)-induced cell injury. These results suggest that polyphenols potato would have protective antioxidant effects.

CB-P30
INHIBITION OF PROLINE-DIRECTED PHOSPHORYLATION SIGNALING AS A POTENTIAL ANTITUMOR STRATEGY

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The reversible phosphorylation of proteins on Ser/Thr-Pro motifs is a key signaling mechanism that controls various cellular processes, including cell division. The intervening peptide bond preceding Pro in Ser/Thr-Pro motifs may adopt two different conformations, cis and trans, whose conversion is specifically catalyzed by the prolyl isomerase Pin1 when Ser or Thr are phosphorylated, inducing conformational changes in target proteins that affect their catalytic activity, protein-protein interactions, subcellular location, and/or turnover. Pin1 is frequently overexpressed in human cancers and its overexpression correlates with poor prognosis. Thus, Pin1 has been proposed as a therapeutic target. In this work, we have analyzed the in vitro antitumor potential of the Pin1 inhibitor Juglone in combination with retinoic acid (RA), a drug used in clinical treatment of neuroblastoma and acute promyelocytic leukemia. Our results show that Juglone cooperates with RA to increase cytotoxicity in human adenocarcinoma cell line, MDA-MB-231. Furthermore, using neuroblastoma cell line Neuro-2a, the effects of Juglone in RA-neuronal

differentiation as well as the effect of Pin1 overexpression have been analysed. Together, our results suggest that Pin1 has an inhibitory effect in RA-induced neuronal differentiation and suggest that its inhibition promotes the differentiation in the absence of RA.

CB-P31
GLUCOCORTICOIDS AS REGULATORS OF GENE EXPRESSION ON HUMAN MACROPHAGES

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Human monocytes THP1 cells were transformed into macrophages by adding PMA in the culture medium. Macrophages THP1 cells were treated with Ox-LDL/cortisol or Ox-LDL/cortisone or Ox-LDL/cortisone/BVT.2733 inhibitor for 24 h. Isolated LDL fraction from human plasma was peroxidized in vitro with Cu++ to transform LDL into Ox-LDL. We evaluated the expression of genes involved in the pro inflammatory (TNFα, FAT/CD36, IL6) and anti inflammatory processes (11ßHSD1, rIL10, MMR). The 11ßHSD1 expression is involved in the reduction of cortisone to cortisol (glucocorticoid active form) and the 11ßHSD2 turns cortisol into cortisone. The mRNA level of different genes was measured by quantitative real-time PCR. Results showed a decrease of pro-inflammatory marker in cells treated with increasing cortisol doses. Also, the treatment with high cortisone concentration (1000nM) showed decrease of pro-inflammatory gene expression. However, cells treated with increasing cortisone concentration and 11ßHSD1 inhibitor (preventing conversion of cortisone into cortisol) did not show pro-inflammatory gene expression decrease. Enhancing cortisol concentration promotes a decrease of 11ßHSD1 and an increase of 11ßHSD2 gene expression. In conclusion, our results showed that only cortisol contributes to an anti-inflammatory response.

CB-P32
LENTIVIRUS-MEDIATED IN VIVO GPAT2-SILENCING IN MOUSE TESTIS IMPAIRS SPERMATOGENESIS

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We have previously shown that the isoform 2 of glycerol-3-phosphate acyltransferase (GPAT2), an enzyme belonging to the GPAT family that has unique expression characteristics compared to the other members (GPAT1, 3, and 4), is expressed almost exclusively in spermatic cells. We have further confirmed GPAT2 as a member of the cancer-testis group of genes having an abnormal overexpression in certain tumor cell types and DNA-methylation-mediated transcription regulation. To understand the role of GPAT2 in relation to spermatic cell maturation and the spermatogenic process, we studied the age dependent expression profile of mouse GPAT2 by Q-PCR and Western blot. We found an expression peak at post natal day (PND) 15. We confirmed these results by immunohistochemistry and in situ hybridization, and observed that GPAT2 is localized preferentially in pachytene spermatocytes and not in spermatogonia. We then performed an in vivo lentivirus-mediated silencing of GPAT2 in mouse testis. Histological and immunohistochemical analysis showed both a strong post-meiotic arrest and a severe decrease in the number of mature sperm cells in those tubules with diminished GPAT2 protein expression. These results show that GPAT2 is important for the normal progress of the spermatic cycle in post-natal testis development.

CB-P33
TWO TYPES OF DNA LESIONS DIFFERENTIALLY AFFECT ALTERNATIVE SPLICING

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UV radiation is a constant source of damage to DNA and other cellular components. This activates a network of specific signaling pathways that regulates transcription and alternative pre-mRNA splicing (AS), among other cellular processes. Deficiencies in these pathways lead to mutation accumulation and, likely, cancer. We have previously shown that UV irradiation promotes hyperphosphorylation of the carboxy terminal domain of RNA polymerase II, slowing down transcriptional elongation and therefore affecting the AS of several genes. UV radiation generates two main types of DNA lesions, the photoproducts cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidones [(6-4)PPs] that differ in terms of the distortion generated on the DNA double helix, their

half-life, and the initial recognition by proteins of the Nucleotide Excision Repair pathway. Here, we used two approaches to dissect the effect of each type of lesion on alternative splicing: 1) two xenogenic lesion-specific DNA photolyases, absent from human cells, that selectively repair CPDs or (6-4)PPs; 2) irradiation with UVB (305 nm) and UVC (254 nm) that differ in the relative proportion of CPD and (6-4)PPs generated. Using these tools, we show that DNA damage in itself is sufficient to cause changes in AS. Moreover, UV-induced lesions, CPDs and (6-4)PPs differentially affect individual AS events.

CB-P34
CHROMATIN STRUCTURE REGULATES ALTERNATIVE SPLICING OF HISTONE METHYLATING ENZYMES

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In mammals G9a and GLP are the primary enzymes for both mono- and dimethylation at Lys 9 of histone H3 (H3K9me1 and H3K9me2). There are different alternatively spliced transcript variants of these genes, but their differences in function are still unknown. During in vitro and in vivo neuronal differentiation we have determined that inclusion of alternative exons 10 in G9a and 12 in GLP are increased. Since it is known that dynamic changes in intragenic chromatin structure could affect RNA processivity and modulate alternative splicing choices, we are currently studying the intragenic chromatin structure of G9a and GLP during cell differentiation. We have seen that treatment of mature neurons with a DNA methylation inhibitor and with a histone hyper-acetylating drug reverts the effect of differentiation on exon 10 inclusion, suggesting that an epigenetic component is involved in this process. Furthermore, RNAi depletion of G9a or GLP in differentiated neurons in culture downregulates G9a exon 10 inclusion, suggesting an important role of G9a in regulating its own splicing. However, depletion of either G9a or GLP has no effect on GLP alternative splicing, pointing out at a different splicing regulation. Finally, we found that pharmacological inhibition of G9a and GLP enzymatic activities, as well as G9a depletion, are able to inhibit neuron differentiation.

CB-P35
NUCLEOTIDE EXCISION REPAIR (NER) FACTORS MODULATE ALTERNATIVE SPLICING IN SKIN CELLS

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The UV radiation that reaches the Earth's surface can damage cellular components such as DNA, RNA, proteins and lipids and is, therefore, the most prominent and ubiquitous carcinogen in our natural environment. Keratinocytes, the major cell type of our skin, are regularly exposed to UV radiation. We have shown that UV irradiation slows down transcriptional elongation and affects alternative splicing (AS) of several genes, some of which are key for survival/apoptosis decisions. Now we have demonstrated that introduction of in vitro damaged DNA into skin cells is sufficient to mimic the UV effect on AS. The major pathway that deals with UV-induced DNA damage is Nucleotide Excision Repair (NER). NER is initiated by two distinct DNA damage-sensing mechanisms: Transcription Coupled Repair (TCR) and Global Genome Repair (GGR). Unlike GGR, TCR depends on RNA polymerase II transcription. We have ruled out TCR and showed that the UV effect depends on the xeroderma pigmentosum proteins typically needed in GGR: using siRNA-mediated knockdown we have demonstrated that XPC and XPE are involved in the signaling from the DNA lesion to the transcriptional and splicing machinery, which underscores a new role for NER in skin cells.

CB-P36
ARGONAUTE-1 BINDS TRANSCRIPTIONAL ENHANCERS REGULATING TRANSCRIPTION AND ALTERNATIVE SPLICING

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Argonaute proteins are well known for their roles in miRNAs and siRNAs cytoplasmatic pathways that lead to post-trascriptional gene silencing. Although Argonaute proteins and other RNAi components are known to be in the nuclei of human cells, their role here is less understood. We performed chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) of Argonaute-1 (AGO1) in the human breast tumor derived MCF7 cell line in order to explore its genome-wide distribution. Surprisingly, we found that 80% of AGO1 clusters are associated with the cell type

specific, estrogen-responsive transcriptional enhancers and that this association correlates with the geneation of enhancer RNAs (eRNAs). AGO1 knock-down revealed that AGO1 is necessary for estrogen-mediated transcriptional activation and for eRNAs generation. In turn, RNA polymerase II is necessary for AGO1 recruitment to chromatin. We also found that AGO1 is involved in the alternative splicing regulation of the cassette exon 107 in the SYNE2 gene by binding to, and regulating the activity of, an enhancer located in the intron downstream of the splicing event.

CB-P37
IN VIVO INTERACTION BETWEEN P-BODIES COMPONENTS IN *Drosophila melanogaster*

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Gene expression is a highly regulated process. Translation regulation controls of cellular proteome, enabling prompt quantitative and qualitative changes in response to the environment. Cytoplasmic processing bodies (PB) are discrete cytoplasmic foci involved in mRNA silencing, surveillance and degradation (Andrei et al., 2005; Cougot et al., 2004; Eystathioy, 2003; Layana et al., 2012; Sheth and Parker, 2003). We study the dynamic of mRNP remodeling in PB and the mechanisms that promote the silencing of mRNA active in polysomes. RNAi assays demonstrated that the presence of some factors, in particular eIF4E is crucial to PB formation (Andrei et al., 2005; Eulalio et al., 2007). We have showed that different isoforms and eIF4E mutants are required for PB localization (Ferrero et al., 2012) Here we analyzed in detail the interactions between some components of PB, namely eIF4E-1, eIF4E-3, Lsm-1, y Me31B in *Drosophila melanogaster*. In vitro interaction was demonstrated by two hybrid assays, and in vivo analysis of interactions in PB in *Drosophila* S2 cells was studied by FRET. Our results demonstrated in S2 cells that the isoforms of the cap binding protein eIF4E-1 and eIF4E-3 interact with Me31B and Lsm-1 and that this interaction has implicances in the control of mRNA silencing in somatic cells and in the germ line of *Drosophila melanogaster*.

CB-P38
PROTEIN LIPOYLATION IN THE NEMATODE *Caenorhabditis elegans*

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Lipoic acid (LA) is a sulfur-containing cofactor derived from octanoic acid required for the function of several multienzymes complexes involved in oxidative metabolism. As lipoylation pathways remain unclear in eukaryotes, we propose the use of *Caenorhabditis elegans* as a model for studying them. The ability of synthesizing LA was confirmed by growing worms in minimal medium supplemented with acetate and succinate (M9sup) and feeding them with a bacterial strain auxotroph for LA. By in silico analyses of the *C. elegans* proteome we found several enzymes possibly involved in lipoylation. M01F1.3 appeared to have homology with bacterial and yeast lipoyl synthases. To analyze its importance in *C. elegans*, we performed RNA interference (RNAi) experiments. When grown at 15°C in M9sup and fed with the *E. coli* strains HT115 or AL100 (a HT115 derivative unable to synthetize LA), the worms arrested in the second generation as L4 larvae. The same phenotype was obtained when RNAi was done in NGM, a rich medium that has peptone, and hence, LA. It was not possible to rescue the arrested larvae by adding neither branched chain fatty acid precursors to M9sup, nor LA. Curiously, worms subjected to RNAi grown at 25°C in M9sup and fed with AL100, did become adults and were capable of laying eggs. It can be concluded that, at least at 15°C, M01F1.3 is essential for the normal development of worms.

CB-P39
CYTOTOXIC EFFECTS OF MEROCYANINE 540 PHOTOPRODUCTS (PMC540) IN HUMAN CARCINOMA CELL LINES

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Photodynamic therapy (PDT), an alternative treatment for cancer, involves a photochemical reaction between a sensitizer localized at a target tissue, light and oxygen to generate cytotoxic reactive oxygen species (ROS) leading to cell death. However, clinical application is limited to accessible regions of light. To circumvent this limitation, light irradiation of the sensitizer before its use could result in photooxidation or breakdown into smaller substances biologically active. The present study was carried out to characterize merocyanine 540 photoproducts (pMC540) properties by spectroscopy (UV-vis, fluorescence and FT-IR) and evaluate their effects in human carcinoma cell lines. Cytotoxicity was determined in human breast (MCF-7), colorectal (Caco-2) and prostate (PC3) carcinoma cell lines treated with different doses of pMC540 or MC540. Our preliminary results suggest that photoactivation breaks

down MC540 generating at least two pMC540 products which cytotoxicity is dependent on the cell line type. pMC-540 photoproducts showed to be dose-dependent cytotoxic for MCF-7 and PC3 cells whereas Caco-2 cells were not susceptible to pMC540. We hypothesize that these differences could be derived from the differential interaction of pMC540 on different cell membranes constituents. Further assays and analysis are ongoing to strengthen our hypothesis.

CB-P40
KINETIC OF CORTICAL REACTION INDUCED BY Sr^{2+} AND IONOMYCIN IN OVULATED AND IN VITRO MATURED OOCYTES

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Meiosis progression from metaphase II to embryo development requires oocyte activation, a set of events triggered by spermatozoa. One of these processes is cortical reaction, which consists in exocytosis of small vesicles called cortical granules and is responsible to block polispermy. In vitro maturation is used in assisted reproduction; however it is unknown how it affects oocyte activation and embryo development. In this work we compare the kinetic of cortical reaction stimulated by $SrCl_2$ and ionomycin between ovulated and in vitro-matured oocytes. Ovulated oocytes were collected from ampulla after PMSG-hCG stimulation. Immature oocytes were obtained from ovaries after PMSG stimulation and in vitro-matured in G-IVF Plus (Vitrolife) media during 16 h. Cortical reaction was parthenogenetically activated in both cell types, ovulated and in vitro matured oocytes, in the presence of lens culinaris agglutinin (LCA)-FITC for labeling of cortical granule´s exudate. Epifluorescence images were collected (1frame /minute during 60 minutes) and recorded on a LUCA-R EMCCD camera. After each experiment, cells were fixed and cortical granules were stained with LCA-rhodamine and quantified using Image J program. Our results show that cortical reaction triggered by $SrCl_2$ and ionomycin have different kinetic patterns and suggest that in vitro maturation affect the kinetic of cortical reaction.

CB-P41
EFFECT OF CARDIAC EGFR SILENCING WITH SIRNA ON THE SLOW FORCE RESPONSE TO MYOCARDIAL STRETCH

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The stretch of cardiac muscle produces an initial fast increase in force, followed by a slow force response (SFR). The SFR seems to be due to an autocrine mechanism that involves the activation of the epidermal growth factor receptor (EGFR) and the consequent generation and release of reactive oxygen species (ROS). We developed a lentivirus carrying shEGFR sequence to inhibit EGFR expression, without affecting cardiac family members ErbB2 and ErbB4, and injected into a rat cardiac left ventricular wall (n=5). As control, a second group of sham rats were injected with phosphate buffer saline (PBS, n=5). Four-week later, EGFR protein expression in the left ventricle of shEGFR expressing rats showed 52 ± 15 % reduction compared to sham animals (100 ± 6 %, P ‹0.05). The SFR, evaluated in isolated left ventricle papillary muscles, was blunted in animals expressing shEGFR (in % of initial phase: 102 ± 1 vs. sham 112 ± 1, P‹0.05). ROS production (evaluated by the lucigenin method) was assessed in cardiac left ventricle stripes after AII (1 nM) and EGF (0.1 µg/ml) stimulation. AII and EGF increased basal ROS production in sham rats by 47 ± 2 and 43 ± 6 % respectively, effect that was significantly reduced in shEGFR expressing rats (5 ± 5 and 17 ± 12 % respectively). We conclude that EGFR activation is crucial for the redox-sensitive mechanism that triggers the SFR to myocardial stretch.

CB-P42
HYPOXIA INCREASES THE EXPRESSION OF KRÜPPEL LIKE FACTOR 6 (KLF6) IN THE PLACENTA

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Preeclampsia (PE) is a pregnancy-specific disorder related to abnormal placental blood perfusion and poor trophoblast invasion. It is an important cause of maternal and fetal mortality and morbidity. While its pathophysiology has not been fully elucidated, placental oxidative stress injury appears as one important mechanism. In addition, normal early placentation occurs in a relatively hypoxic environment and throughout this period, HIF-1α mediates low oxygen tension effects. KLF6 is a transcription factor highly expressed in placenta, involved in

carcinogenesis and cell differentiation in various systems. Interestingly, in mouse kidney KLF6 expression is up-regulated following ischemia/reperfusion. Our aim was to elucidate whether placental KLF6 expression depends on oxygen level. We found that hypoxia increases KLF6 expression early and transiently in placental cells and tissue. KLF6 up-regulation was partially dependent on HIF-1α as revealed by siRNA knockdown and western-blot assays. Furthermore, immunohistochemical analysis showed a decrease in the nucleo/cytoplasmic KLF6 expression ratio in PE placentas compared to control. According to these data, KLF6 is a potential mediator of hypoxic effects participating in normal and/or pathological placental development. Supported by CONICET, FONCyT, SECyT-UNC, and IUBMB and JCS fellowships.

CB-P43
ASSESSMENT OF MECHANISMS INVOLVED IN TROPHOBLAST RESPONSE TO CHLORPYRIFOS (CPF) PESTICIDE EXPOSURE

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Prior studies have shown that CPF induces ROS production in trophoblastic cells; however, they are quite resistant to cytotoxicity. In this context, JEG3 cells increase ABCG2 xenobiotic transporter and βhCG expression, and trigger an antioxidant response with augmented Nrf2 expression and nuclear translocation. KLF6 is an early response factor enriched in placenta, involved in βhCG transcription and upregulated by ROS. Here, we addressed whether these transcription factors mediate CPF effects on JEG3 cells. KLF6 protein level increased in cells exposed to CPF for 3h, preceding βhCG and ABCG2 activation observed after 24h of treatment. When KLF6-silenced cells were treated with 50 μM CPF for 24 and 48h, cell viability was not affected; whereas βhCG and ABCG2 transcript and protein levels, measured by real time PCR and western-blot respectively, were increased to similar levels than in non-silenced cells. Compared to controls, Nrf2-silenced cells presented lower ABCG2 level but its expression as well as βhCG remained inducible by CPF. In summary, CPF increase Nrf2 and KLF6 levels though they are not the main factors mediating CPF-induced upregulation of ABCG2 and βhCG in trophoblast cells. We are investigating if the AhR, involved in xenobiotic toxicity and recently described as a novel KLF6 partner, participates in JEG3 cell response to CPF. Supported by CONICET, FONCyT & SECyT-UNC.

CB-P44
SELECTION OF SINGLE DOMAIN ANTIBODIES AGAINST SURFACE HUMAN MELANOMA ANTIGENS

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Cutaneous melanoma is a malignant tumor whose incidence and mortality rates have been increasing worldwide. Although new therapeutic options have improved therapy of advanced melanoma, the long-term prognosis remains poor. Therefore, the discovery of new targets is essential to design effective therapies. We have developed a phage display library constituted by variable domains of llama heavy-chain antibodies (VHH). VHHs possess small size, ability to recognize epitopes inaccessible to conventional antibodies, low immunogenicity and good tissue penetration. After enrichment of the library in VHHs that recognize melanoma antigens (Ags) through negative (using red blood cells) and positive (melanoma cells) steps, groups of phages-VHHs showed reactivity against melanoma patients tissue microarrays. Dry cell ELISA enabled us to identify 3 clones with strong binding against melanoma cells. Flow cytometry assays allowed us to select 4 high reactivity clones, one of them recognizing melanoma cells (MC) but not breast cancer cells (BC) or lymphocytes. The 3 others clones, which overlap those identified by ELISA, showed high reactivity to MC and BC. VHH clones with differential cell type recognition probably bind different antigens. The four binder clones were isolated and soluble VHHs produced. Binding assays of soluble VHHs and future perspectives for Ags identification will be presented.

CB-P45
BIOCHEMICAL AND BEHAVIORAL PARAMETERS AFTER ECLOSION IN *Ceratitis* AND COMPARISON WITH *Drosophila*

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Despite numerous studies on the life cycle of Cychlorraphan flies and other Dipterans, many aspects related to holometaboulous metamorphosis are poorly known. Several details on the emergence of the immature adult remain

unknown, both in the *Ceratitis capitata* and in *Drosophila*. We focused our interest in the extrication behavior of both flies, and in the subsequent body maturation. Our aim was to correlate behavioral performance with metabolic parameters and with the sclerotization machinery. To analyze in detail a significant number of flies and parameters, different arenas and a novel imaging recording system was set up. An image and movement analytical software was *de novo* implemented. Data on the timing of events during extrication of both flies indicated that the respective programs are very similar, although emergence is differently timed. However, from just exarate adults to fully body maturation, the body maturation phase, significant differences were detected. The timing was normalized to understand the equivalent developmental times. Rapid recovery of glycogen and lipid reserves occurs immediately after emergence. Variation of the sclerotization/tanning machinery (N-B-alanyldopamine synthesis) was also analyzed. Other events were recorded in *C. capitata* and for the first time the above behavioral parameters were related to wet/dry weight, water content and O_2 consumption.

CB-P46
ROLE OF PROTEIN UBIQUITYLATION IN REGULATING TUMOR-CELL INVASION AND MIGRATION

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Metastatic disease is the most common cause of cancer-related death in patients with solid tumors. A considerable body of evidence indicates that tumor cells are shed from a primary tumor mass at the earliest stages of malignant progression, and that the invasive potential of these determines, to some extent, the aggressiveness of newly formed secondary tumors. Despite characterizations on metastatic cells, there is yet not an effective treatment for this disease. Ubiquitylation is a post translational modification that has recently became of great interest due to its therapeutic potential regarding cancer treatment. Alterations in the ubiquitylation cascade have been shown to be associated with malignant transformation, invasive potential of cells and metastasis. Hence, we sought to investigate the role of the Ubiquitin Proteasome System (UPS) in the regulation of tumor-cell invasion and migration. In order to find new UPS genes that are related to invasion and migration, we set out to perform a genetic screen using a shRNA library against UPS genes, and Boyden chambers to analyze the migrating/invasive potential of cells infected with this library. After the selection process, the relative abundance of the shRNA present in the different isolated populations will be assessed by next generation sequencing and the putative candidates will be further validated in vivo.

CB-P47
IDENTIFICATION OF NOVEL SUBSTRATES OF THE UBIQUITIN PROTEIN LIGASE CRL4^{CDT2}

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E3 ubiquitin ligases represent the most important regulation level of the Ubiquitin-Proteasome System. The E3 ligase CRL4^{Cdt2} plays fundamental roles in the control of cell proliferation and DNA repair processes. Different lines of evidence demonstrate that alterations in the expression and activity of CRL4^{Cdt2} induce genomic instability. Therefore, it is not surprising that Cdt2 protein levels are increased in many tumor cells and its expression correlates with tumor grade, metastasis and poor survival. The purpose of this project was to identify and characterize novel CRL4^{Cdt2} substrates in an effort to contribute to the identification of potential molecular targets for the treatment of cancer. To this end, we performed Cdt2 immunoprecipitation followed by Tandem Mass Spectrometry (MS/MS) analysis. Hek293t cells were transfected with Cdt2 containing vector (Cdt2) or empty vector (PCDNA) and were treated with different DNA damage agents (Hydroxyurea, Camptothecin and UVC), alone or in combination with the proteasome inhibitor MG132 and an inhibitor of NAE1 (MLN4924). From each sample both the CSK buffer soluble and chromatin fraction were analyzed and several novel putative Cdt2 interactors were identified. We are in the process of validating and characterizing the functional interaction between Cdt2 and the most abundant putative binding partners identified in both fractions.

CB-P48
CHANGES IN TUBULIN TYROSINE LIGASE OF ERYTHROCYTES DURING ESTABLISHMENT OF HYPERTENSION

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In previous work we demonstrated that Tubulin Tyrosine Ligase (TTL) activity is reduced in human erythrocytes from hypertensive subjects. In this work content, distribution and activity of this enzyme was evaluated before and after development of hypertension in spontaneously hypertensive rats (SHR). Since hypertension develops after 8-weeks old in SHR these parameters were evaluated in 4 and 12-weeks old rats. After hypertension establishment, TTL activity was 50 % lower in erythrocytes from SHR than Wistar, which were normotensive controls. In according, Western blot analysis shows that TTL content was lower in erythrocytes from than normotensive ones. Distribution of TTL along the cell was also different between both groups. In opposite, before hypertension is established, neither activity nor content of TTL were statistically different between erythrocytes from SHR and Wistar rats. Moreover, TTL activity was modified in other tissues, such heart, kidney and brain, which are relevant in the hypertension development. In conclusion, results suggest that during establishment of hypertension changes in TTL content and activity in different tissues are induced, since detyrosinated tubulin, substrate of TTL, is involved in NKA regulation, this changes could be involved in alteration of NKA activity observed in hypertensive subjects.

CB-P49
OSMOTIC FRAGILITY IN GLUCOSE-TREATED OR DIABETIC ERYTHROCYTES IS MEDIATED BY ACETYLATED TUBULIN

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In previous work we demonstrated that high glucose concentration induces translocation of tubulin from sedimentable fraction to the plasma membrane in erythrocytes from diabetic subjects and this change affected cellular deformability. In this work osmotic fragility of erythrocytes was analyzed. We observed a significant increase of erythrocyte osmotic fragility in diabetic subjects and a similar result was observed in erythrocytes from control subjects treated with high glucose concentrations. Due to glucose induces increases in acetylated tubulin levels and its translocation to the membrane in erythrocytes; we evaluated the effect of acetylated tubulin levels on osmotic fragility. The erythrocytes pretreated with trichostatin A, to increase acetylated tubulin levels, and then incubated for 3 hours with glucose have an increased hemolysis compared to those that were only treated with glucose. Following acetylation capacity was analyzed in each case. The results suggest that glucose decreases de deacetylation capacity, which was corroborated by treating erythrocytes with tubacin as a specific inhibitor of HDAC6. This work allows us to conclude that the increased level of acetylated tubulin in erythrocyte membrane affects its properties and that this increase occurs in diabetics by the decrease in deacetylation capacity.

CB-P50
CHARACTERIZATION OF *Trypanosoma cruzi* SIRTUINS

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T. cruzi is the protozoan pathogen responsible for Chagas disease. Current therapies rely on a very small number of drugs, most of which are inadequate because of their severe host toxicity. To determine efficient therapeutic alternatives, the identification of new biotargets is essential. Class III KDACs, sirtuins, are highly conserved from Archaea to higher eukaryotes, and they have been recently considered as promising targets for the development of new treatments for Chagas disease. Genes encoding two Sir2 related proteins (SIR2RPs) were found in *T. cruzi*. Here we report their first characterization. To study the expression of TcSIR2RPs, antibodies were raised in rabbit against the recombinant proteins. We also cloned the sirtuins with an HA tag in a plasmid which allows inducible expression and transfected epimastigotes. The expression of these constructs after Tetracycline addition was tested using antiHA antibodies, no leaky expression was observed in the uninduced cultures. We monitored the effect of sirtuins overexpression on epimastigote growth by counting cell numbers daily, on in vitro metacyclogenesis and on mammalian cell infection. Furthermore, we tested the effect of NAM (a sirtuin inhibitor) treatment in Dm28wt, uninduced and induced Dm28 pTcIndexSIR2RP1 and Dm28 pTcIndexSIR2RP3.

CB-P51
THE EFFECT OF ESTRADIOL ON THE EXPRESSION AND DISTRIBUTION OF CD-MPR IN BREAST CANCER CELLS

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Cathepsin D (CatD) is involved in the invasion and metastasis of breast cancer cells. This protease is normally transported to lysosomes via mannose-6-phosphate receptors (MPRs). Two types of MPRs have been described to date: the cation-dependent (CD-MPR) and the cation independent (CI-MPR). The CD-MPR could also participate in exocytosis of lysosomal proteins. In mammary carcinomas, CatD is highly secreted as a pro-enzyme, although the mechanism is still poorly understood. Here, we studied the expression and distribution of CatD and CD-MPR in breast tumor cells and a possible hormonal regulation. For this, two breast cancer cell lines were used (MCF-7 and MDA-MB 231, which express or not estrogen, EST, receptors respectively) and compared to normal breast cells (MCF 10A). They were cultured either in the presence or absence of 17β-EST and/or tamoxifen (TX). It was observed that both proteins are highly expressed in MCF-7 cells. In turn, this expression (in MCF-7) was increased in the presence of EST and was reverted by TX. We also observed that CD-MPR is redistributed toward light fractions in a gradient and from a supranuclear location to a more dispersed cytoplasmic signal (by IFI). We concluded that EST could regulate the expression and distribution of CatD and CD-MPR and the interplay of both proteins could be responsible for the high secretion of the enzyme in these cells.

CB-P52
INTERACTION OF LYSOSOMAL ENZYMES WITH SPERMATOZOA FROM BOVINE EPIDIDYMIS. POSSIBLE IMPLICATIONS OF MANNOSE-6-P-RECEPTORS

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Mammalian epididymis participates in sperm maturation through its endocytic and secretory activity. In bulls, as in other mammals, many lysosomal enzymes (LE) are secreted to the epididymal fluid. Here, we proposed to study the interaction LE secreted by the epididymal epithelium with spermatozoa, in order to evaluate the participation of mannose-6-phosphate receptors (MPRs). We observed that β-galactosidase (β-Gal), β-glucuronidase (β-Glu), and N-acetyl-β-D-glucosaminidase (β-NAG) associated to sperm membrane are released easily with increasing ionic strength or M6P, meanwhile α-mannosidase (αMAN) and α-fucosidase (α-Fuc) mostly remained associated to the gametes after treatments. The values of KD and Bmax for MPRs were estimated from curves of binding assays using an exogenous enzyme, and showed a higher number of active CI-MPR sites and with more affinity to phosphomannosyl ligands. We also observed that both MPRs distribute differently on the sperm surface and that CD-MPR is redistributed during epididymal transit. Instead, the CIMPR is mostly concentrated at the acrosomal region along the epididymal duct. We concluded that both MPRs are present in bull spermatozoa and they interact differently with LE in the epididymal lumen. Thus, MPRs may interact with LE for transport to the female reproductive tract, suggesting new roles for these receptors.

CB-P53
EFFECT OF N-ACETYLCYSTEINE AND IDEBENONE DURING ANAEROBIC INCUBATION OF *Saccharomyces cerevisiae*

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There are circumstances in which cells are subjected to the absence or reduction of oxygen level. Re-oxygenation may exceed the capacity of the cell to metabolize the received oxygen. Thus ROS increase can lead to diverse cellular alterations. We used yeast S.cerevisiae as a model system to study cellular processes involving mitochondrial metabolism since its ability to grow under aerobic and anaerobic conditions. The aim of this study was to: a) assess cell viability under absence of oxygen, b) relate viability observed with the ROS formation and mitochondrial membrane potential and c) to study N-acetylcysteine (NAC) or idebenone effect in cycles of absence/presence of oxygen. Cells were incubated at 30 °C using an anaerobic jar and a Oxoid generation system. Incubation under aerobic and anaerobic conditions, was performed in the presence of NAC or idebenone. We measure ROS using diclhorofluorescein 2,7-diacetate signal and mitochondrial membrane potential with rhodamine 123. NAC reduces mitochondrial membrane potential in anaerobiosis and ROS production in aerobiosis. Idebenone, increases only the membrane potential in anaerobiosis. Results shows that NAC would be more advantageous as an antioxidant

protector under both conditions. Instead, idebenone may be appropriate to enhance the functioning of mitochondria under low oxygen supply or deficient functioning of the organelle.

CB-P54
YACON ROOTS AMELIORATE NON-ALCOHOLIC FATTY LIVER

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Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological change character¬ized by the accumulation of triglycerides in hepatocytes and has frequently been associated with obesity, type 2 diabetes mellitus, hyperlipidemia, and insulin resistance. In the present study we determine the effects of yacon roots on hepatic steatosis induced by a high-fructose-diet (HF) in rats and elucidate the underlying mechanism. Yacon flour supplementation significantly lowered plasma triglycerides, free fatty acids and elevated adiponectin levels. Yacon also reduced hepatic weight, hepatic triglyceride content and ameliorated hepatocyte degeneration decreasing the inflammatory cell infiltration in HF-livers. Yacon significantly increased the AMP-activated protein kinase (AMPK) expression and restored the HF-induced inhibition of the AMPK phosphorylation. Both processes are related to fatty acid β-oxidation, in the hepatic tissue. Dietary supplementation with yacon roots also up regulate the expression of the peroxisome proliferator-activated receptor alpha (PPARα) and decreased the expression of the lipogenic genes fatty acid synthase (FAS), glycerol-phosphate acyltransferases (GPAT) and HMG-CoA reductase (HMGR) in HF-livers. These results suggested that yacon roots improve liver fatty degeneration regulating lipid metabolism through an elevated AMPK protein expression and phosphorylation.

CB-P55
PARACRINE REGULATION DURING *Xenopus laevis* OOGENESIS

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Oogenesis in vertebrates has been characterized as the result of a regulation between different signaling systems such as endocrine and juxtacrine. Paracrine factors belonging to the TGF-β superfamily -like BMPs- have been identified as fundamental for the progress of oogenesis. Not much is known about paracrine regulation during oogenesis in oviparous vertebrates, which has the complexity of the three different stages: previtellogenesis, vitellogenesis and maturation. Amphibian vitellogenesis is a well-controlled process characterized by hepatic production of the glycoprotein vitellogenin (VTG) that is transported via the bloodstream to the ovary where it is incorporated by oocytes. In previous work we have demonstrated that in *Xenopus laevis* ovary, VTG uptake is regulated not only by endocrine and juxtacrine signaling but also by an interplay between them. In this work we delve deeper into the study of paracrine regulation assessing the role of the TGF-β signaling via Smad2/3 during *X. laevis* oogenesis. To analyze the role of this pathway in the progression of oogenesis, we performed different assays with the anti-TGFβRII antibody, a BMP pathway inhibitor and a gap junction uncoupling agent. The results showed that TGF-β signaling might be implicated in *X. laevis* oogenesis, a complex process coordinated by a cross talk between the molecules of different signaling pathways.

CB-P56
DIFFERENTIAL EXPRESSION OF MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS IN DIABETIC COLON

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Diabetes is characterized by alterations of intestinal wall with the thickening of the smooth muscle layer and excessive extracellular matrix (ECM) deposition. The cytokine TGF-β1 appears critical in this process regulating ECM production and turnover. We have recently shown that smooth muscle cells from diabetic intestine express significantly higher amounts of TGF-β1, TGFβ RII and the intracellular effector p-Smad2/3. Matrix metalloproteinases (MMPs) are important enzymes of ECM remodeling and growth factors activity. Now, we report that muscle layer of diabetic intestine have significantly lower constitutive expression of MMP-2 and MMP-9 transcripts together with localized collagen III and fibronectin deposition. Also diabetic smooth muscle layer expressed lower amounts of tissue inhibitor of metalloproteinase (TIMP)-1 compared to normal tissue. Interestingly, the expression of specific contractile markers of smooth muscle cells (α-SMA, Smoothelin and MYH-11) was down regulated in diabetic colon indicating a phenotypic change. These studies illustrate a potential mechanism by which differential expression of TGF-β may lead to excessive deposition of ECM in the intestinal smooth muscle layer via

the imbalance between MMPs and TIMPs activities and changes in cell phenotype that result from hyperglycemic environment.

CB-P57
NOVEL STRATEGY TO STUDY ARGININE DEIMINASE PARTNERS IN THE PARASITE *Giardia lamblia*

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Giardia lamblia is a ubiquitous unicellular parasite of humans and other vertebrates that commonly causes diarrhea and gastrointestinal upset. During the last years, we have been studying the multiple roles played by the enzyme arginine deiminase (ADI) during growth and differentiation of this parasite. An interesting feature is the capacity of ADI to interact with different partners from surface protein to histones depending on its localization during the cell cycle of *Giardia*. In this study, we develop a novel strategy to further analyzed ADI partners, by generating monoclonal antibodies (mAb) against in vivo immunoprecipitated *Giardia* ADI. By using ADI-HA as immunogen, obtained from immunoprecipitation using anti-HA mAb, we obtained not only specific mAbs against ADI but also to other proteins that co-immunoprecipitated with it. It is important to highlight the reproducibility of this method using different protein baits, which, in combination with MS-MS, allowed to obtain specific mAbs and also to disclose the architecture of particular protein networks.

CB-P58
HIPPOCAMPAL NEURONAL RESPONSE TO AMYLOID β PEPTIDE OLIGOMERS. BIOLOGICAL AND BIOPHYSICAL INSIGHTS

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We have previously demonstrated that oligomeric amyloid β peptide (oAβ), known as the most harmful species of Aβ, concomitant with iron overload led to synaptic injury and local activation of several signaling cascades. In this work, we characterized hippocampal neuronal response to oAβ exposure both in the presence and absence of iron. HT22 neurons exposed to iron overload displayed increased lipid peroxidation, slight loss of mitochondrial function, and activation of ERK and Akt pathways. oAβ neither induced an increase in lipid peroxidation nor altered mitochondrial function. However, oAβ alone triggered the activation of ERK and Akt, and the coincubation with oAβ/iron restored pAkt and pERK to the control levels. In addition, we also studied the effect of iron, oAβ and both conditions together, on the biophysical state of the plasma membrane by measuring the generalized polarization of the fluorescence probe Laurdan and the fluorescence anisotropy of DPH and TMA-DPH. Both studies showed that the presence of iron (even at the highest concentration tested), oAβ, or both conditions together, did not perturb the lipid order of the membrane. We conclude that oAβ activates signaling pathways in the absence of oxidative stress or membrane disturbances in hippocampal neurons.

CB-P59
ANTITUMORAL EFFECTS OF BIOENERGETIC MODULATION IN FELINE MAMMARY CARCINOMA CELLS

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Feline mammary carcinoma (FMC) is a highly aggressive pathology that has been proposed as an interesting model of breast cancer disease. Most tumor cells metabolism rely in an increase of glycolysis that enhances the malignant phenotype. The aim of the present work was to investigate the effects and mechanisms of metformin (MET, antidiabetic drug), 2-deoxyglucose (2DG, hexokinase inhibitor) and dichloroacetic acid (DCA, piruvate deshydrogenase kinase inhibitor) in ALRB, an established FMC cell line. The antitumor effects of glucose metabolism modulation were evaluated by the acidic phosphatase assay (APH), 5 days after treatments. While all treatments significantly diminished cell viability ($p<0.05$), the combination MET+2DG displayed a potentiated effect that was significantly higher than the addition of the single treatments ($p<0.01$). In addition, MET+2DG caused an increase in both intracellular oxidants and G0/G1 subpopulation (as determined by DCFH-DA and propidium iodide flow cytometry). The drugs here evaluated displayed an increase of autophagic vacuoles (revealed by expression of lipofected RFP-LC3 plasmid). Finally, glucose consumption and lactate concentration were increased only by MET

treatment (commercial kits, Weiner Lab). The results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

CB-P60
CHARACTERIZATION AND EFFECTS OF GENE/CHEMOTHERAPY ON NEW ESTABLISHED CELL LINES DERIVED FROM MELANOMA

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The aim of the present study was to evaluate the in vitro effects of a combination of bleomycin and human interferon-B (hIFN-B) lipofection on cell lines derived from spontaneous human melanoma. Three new cells lines (MLUTG-1, MLUTG-2 and MLUTG-4) were established from surgically excised metastatic tumors by mechanical disruption and serial passages. The growth duplication times were respectively: 24.4 ± 0.6; 33.3 ± 0.4 and 59.4 ± 0.9 h, for MLUTG-1, MLUTG-2 and MLUTG-4. As determined by immunochemistry, the three cell lines presented the specific melanoma markers S-100, HMB-45 and Melan-A. The antitumor effects of hIFN-B lipofection in the absence or presence of bleomycin were evaluated by the acidic phosphatase assay (APH). Lipofection with hIFN-B sinificantly decreased cell viability ($p<0.001$) of MLUTG-1 and MLUTG-2 (36.5 ± 0.7% and 35 ± 5% respectively) and increased the concentration of intracellular oxidants in both cell lines (as determined by DCFH-DA assay). In the case of MLUTG-1, the combination of hIFN-B lipofection plus bleomycin significantly increased ($p<0.01$) the antitumor effects (22.8 ± 1.7% of viability). The results reported here support further studies to investigate the potential use of this gene/chemotherapy approach in a clinical setting.

CB-P61
DETOXIFYING METABOLISM FOR THE PESTICIDE AZINPHOS METHYL IN THE PATAGONIAN SILVERSIDE

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The intensive fruit production in Río Negro valley leads to the massive use of organophosphate (OP) pesticides. Our objective was to study the detoxifying metabolism of the OP azinphos methyl (AZM) in the Patagonian silverside (*Odontesthes hatcheri*). Juvenile fish were exposed to 0.1-10 µg/L AZM during 96h. The LC50 was 0.174±0.076 µg/L AZM, 10 times lower than maximum environmental concentrations. Target organs showed diverging toxodynamics; while brain acetylcholinesterase (AChE) was inhibited (IC50 = 0.193±0.063 µg/L), with low carboxylesterase (CabE) activity unaffected by AZM, the opposite was found in muscle, with buffer CabE (40% inhibition, $p<0.001$) that prevented AChE inhibition. GSH increase as antioxidant response was elicited in both (2X, $p <0.05$). CabE was also inhibited in intestine (70%, $p<0.001$), but hepatic detoxifying response was induced through CabE (150%, $p<0.005$) and GSH increase. GSH-S-transferase (GST) activity was inhibited by 50% in liver and intestine ($p<0.05$), showing that oxidative stress indeed affected the detoxifying capacity. Multiple Xenobiotic Resistance (MXR) capacity was analyzed by ex vivo assay of conjugated DNP-S-GSH transport dependant on GST conjugating activity. AZM caused an increase of 182% ($p<0.001$) in liver slices, while 50% of decrease was found in intestine ($p<0.05$), suggesting organ-specific effects on the excretory capacity.

CB-P62
Tl(I) AND Tl(III) DIFFERENTIALLY AFFECT THE METABOLISM OF EXTRACELLULAR ATP IN PC12 CELLS

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We previously demonstrated that Tl(I) and Tl(III) (100 µM) induced PC12 cell apoptosis. In this study, we investigated the hypothesis that a misbalanced homeostasis of extracellular ATP (eATP) may participate in the initial steps of Tl-mediated apoptosis. Homeostasis of eATP depends on the balance between the rates of ATP release and extracellular ATP hydrolysis (i.e. ectoATPase activity). Signaling of eATP is effected through activation of purinergic receptors. Release of ATP was negligible with Tl(I) and amounted to 10.6+/-2.2 pmol ATP/min/10^6 cells with Tl(III). This release was inhibited 70% by carbenoxolone (pannexin 1 inhibitor) and 50% by brefeldin A (exocytosis inhibitor). EctoATPase activity was increased 65% by Tl(I) and reduced 68% by Tl(III). Together, results suggest that even when Tl(I) does not promote per se the release of ATP, it accelerates eATP hydrolysis which would limit the duration of eATP-mediated cell signaling. In contrast, Tl(III) promotes ATP release and inhibits eATP

hydrolysis by ectoATPases, which would increase the duration of eATP cell signaling. *Supported by grants of UBA (20020100100112) and CONICET (PIP112-201101-00639).*

CB-P63
REGULATION OF THE 2-OG DEPENDENT DIOXYGENASE FATIGA IN RESPONSE TO CHANGES IN NUTRIENT AVAILABILITY

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The study of 2-oxoglutarate (2-OG) dependent dioxygenases is acquiring an increasing interest, given that these enzymes use 2-OG as co-substrate, being the metabolite concentration an indicator of the cell metabolic condition. The objective of this work consisted on analyzing if 2-OG dependent dioxygenases have the capacity of acting as molecular sensors of the metabolic condition by sensing fluctuations in 2-OG levels. In particular, we studied if the *Drosophila* HIF-α prolyl-hydroxilase, Fatiga (Fga), reacts to changes in nutrient availability. We used a transgenic fly line that expresses a reporter gene of Fga activity, which consists on the fusion of GFP with the ODD of HIF-α. Hence, the ODD hydroxylation leads to the proteasomic degradation of the fusion protein, lowering its levels. Both the exposure of the transgenic line to hypoxia, as well as the Fga levels reduction by RNAi dependent silencing, were able to stabilize the GFP-ODD reporter, showing that this construct responds to Fga activity. However, the reporter exposure to several nutrient conditions did not altered its stability, even in fly lines whose capacity to mobilize nutrients was impaired. Therefore, it was not possible to corroborate the existence of a Fga activity regulation on response to changes in nutrient availability.

Enzymology

EN-P01
MICROCIN J25 INHIBITS CYTOCHROME *BD* OXIDOREDUCTASE ACTIVITY

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Microcin J25 (MccJ25) is a plasmid-encoded antimicrobial lasso peptide produced by *Escherichia coli*. It displays antibiotic activity against a range of Gram-negative pathogens including *E. coli*, *Salmonella* and *Shigella*. MccJ25 has two cellular targets, the RNA polymerase and the membrane respiratory chain. MccJ25 targets the respiratory chain enzymes on the bacterial membrane with the consequent inhibition of oxygen consumption. This effect is mediated by an increase in superoxide production during the membrane respiratory processes. *E. coli* has two terminal oxidoreductases, the cytochrome *bd* and cytochrome bo_3 in their respiratory system. The *E. coli* C43 strain (wild type) was sensitive to the peptide whereas the Δ*bdI* Δ*bdII* double mutant became fully resistant. In our laboratory, cytochrome *bdI* was purified and the effect of MccJ25 on the activity of this terminal oxidase was studied. The ubiquinol oxidase activity was significantly inhibited in presence of MccJ25. This inhibition was dose dependent. Furthermore, the analysis of the kinetic constants Km and V_{max} allowed us to conclude that MccJ25 would act as a non-competitive inhibitor. This finding indicates that this lasso peptide would be capable of inhibiting the ubiquinol activity *in vitro* suggesting that the cytochrome *bd* might constitute a main target in *E. coli* membrane chain.

EN-P02
FIDELITY OF DNA RECOMBINATION CATALYZED BY HUMAN DMC1 RECOMBINASE

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Dmc1 is a meiosis-specific recombinase that carries out the search for DNA homology and catalyzes the strand exchange (StEx) reaction during homologous recombination (HR). Previous works have suggested that heterologies in DNA affect the StEx reaction catalyzed by recombinases and therefore the fidelity of HR. To study the influence of heterologies on HR promoted by human Dmc1, we analyzed the efficiency of StEx using oligonucleotides containing different types, frequency and distribution of mismatches (MM). We examined by fluorescence resonance energy transfer the StEx reaction of 12 different types of single-MM in three positions of the incoming oligonucleotide strand. We found that some types of MM had a stronger inhibitory effect on StEx when they were

near the 5' end of the incoming strand. This effect was not observed in the middle or 3' end position. A similar trend was obtained when the frequency of MM was increased. In addition, we examined if the presence of Hop2-Mnd1 complex, which interacts with Dmc1 and enhances HR, affects the discrimination of DNA heterologies. We observed that Hop2-Mnd1 only stimulated Dmc1-StEx reaction in the presence of a single MM. Finally, analysis of different mutants of Hop2 and Mnd1 revealed that Mnd1 DNA binding site is important to stimulate Dmc1. Thus, our data provide new insights on the fidelity of HR catalyzed by human Dmc1.

EN-P03
BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF A MONOTHIOLIC GLUTAREDOXIN FROM *Trypanosoma cruzi*

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Glutaredoxins (Grx) are small proteins linked to the cellular iron and redox metabolism. *Trypanosoma cruzi* is the protozoan parasite that causes the Chagas disease. Despite a putative monothiolic glutaredoxin (*Tcr*PICOT80) is encoded in the genome of *T. cruzi*, it was not yet characterized. In this work, we present the functional and structural properties of the recombinant *Tcr*PICOT80. The protein was able to catalyze the *in vitro* reduction of GSSG using $T(SH)_2$ as electron donor. In addition, the protein was subjected to *in vitro* iron-sulfur cluster (ISC) reconstitution and the resultant absorption spectra revealed two characteristic peaks (at 320 and 420 nm) of ISC-Grx complexes. Furthermore, gel filtration chromatography profile indicated that the ISC Grx complex eluted with retention volume nearly corresponding to that expected for dimeric Grx specie. On the other hand, we employed a yeast-based functional complementation assay to rescue phenotypes of Grx mutants. The complementation experiments demonstrate that Δgrx5 and Δgrx4Δgrx5 can rescue phenotype in SD medium and suppressed the sensitivity of Δgrx5 cells to oxidants. These results suggest that *Tcr*PICOT80 may have important functions in redox metabolism and the biogenesis of ISC in *T. cruzi*.
Granted by UNL, CONICET (PIP112 2011 0100439, PIP114-2011-0100168) and ANPCyT (PICT2012-2439, PICT2013-0253).

EN-P04
CHARACTERIZATION OF A PROMISCUOUS GLUTAMATE CYSTEINE LIGASE FROM *Leptospira interrogans*

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Glutathione (GSH) is the most abundant low molecular weight thiol in almost all eukaryotic cells as well as in proteo and cyanobacteria. It is synthesized enzymatically in two ATP–Mg^{+2}dependent steps. First, glutamate cysteine ligase (GCL) establishes a peptide bond between cysteine and glutamate, forming γ-glutamylcysteine (Y–GC). Second, glutathione synthetase (GS) adds a glycine residue to the carboxy-terminus of Y–GC, producing glutathione. Analysis of the genome sequences of *Leptospira interrongans* (the casusative agent of the leptospirosis) indicates the absence of the encoding-gene to GS. However, the presence of the gene that encodes GCL (LIC11812) leads us to believe that *L. interrogans* could has Y–GC as redox buffer similar to *Halobacteria* and *Lactic acid bacteria*. Therefore, we cloned, expressed in recombinant form and characterized the functionality of GCL of *L.interrogans*. We showed that the enzyme is active in presence of their physiologic substrates (glutamate, cysteine and ATP), and also has the ability to use GTP, aspartic and serine, although in lower proportion. The enzyme has optimal activity at pH~7.5. Our results add value to the genome project information and contribute to the understanding of the redox metabolism present in this bacterium. Granted by UNL, CONICET (PIP112-2011-0100439, PIP114-2011-0100168) and ANPCyT (PICT2012-2439, PICT2013-0253).

EN-P05
MULTIPLE STARCH SYNTHASE III ISOFORMS IN THE SMALLEST FREE–LIVING EUKARYOTE *Ostreococcus tauri*

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Starch synthase III from *Arabidopsis thaliana* contains an N–terminal region, including three in–tandem starch–binding domains (SBD), followed by a C–terminal catalytic domain. These SBDs are essential for starch–binding and are involved in the regulation of catalytic activity. In *Ostreococcus tauri*, a unicellular green alga, there are three SSIII isoforms. Using *in silico* characterization techniques, we have shown that these three isoforms contain two, three and no N–terminal SBD.
In this work, we characterized the starch synthase activity of the three isoforms, and the involvement of SBDs in this function. For this purpose, the full–length enzymes as well as truncated isoforms lacking SBDs was cloned, expressed, and purified from *Escherichia coli* cells, and their kinetic parameters were determined using a new discontinuous colorimetric assay. Our results indicate that the *O. tauri* SSIII SBDs present polysaccharide binding ability and modulate the catalytic properties of SSIII. In addition, using co–sedimentation binding assays, we observed that these domains displayed some promiscuity in binding to different polysaccharide substrates.
These results not only reveal significant information concerning functional aspects of SSIII and SBD domains, but are also crucial to understand the evolutionary conservation of multiple SSIII isoforms in this small picoalga.

EN-P06
INTERACTION OF *Leptospira interrogans* FERREDOXIN-NADP(H) REDUCTASE WITH FERREDOXIN

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Leptospira interrogans is a parasitic bacterium that infects humans and causes leptospirosis. *L. interrogans* contains a plastidic-type ferredoxin-$NADP^+$ reductase (LepFNR) similar to the enzymes present in plant plastids and different from the bacterial-type FNRs. A unique feature found in LepFNR structure is a loop that ranges from I73 to R94. By structural modeling we noticed that this structure may interfere with the ferredoxin (Fd) binding. Also, a conserved lysine essential for productive binding of Fd to plant FNRs, corresponds to a threonine residue (T92) in LepFNR. This allow us to suggest that the binding of Fd LB107, identified as a substrate of LepFNR should take place by a different arrangement from the observed in plant enzymes. We designed and constructed different site-directed mutants of LepFNR altering the subdomain I73-R94. We succeeded in expressing and purifying the mutants proteins properly folded and with the FAD bound. We measured the diaphorase activity catalyzed by the LepFNR variants. We found that the substitutions did not lead to mayor changes in this activity and in the NADP(H) binding. We were also able to measure Fd-dependant cytochrome *c* reductase activity with the mutants. Our results indicate that the LepFNR subdomain I73-R94 participates in Fd binding and intervenes in substrate specificity.

EN-P07
ON THE REGULATORY PROPERTIES OF THE *Rhodococcus jostii* ADP-GLC PPASE

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Rhodococcus jostii is an actinobacterium (Gram-positive, GC rich) accumulating lipids as a main carbon and energy storage, although it also produces glycogen as a temporal reserve molecule. To shed light into relationships between the polyglucan synthesis and lipids metabolism, we performed the molecular cloning of the *glgC* gene, encoding ADP-glucose pyrophosphorylase (EC 2.7.7.27, ADP-Glc PPase) from *R. jostii*. The gene was heterologously co-expressed with GroEL/GroES in *Escherichia coli* BL21 (DE3) to achieve the production of recombinant *R. jostii* ADP-Glc PPase with high purity level. The purified enzyme depicted a V_{max} of 0.39 U/mg in the ADP-Glc synthesis direction, with $S_{0.5}$ values for both substrates around 1 mM and exhibiting sensitivity to allosteric regulation by metabolites. Glucose-6P, fructose-6P, mannose-6P, ribose-5P and PEP are activators enhancing V_{max} and the affinity of the enzyme towards Glc-1P; whereas NADPH, pyruvate and 6P-gluconic acid behaved as main inhibitors. Analysis on the interplay between the different effector molecules suggest a fine tuning of the *R. jostii* ADP-Glc PPase activity in a metabolic frame relating in an opposite form the pathways for glycogen and lipids synthesis.

Results support a model where allosteric regulation of ADP-Glc PPase is critical for determining a role of glycogen as a temporal carbon storage molecule in *R. jostii*.

EN-P08
ON THE ROLE OF GENES CODING FOR SUCROSE SYNTHASE IN *Anabaena variabilis*

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Sucrose (Suc) is mainly found in organisms performing oxygenic photosynthesis. The disaccharide plays a central physiological role for carbon metabolism, nitrogen fixation and stress tolerance in filamentous cyanobacteria. It has been described that plant Suc synthase (SucSase; EC 2.4.1.13) is mainly involved in catalyzing the reversible conversion of Suc and UDP into fructose (Fru) and UDP-glucose (UDPGlc). *Anabaena variabilis* has two genes (*susA* and *susB*) respectively encoding for forms A and B of SucSase. These genes were synthesized *de novo* for optimal expression in *Escherichia coli* and cloned in pET28c vector to produce active SucSaseA and SucSaseB purified to homogeneity. To catalyze synthesis of Suc, SucSaseA showed 3-fold higher affinity towards ADPGlc ($S_{0.5}$ = 0.8 mM) than for UDPGlc, with similar values of V_{max}. The $S_{0.5}$ for Fru (3.6 mM) was 33-fold lower when using ADPGlc compared with UDPGlc as the co-substrate. SucSaseB mutated in key amino acid residues exhibited 2.5 mU/mg of activity (100-fold lower than that of SucSasaA) when assayed with 20 mM ADPGlc and 40 mM Fru. No activity was observed with UDPGlc (up to 400 mM). Genes *susA* and *susB* were subcloned in vectors allowing co-expression (pDUET) to explore for the production of heterooligomeric forms of the enzyme. Results contribute to a better understanding on the occurrence, regulation and evolution of Suc metabolism.

EN-P09
KINETIC CHARACTERIZATION OF RECOMBINANT PPI-DEPENDENT PHOSPHOFRUCTOKINASE FROM ORANGE

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Pyrophosphate-dependent phosphofructokinase (PPi-PFKase; EC 2.7.1.90) is a glycolytic enzyme present in higher plants, some bacteria and protozoa. The enzyme performs the reversible phosphorylation of fructose-6-phosphate (Fru6P) using PPi, as an alternative for the reaction catalyzed by the ATP-dependent enzyme (ATP-PFKase, EC 2.7.1.11). PPi-PFKase purified from plant extracts was found composed by two different subunits. In this work, we carried out the molecular cloning and expression of the genes encoding for α and β subunits of PPi-PFKase from *Citrus sinensis*. Both genes were expressed and co-expressed in *Escherichia coli* to obtain different active forms of the enzyme that were kinetically characterized. Fructose-2,6-bis-phosphate (Fru2,6bisP) activated the hetero-oligomeric PPi-PFKase by increasing V_{max} from 40 U/mg to 90 U/mg and the affinity toward Fru6P and Mg^{2+} by ~7- and ~2-fold, respectively. The homo-oligomeric enzymes were 570-fold (homo-α) and 2350-fold (homo-β) less active than the hetero-oligomer. Fru2,6bisP showed no effect on the homo-α enzyme and inhibited the homo-β PPi-PFKase, mainly by decreasing ~21-fold the affinity toward Fru6P. Results suggest that plant PPi-PFKase could be finely regulated by an allosteric mechanism, and they also support the view that regulation could be complemented by the differential expression of the enzyme subunits

EN-P10
ADP-GLUCOSE PYROPHOSPHORYLASE ALLOSTERIC REGULATION INVOLVES CHANGES IN SUBSTRATES SPECIFICITY

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ADP-glucose pyrophosphorylase (ADP-Glc PPase) is the enzyme that catalyzes synthesis of ADP-Glc and PPi from ATP and Glc-1P, through a reaction that requires a divalent metal ion (physiologically Mg^{2+}). Several studies established that this is the regulatory step in the synthesis of glycogen and starch in bacteria and plants, respectively. Within the family of PPases, the ADP-Glc PPase is the only that is allosterically regulated. In particular the enzyme from *Escherichia coli* is mainly activated by fructose-1,6-bisP (FBP). We observed that the *E. coli* ADP-Glc PPase exhibits a degree of promiscuity toward substrates and the essential cofactor. Such a promiscuous behavior was found

affected by FBP, which markedly increased the affinity of the enzyme toward ATP as well the catalytic efficiency when using Glc-1P. Co^{2+} and Mn^{2+} also behaved as cofactors of the enzyme in reactions with both ATP and UTP. Although only with ATP the FBP increased the catalytic efficiency for divalent cation. Results support the view that the allosteric regulator would play a critical role in determining the specific use of ATP as a substrate. The action of the activator in a mechanism to increase the specificity of the enzyme is a new view. It opens perspectives for understanding device procedures for allosteric regulation of ADP-Glc PPases (and other enzymes?) in the context of the metabolic scenario.

EN-P11
FUNCTIONAL CHARACTERIZATION OF PROTEINS INVOLVED IN A THIOREDOXIN SYSTEM OPERATING IN PEACH FRUIT

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Redox regulation plays a significant role in a wide range of biological processes. Thus, in order to maintain redox equilibrium, cells have developed many compartmentalized enzymatic antioxidant systems. NADPH-dependent thioredoxin reductases (NTRs) are regulatory enzymes determining the redox state of thioredoxins (TRXs): once reduced, TRX is able to reduce target proteins. We cloned the genes coding for NTR (*Ppe*NTR) and cytosolic TRX (*Ppe*TRX*h*) in peach fruit and purified the recombinant proteins. *Ppe*NTR was unable to directly reduce the chemical DTNB, but in presence of recombinant *Ppe*TRX*h* the system was fully active (with a maximum at pH 7.5). Interestingly, NADPH and NADH were used as electron donor with a similar catalytic efficiency. *Ppe*NTR showed a high catalytic efficiency for reducing the homologous *Ppe*TRX*h* but the ability to reduce the ortholog *E. coli* NTR was significantly lower. The capacity of *Ppe*NTR to catalyze the reversible transference of reduction equivalents from NADPH to oxidized TRX was utilized to determine the reduction potential of the enzyme at −296 mV. There is a thermodynamic coherence between the calculated reduction potential of *Ppe*NTR, the corresponding potential for NAD(P)H (−320 mV), and *Ppe*TRX*h* (−265 mV), which is indicative of the potential physiological operation of the system for the transference of reduction equivalents in plants.

EN-P12
EXPRESSION AND ACTIVITY OF PROTEINASES IN THE GUT OF STINK BUGS, *Nezara viridula*

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The southern green stink bug (SGSB) is a major pest of several crops world-wide, including Argentina. SGSB feeds on the seeds, reducing crop yield and seed quality. Although SGSB is well studied, little is known about their digestive physiology or how it overcomes soybean anti-herbivore defenses. Our study aimed to characterize the cysteine proteinases (CPs) responsible for digestive activity in the SGSB's gut. Insects were fed either on artificial diet or soybean pods for 24-72h and then dissected. To clone the unknown genes, total mRNA was extracted and cDNA was obtained by RT-PCR, and degenerate primers were used for PCR amplification. Fragments were multiplied on *E. coli* after vector ligation (pGEM-T). Fragments were then sequenced using an ABI PRISM DNA Sequencer. Expression of sequences was analyzed by semi-quantitative PCR. In addition, CP activity was assayed with chromogenic substrates. Cathepsins L and B obtained had high similarity to reported bug sequences. Cathepsins L showed higher specific activity when compared to cathepsins B; activity and expression were not affected by diet. However, cathepsin B activity was reduced when insects fed on pods. Our results suggest that cathepsin B-like enzymes are inhibited by soybean proteinase inhibitors. This is the first step in identifying CPs in the gut of the SGSB and unveiling its complex digestive system.

EN-P13
MOLECULAR AND PROTEOMIC ANALYSIS OF PSYCHROTOLERANT *Brevundimonas* sp. CI19

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Brevundimonas sp. Ci19 was isolated from seawater samples from sub-Antarctic marine ecosystems. In preview studies showed production of α-L-rhamnosidase with a broad range of properties that allows its application in several biotechnological processes. The aim of this work was to analyze the differential protein expression by *Brevundimonas* sp. Ci19 in the presence of rhamnose or glucose as only carbon source under cold-adapted conditions by two-dimensional polyacrylamide gel electrophoresis. Simultaneously, primers were designed for amplification of α-L-rhamnosidase sequence. For proteomic analysis, the bacteria were incubated in LBM medium containing glucose or rhamnose, during 48 h at 15°C and 200 rpm. The cells were recollected and broken using press French. The supernatants of the cell lysates were used as protein samples. Two-dimensional gel electrophoresis of the whole proteins pattern of *Brevundimonas* sp. Ci19 revealed protein differential expression by rhamnose and glucose presence. Sequencing by MALDI-TOF of the more relevant proteins suggested that different regulation schemes are involved in response to carbon source. In relation of molecular study, 542 bp were amplified with designed primers and the sequence presented 57% identity to the conserved domain pfam05592, belonging to the superfamily cl18469 of rhamnosidases.

EN-P14
THE ALLOSTERIC NATURE OF HUMAN GLYCOGEN BRANCHING ENZYME

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Glucose is the principal source of energy for most cells. In mammals and bacteria, glucose is stored as glycogen, the branched polymer formed by linear α-1,4-oligoglucan chains linked by α-1,6-glucosidic bonds. The *de novo* biosynthesis of glycogen starts with glycogenin autoglucosylation, that produces a protein-bound oligoglucan that serves as primer for other two enzymes, glycogen synthase elongating the chains, and the glycogen branching enzyme (GBE) catalyzing the cleavage of a linear segment and transferring it to the 6-position of a non-terminal glucosyl unit. GBE belongs to the Glycosil Hydrolase family 13, and has been isolated and characterized in some bacteria, rabbit and rat. In this study we report the production of human GBE (HBE) in insect cells using the baculovirus expression system, and the activity analysis of the recombinant enzyme. The branches introduced by HBE were analyzed after degradation with isoamylase. The new reducing oligosaccharides generated are subjected to fluorophore-assisted carbohydrate electrophoresis (FACE), where saccharides are derivatized at their reducing ends with a fluorophore and then separated by polyacrilamide gel electrophoresis (PAGE). Using this method we could measure HBE activity by quantifying the specific reaction products, and perform the kinetic studies that reveal the allosteric nature of this enzyme.

Lipids

LI-P01
POLYPHENOLS-MEMBRANE INTERACTION AND PROTECTION AGAINST LIPID OXIDATION. A BIOPHYSICAL STUDY

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Polyphenols are a heterogeneous group of secondary metabolites, characterized by the presence of two or more phenolic groups. The characterization of the interaction of polyphenols with membranes may provide insights on the mechanism of action of the antioxidant and potentially protective activity of polyphenols on biological membranes. The aim of the present work was to determine the membrane interaction of resveratrol, naringenin, epigallocatechin gallate and enterodiol by means of biophysical techniques in model systems. Also, the extent of lipid oxidation in the presence of polyphenols was evaluated by means of an *in-vitro* Fenton assay. Gibbs monolayer experiments indicated that polyphenols readily interacted with lipid films. Also, the exclusion surface pressure of the polyphenols in

different monolayers indicated that this interaction is dependent on the composition and phase state of the membrane. Furthermore, fluorescence anisotropy studies showed that polyphenols were able to modulate membrane viscosity depending on their composition and phase state. However, polyphenols were unable to inhibit lipid oxidation induced by Fenton reaction with the exception of resveratrol. The results presented in this work indicate that polyphenols are able to closely interact with biological membranes but the antioxidant activity is not related to their membrane affinities.

LI-P02
FATTY ACID BIOSYNTHESIS ENZYMES HAVE A CRUCIAL ROLE IN *Caenorhabditis elegans* DEVELOPMENT

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Fatty acids are structural components of cell membranes and have central roles in selective permeability, membrane fluidity and signaling. *Caenorhabditis elegans* obtains fatty acids from its bacterial diet and also synthesizes them *de novo* using two multifunctional enzymes: acetyl CoA carboxylase (ACC) and fatty acid synthase (FASI) yielding palmitic acid, which can be modified by fatty acid elongases and desaturases to form polyunsaturated fatty acids (PUFAs). In order to understand the role of fatty acid synthesis in nematode development, we performed interference experiments against ACC (pod-2) or FASI in different developmental stages (eggs, L1, L2 and L3). We observed that the development and the *de novo* synthesis of fatty acids are affected and vary according to the stage, being more important in L1. In this stage worms are arrested in their growth and PUFAs are not synthesized in spite of incorporating the necessary precursors from the diet. Moreover, experiments supplementing interfered worms with free fatty acids, *E. coli* NA22 or *B. subtilis* JH642 could not rescue the wild type phenotype or their fatty acid profiles. These results suggest the existence of an interaction between ACC and FASI with the enzymes acting downstream in the pathway.

LI-P03
CERAMIDE 1-PHOSPHATE PROMOTES INTRACELLULAR CALCIUM INCREASE AND ACROSOMAL EXOCYTOSIS IN HUMAN SPERM

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The acrosome is a membrane-limited granule that overlies the sperm nucleus. In response to physiological stimuli, sperm undergo a calcium-dependent exocytosis of this granule termed the acrosome reaction (AR). Ceramide´s role in exocytosis is still in discussion, since it´s been reported that in some systems it positively regulates membrane fusion while in others has the opposite effect. Here, by using biochemical and exocytosis assays we evaluated the role of ceramide and its metabolites in our model. Western blot analysis of sperm extracts showed the presence of enzymes of the sphingolipid metabolism such as neutral and alkaline ceramidase, and neutral sphingomyelinase. Measurements of AR by flow cytometry demonstrated that C6-ceramide induces exocytosis. Using a ceramidase inhibitor, in order to increase endogenous ceramide levels, resulted in an induction of the AR. Exocytosis and biochemical assays led us to the conclusion that probably ceramide 1-phosphate (C1P) is regulating AR after a ceramide increase. Human sperm loaded with Fluo3-AM, a fluorescent calcium sensor, responded both to C6 ceramide and C1P with a transient increase in calcium concentration whose profiles were similar to that elicited by progesterone. Further, C1P triggered exocytosis as efficiently as progesterone. Here, we identified C1P as a possible mediator of membrane fusion during acrosomal exocytosis.

LI-P04
PTERIN ENHANCE LIPID PEROXIDATION OF SOYBEAN PHOSPHATIDYLCHOLINE LIPOSOMES UNDER UVA LIGHT

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Sonicated liposomes prepared with soybean phosphatidylcholine (SoyPC) were submitted to lipid peroxidation, under air atmosphere at RT, with pterin (Ptr) as initiator. Pterins are heterocyclic compounds widespread in nature and have been previously identified as good photosensitizers under UVA irradiation. In this work, we have investigated the ability of Ptr to photosensitize the oxidation of SoyPC in aqueous solutions under UVA irradiation. Conjugated

dienes and trienes, determined by absorption at 234 and 270 nm respectively were measured as a function of time. Lipid peroxidation of SoyPC resulted in an increase in conjugated dienes production, reaching a maximum at around 50 min and then decreasing. In the absence of Ptr a significant smaller production of conjugated dienes, after a lag of 30 min, was observed. Mass spectrometry measurements were performed to identify peroxidation products. As a conclusion, steady UVA irradiation of solutions containing Ptr and SoyPC led to peroxidation of polyunsaturated fatty acids, whereas the Ptr concentration remained unchanged. Ptr showed a clearly enhanced effect on peroxidation rate and steady state concentration of oxidation products. This model constitutes a useful system to study formation of lipid peroxidation intermediaries and products in an aqueous environment to determine the effect of Ptr as photoinducers.

LI-P05
SK1 INHIBITION AS A REGULATOR OF A RENAL CELL PROLIFERATION AND β-CATENIN DISTRIBUTION

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Sphingosine Kinase (SK) is a key regulator enzyme that regulates sphingosine-1-Phosphate (S1P) synthesis, a lipid involved in several cellular processes. SK is a key regulator of sphingolipids de novo synthesis, for these reasons we evaluate the importance of SK activity in renal epithelial cell cycle modulation. For this purpose, MDCK cells were cultured at low density, to allow cell cycle progression and were treated with D,L-threo-dihydrosphingosine (DHS), a SK1 inhibitor. SK inhibition induced a decrease in cell number after 24 hs of incubation with no alteration in cell viability. It has been reported that intranuclear β-catenin translocation is involved in cell cycle modulation. So, we evaluated β-catenin distribution by inmunofluorescence. In control cells, β-catenin was distributed mainly at cell-cell contacts, whereas DHS treatement induced a β-catenin intranuclear distribution. SK1 inhibition induces an increase in de-novo ceramide synthesis. To study if β-catenin mobilization was due to a ceramide increase, we used myriocine (Myr - de novo synthesis inhibitor) that avoid Cer accumulation. Myr prevented DHS effect suggesting ceramide was involved in this process and β-catenin intranuclear distribution. In summary we propose that SK1 inhibition modulates cell cycle by mechanism that involves β-catenin intranuclear distrubution as consequence of ceramide accumulation.

LI-P06
HIGH NaCl DIFFERENTIALLY REGULATES PL AND TAG HOMEOSTASIS IN RENAL CELLS

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Renal papillary cells are surrounded by the highest osmolarities of the body due to their function in concentrating urine. In order to survive in these adverse conditions, they must have protective mechanisms. We previously showed that this kidney zone has the highest synthesis and phospholipid (PL) turnover, and that this helps to preserve membrane structure and cell viability. Fatty acids, necessary for PL synthesis, are stored as triacylglicerols (TAG) in lipid droplets. We showed that hyperosmolarity increased TAG content and synthesis in MDCK cells. In this work we evaluate which signaling pathway mediates hyperosmolarity-regulated lipid metabolism. MDCK were subjected to hyperosmolality (298-512 mOsm/kg H2O) for 48h, treated with different phospholipases (PLA2, PLD, PLC-PI and PLC-PC) or kinases (PI3K, PKC or MAPKs) inhibitors and labeled using [14C]-Glycerol. After treatments, lipids were extracted, separated by TLC and quantified. PLC-PI and PLC-PC inhibitors increased PL and TAG synthesis, whereas cPLA2 and iPLA2 inhibitors prevented hyperosmotic-induced lipid synthesis. PLD activity was only involved in PL homeostasis. MAPKs inhibitors did not affect PL and TAG synthesis. PKC and PI3K were mediating TAG, but not PL, synthesis. These data show that hyperosmolarity differentially regulates PL and TAG homeostasis in renal cells.

LI-P07
CHANGES IN MEMBRANE LIPIDS OF RAM SPERMATOZOA AFTER CRYOPRESERVATION

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Cryopreservation is known to affect spermatozoa structure and functions. Ram spermatozoa are among the most highly sensitive mammalian gametes to freezing, with frozen-thawed ram semen producing very low pregnancy rates. The aim of this study was to find, in ram spermatozoa, possible correlations between effects of cryopreservation on sperm functionality on the one hand, and quantifiable changes in lipid profile and membrane properties on the other. As expected, freeze-thawing decreased sperm quality, as indicated by post-thaw parameters related to membrane integrity and functionality, mitochondrial viability and sperm motility. Chlortetracycline staining revealed that the percentage of capacitated spermatozoa increased in cryopreserved versus control fresh semen. The most relevant lipid change after cryopreservation was a remarkable increase of lysophosphatidylcholine (LPC) and free fatty acid (FFA) levels, suggesting that a form of phospholipase A2 is activated during this process. Interestingly, in fresh spermatozoa the LPC content was very low in autumn (the reproductive period) and far higher in summer and spring time. Since LPC and FFA display inhibitory effects on sperm motility, in addition to inducing acrosomal damage, our data suggest that low LPC levels may be useful as markers of sperm quality and potential predictors of sperm suitability to cryopreservation

LI-P08
In vivo AND in vitro EVALUATION OF LIPID PEROXIDATION BY GERANIOL

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We have previously demonstrated antitumor activity and antiatherogenic properties of geraniol (G). To evaluate the anti or pro-oxidant effect of G and its relationship with those processes we studied the most sensitive oxidative damage biomarker (lipid peroxidation) in tissues obtained from mice and also in human plasma LDL. To analyze the impact of dietary G *in vivo* we implanted A549 cells in nude mice and animals were fed a control or experimental (25 , 50 or 75 mmol G/kg food) diet for 21 days after tumor implant. Liver, adipose tissue and tumors were removed from sacrificed mice. Additionally, plasma LDLs isolated from healthy human donors were preincubated with G (20-5000 μM) and oxidized with $CuSO_4$. Lipid peroxidation in mice tissues and plasma LDL was measured by TBARS assay. Our results showed that G significantly decreased lipid peroxidation in adipose tissue (20-35%) but enhanced it in tumor (300%). Nevertheless, G did not modify this parameter in mice livers. In vitro tests showed that low concentrations of G inhibited 30% of LDL lipid peroxidation whereas LDL treated with high doses showed no significant changes from control samples. These data demonstrated that G possesses anti or pro-oxidative effects depending on doses used as well as cell type. It suggests that G has a dual effect on redox balance with a great potential as cytotoxic and/or an antiatherogenic agent.

LI-P09
ROLE OF LIMONENE IN CELL PROLIFERATION, LIPID SYNTHESIS AND OXIDATIVE STRESS

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D-limonene (L) is one of the most frequent terpenes in nature and a major constituent in several citrus essential oils. Many terpenes have antitumor and hypolipidemic activity with multiple effects on mevalonate pathway (MP). Also, they have attracted interest for its potential antioxidant properties. The aim of the present work was to study the effects of L on cell proliferation (CP), MP and redox balance. CP was evaluated in human tumor cell lines (HepG2 and A549) incubated with different concentrations of L (0.05-0.4 μl/ml) by MTT and neutral red assays , and cell cycle progression was analyzed by flow cytometry. ^{14}C-acetate lipid incorporation, particularly in intermediates and products of MP, were determined by radio-TLC. Anti or pro-oxidative effect of L was studied by lipids peroxidation analyses (TBARS) in culture cells and human plasma LDL, and by spectrophotometric analyses of superoxide dismutase, catalase and glutathione-S-transferase activities. Our results showed that L inhibits CP in both cell lines. Cholesterol synthesis decreased in a dose-dependent manner but ubiquinone and some MP intermediates showed a dose-dependent increase. Total lipids synthesis decreased with the highest doses. No changes were observed in

saturated/unsaturated fatty acids ratio. Lipid peroxidation and antioxidant enzymes showed that L has anti or pro-oxidant effect depending on doses used.

LI-P10
VERSATILITY OF STEROL BIOSYNTHESIS DURING THE LIFECYCLE IN A CLOSE UNICELLULAR RELATIVE OF ANIMALS

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Sterols are essential for several physiological processes in eukaryotes. Here, we provide strong genetic and biochemical evidence about the biphasic nature of the sterols metabolism in *Capsaspora owczarzaki*, a close unicellular relative of metazoans. The life cycle of *C. owczarzaki* presents an amoeboid stage, a multicellular aggregative stage and a cystic stage, assumed as a resistance form. The *C. owczarzaki* genome shows the presence of a complete set of genes for the canonical biosynthesis pathway of ergosterol, the typical sterol of Fungi, as well as the presence of an ortholog of the Rieske sterol C7(8)-desaturase, previously described by our group in the unrelated ciliate species *Tetrahymena thermophila*. However, the sterols profile obtained by GC/MS suggests the cells are able to convert exogenous cholesterol into ergosterol by using a novel pathway. This was further confirmed by supplementation studies with radioactive precursors. Moreover, we demonstrate the lack of de novo sterol synthesis during the amoeboid and aggregative stages. Nevertheless, we were able to detect de novo produced 7-dehydrocholesterol in the cystic stage. Conversion of cholesterol into ergosterol involves the action of three enzymes from the ergosterol canonical pathway, as well as the sterol C7(8)-desaturase. These findings are supported by available transcriptomics data of the *C. owczarzaki* lifecycle.

LI-P11
THE CIRCADIAN CLOCK AND PHOSPHOLIPID METABOLISM FUNCTION IN THE GLIOBLASTOMA CELL LINE T98G

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The circadian system temporarily regulates diverse behavioral, physiological and metabolic processes and comprises central and peripheral oscillators distributed in organs, tissues and even in immortalized cell lines. The disruption of circadian clocks leads to severe metabolic disorders (hyperlipidemia, fat liver, obesity) and higher cancer risk. Our aims were to evaluate whether the immortalized human glioblastoma T98G retains the molecular clock machinery functional regulating temporally clock gene (Bmal1 and Per1) and clock controlled gene (CCG) (choline kinase (CK) alpha1) expression and the metabolic labeling of total phospholipids (PLs). To this end, T98G cells were grown in 10% FBS-supplemented DMEM for 48-72 h. After synchronization with dexamethasone (100 nM), T98G cells were maintained with or without 10% FBS-DMEM in a proliferative condition (P) or a partial arrest (PA) respectively for 48 h. Results showed that Bmal1, Per1 and the CCG CKalpha1, conserved a temporal expression under both experimental conditions (P and PA) with a period ~ 24-28 h. Also, it was found that T98G cells displayed a circadian rhythmicity in the labeling of 32P-PLs with highest levels at 20 and 48 h for both culture conditions. Results strongly suggest that the tumor cells preserve the temporal control of the molecular clock and the PL biosynthesis under the experimental conditions studied.

LI-P12
MEMBRANE FLUIDITY OF *Pseudomonas putida* AND ITS RELATION WITH THE TOLERANCE TO CATIONIC SURFACTANTS

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Adaptive response of *P. putida* to tetradecyltrimethylammonium (TTAB) involves changes in the content of phospholipids (PL) and fatty acid (FA) of the membrane. Phosphatidylglycerol (PG) and phosphatidic acid increased and cardiolipin (CL) decreased. The levels of unsaturated FA (UFA) decreased significantly while the amount of saturated FA (SFA) increased. Consequently, the SFA/UFA ratio increased 6 fold, indicating decreased the degree of fluidity of the cell of membrane. CL, one of the major PL in *P. putida*, carries highest content of UFA (18%). In order to assign a role to CL in the response to TTAB, a double mutant strain affected in the genes responsible for the synthesis of CL was obtained and characterized. Compared with the wild-type, the fluorescence polarization showed that the membrane of the mutant strain was more rigid (P=0.22), with high reduction in CL content (1.9% of total PL)

and with UFA content similar to detected in the parental strain (9.8% and 11%, respectively), although distributed mainly in PG. When the mutant strain was exposed to TTAB, the *P* value was 0.14, the UFA content increased (16.5%) and the amount of viable cells decreased from 10^{12} ufc ml^{-1} to 10^{6} ufc ml^{-1}, demonstrating that the fluidizing effect of surfactant cannot be counteracted. The set of results indicate that an adequate level of CL is indispensable in the cell's response to TTAB

LI-P13
LIPID PROFILE IN BRAIN MITOCHONDRIA DURING DEVELOPMENT: SEXUAL DIFFERENCES

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During perinatal development, testosterone (Te) released by male testes has organizational functions responsible for sexual differences in mce adult brain. As neuronal remodeling during this period depends on mitochondrial metabolism, we studied mitochondrial lipid composition during postnatal development and it relationship with Te. We used C57BL/6 pregnant dams to separate pups by sex at postnatal day 0, 2, 4, 6, 8 and 10. At each time point we obtained blood samples and mitochondrial fraction (MF) from cerebral cortex. Plasma Te levels were measured by RIA, total lipids from MF were extracted, phospholipids (PL) separated by 2D-TLC and fatty acid composition quantified by c-GLC. We found a sex-independent variation of PC, PE and CL content during the analyzed period. In particular, CL, showed a differential fatty acid (FA) profile within sex, it unsaturation index (UI) is higher in males than in females at PND 0 and 2, due to the higher content of 20:4 and 22:6. The UI correlated well with Te levels (r2=0.76). We confirm that the differences in cardiolipin FA composition were due to a difference in Te levels androgenizing females with Te propionate (100 µg of 2 mg/mL in corn oil) at PND 0. The sexual dimorphism we have found would be relevant for understanding the long-lasting deleterious effects in brain of the exposure to endocrine disruptors during development.

LI-P14
EXPRESSION OF FATTY ACID ELONGASES IN CELLS OF THE SEMINIFEROUS EPITHELIUM

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Fatty acid elongases play a crucial role in the biosynthesis of long-chain polyunsaturated fatty acids (PUFA) and in their further elongation to very long chain (VLC) PUFA. Male rat germ cell membranes contain glycerophospholipids with C18-C24 PUFA and sphingolipids with C26 to C32 PUFA. In the present study, the expression of seven members of the *Elovl* (elongation of very long chain fatty acids) gene family that encode elongases was surveyed in pachytene spermatocytes, round spermatids and Sertoli cells. The mRNAs of *Elovl1*, *Elovl2*, *Elovl4*, *Elovl5*, and *Elovl6* were detected in all of these cells , all of them lacked *Elovl3* expression, and *Elovl7* was expressed only in the latter. As the ELOVL4 protein was previously shown to be responsible for the elongation of >C24 PUFA in the retina, the expression of this protein was also evaluated. During postnatal development, ELOVL4 was not detectable in testis up to P21, i.e., its time of appearance concurred with that of the first spermatocytes. Thereafter, the protein was evidently present in spermatocytes and spermatids, and was also faintly detected in Sertoli cells. As *Elovl2* and *Elovl5* are essential to form PUFA, and *Elovl4* is required to elongate them, the joint expression of these elongases in spermatocytes and spermatids implies that they are functionally related, probably acting in sequence to produce the VLCPUFA of sphingolipids. This work was partially supported by Fondecyt 1140758(JRG).

LI-P15
A53T α-SYN REGULATES TRIACYLGLYCEROL CONTENT IN DOPAMINERGIC NEURONS EXPOSED TO OXIDATIVE STRESS

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Iron-induced oxidative stress and pathological α-synuclein (α-syn) aggregation contribute to the loss of dopaminergic neurons in Parkinson Disease (PD). In this work, we characterized the status of lipid metabolism in N27 dopaminergic neurons and in neurons stably expressing A53T α-syn (a dominant mutation found in familial early onset PD) exposed to iron-induced injury. N27 dopaminergic neurons incubated with iron for 24 hs (Fe, 1mM) displayed increased levels of reactive oxygen species (ROS), lipid peroxidation and elevated plasma membrane

permeability. A different lipid acylation profile was observed in fatty acid prelabeled N27 neurons exposed to Fe. [3H] arachidonic acid (AA) uptake was increased in triacylglycerols (TAG) whereas the incorporation of [3H] oleic acid into TAG showed no changes. AA incorporation increased in phosphatidylcholine and diminished in phosphatidylserine and phosphatidylinositol. Coincidently, TAG content was 40 % higher in Fe-exposed neurons than in controls. The accumulation of TAG was also observed by the appearance of Nile red positive lipid bodies. On the contrary, A53T α-syn neurons exposed to iron-injury showed no increase in TAG levels and diminished ROS content. Our results suggest that TAG accumulation could be a mechanism for AA storage and that α-syn could act as an iron scavenger during oxidative stress in dopaminergic neurons.

LI-P16
THE ORIGIN OF METAZOAN LIPOATE METABOLISM CAN BE TRACED BACK TO HOLOZOAN PROTISTS

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As cofactor in the glycine cleavage system and in several oxoacid dehydrogenases, lipoate is an essential component for the cell. There are multiple strategies in Nature to assure its acquisition. We have explored the presence of the enzymes involved in lipoate synthesis and/or salvage in the protozoan Capsaspora owczarzaki, a symbiont of Biomphalaria snails (vector of the human parasites Schistosoma spp.). Capsaspora was recently highlighted as a model organism to study the origin of multicellularity in Metazoan. Phylogenetic analysis carried out on these enzymes and those from other interrelated pathways, like Krebs cycle and mitochondrial fatty acid synthase system, are all in concordance with the closeness relationship of Capsaspora and other holozoan organisms, like choanoflagellates and metazoans and with a more distant relationship to fungi, together to which they share the Opistokonta. The fact that humans and Capsaspora share similar pathways for lipoic acid acquisition makes this protozoan model very attractive in the study of metabolic defects associated to severe clinical traits in humans.

LI-P17
EUCALYPTOL AND LINALOOL: MECHANISMS UNDERLYING THEIR ANTIPROLIFERATIVE EFFECTS ON HUMAN TUMOR CELLS

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Eucalyptol (Eu) and linalool (Ln) are natural isoprenoids with multiple effects on the mevalonate pathway (MP), a branched pathway essential for cholesterol synthesis and cell proliferation. In previous studies we demonstrated that Eu and Ln were capable of inhibiting cell proliferation in human liver (HepG2) and lung (A549) carcinoma cells. We herein studied the mechanisms involved in those inhibitory effects. Eu and Ln impaired HMG-CoA reductase (HMGCR, the rate-limiting enzyme in the MP) levels , however, the addition of exogenous mevalonate (the HMGCR product) was unable to restore cell growth. Cell cycle analysis showed a G0/G1 arrest produced by Eu and Ln in HepG2 and A549 cells. IC50 of Ln induced apoptosis in HepG2 cells as determined by caspase-3 activity and TUNEL assays, whereas at higher concentrations Eu and Ln triggered apoptosis in both cell types. Ras translocation to the membrane was inhibited by Ln without altering total Ras levels. Our results suggest that in our conditions Eu and Ln at their IC50 exert antitumor activity by inhibition of cell cycle progression in both cell types, meanwhile Ln is also able to induce apoptosis in HepG2 cells. HMGCR inhibition alone is not responsible for the antiproliferative activity of Eu and Ln. The inhibition of Ras translocation to the membrane could be one of the reasons for cell cycle arrest and apoptosis induction.

LI-P18
SYNERGISM OF *Lippia alba* ESSENTIAL OILS WITH STATINS IN ANTIPROLIFERATIVE AND HYPOLIPOGENIC EFFECTS

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Lippia alba (Miller) N.E. Brown is an aromatic shrub which has many chemotypes (CHMs) used in folk medicine. Its essential oil (EO) has a large number and variety of monoterpenes (Mts). Mts have shown to inhibit tumor cell proliferation (CP). This event as well as potential lipid lowering effect is associated with the action exerted by Mts in

different lipid metabolic pathways. Statins, as simvastatin (SV), are HMG-CoA reductase (HMGCR) inhibitors used primarily for hyperlipidemia, but have also been studied as anticancer agents. In the present work we studied the antiproliferative and hypolipogenic action of the combination of SV and *L. alba* EO (LEO) in HepG2 and A549 cells. CP was evaluated using MTT and neutral red assays , cells were treated with LEOs of four CHMs alone or combined with SV. All LEO-SV associations induced synergistic CP inhibition rather than treatments alone. For tagetenone LEO (TLEO)-SV combination, lipid composition and incorporation of ^{14}C-acetate into total, unsaponifiable and phospholipids (PL) were evaluated. The amount of HMGCR was also quantified by western-blot. TLEO-SV increases HMCR quantity, decreases cholesterol, PL and triglycerids synthesis and lipid content, instead of each drug separately. Our results suggest that LEO-SV synergism may be used as a novel cancer therapeutic regimen and as a more effective dyslipidemia control than statins alone.

LI-P19
METABOLIC LINK BETWEEN LIPID SYNTHESIS AND LIPID DROPLETS MORPHOLOGY INDUCED BY OLEIC ACID

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Nuclear lipid droplets (nLD) are a new nuclear (N) domain where neutral lipids are stored and organized. These droplets would be built up around a hydrophobic core of TAG and CE enriched in oleic acid and surrounded by a monolayer of polar lipids along with C (cholesterol) and associated proteins. nLD could be involved in nuclear-lipid homeostasis and provide or incorporate lipids and proteins involved in signaling pathways and transcription factors. The aim of the present work was to determine if nLD reversibly respond to external stimuli. The addition of oleic acid (OA) to HepG2 cells or primary culture of rat hepatocytes determined cellular shape modification, thus increasing TAG and CE content and cLD and nLD (number + size) , LD increments would be reverted if OA was excluded. cLD and nLD induction by OA was prevented by ACS (acyl-Co-A synthetase) inhibitor (TriacsinC) involved in lipid biosynthesis. nLD and cLD were stained with BODIPY and N with DAPI.
In conclusion, 1) nLD are a dynamic nuclear domain, 2) nLD number, size and lipid content are reversibly induced by OA, 3) nLD and cLD have a coordinated metabolism with a cellular regulation, 4) nLD and cLD are induced by OA by a reversible mechanism that involves ACS activity and TAG and CE biosynthesis.

LI-P20
INDOMETHACIN EFFECT ON BMMC MIGRATION: PROSTAGLANDIN SYNTHESIS INHIBITION OR PPARγ ACTIVATION?

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We have previously described the reorganization of major myelin and axonal proteins during sciatic nerve demyelination, as well as the migration of bone marrow mononuclear cells (BMMC) exclusively to the injured nerve. Once in the ipsilateral nerve, some BMMC colocalize with Schwann cell and nerve fiber markers. In this context, BMMC accelerate the degeneration process and, as a consequence, promote the onset of regeneration. The aim of the present work was to determine whether lesion-associated inflammation is involved in cell recruitment. To that end, animals subjected to sciatic nerve crush and transplanted or not with BMMC, were treated with indomethacin or vehicle and sacrificed at different survival times. Then BMMC migration, COX expression and prostaglandin (PGs) biosynthesis were evaluated. Results showed an injury-mediated induction of COX-2 expression. Treatment with indomethacin blocked BMMC migration but did not inhibit PG synthesis as evidenced by an increase in PGJ2. In the light of these results we can suggest that indomethacin action on BMMC migration occurs through an independent PG-mediated mechanism such as the PPARγ pathway. Further experiments are necessary to elucidate indomethacin effect on BMMC migration.

LI-P21
HEAT STRESS AFFECTS THE METABOLISM OF LIPIDS OF EPIDIDYMAL EPITHELIAL CELLS

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Frequent exposures of testes to hyperthermia are known to result in germ cell death, and, although not lethal to Sertoli cells, we previously showed that such exposures damage them structurally and functionally. Here we studied the

consequences of heat stress on the epididymis by assessing its effects on epithelial cells from its three regions (caput, corpus, and cauda) in primary culture. All of them had abundant lipid droplets (LD) rich in triacylglycerols (TAG), similar in size and numbers in vivo and in culture. Temperature-treated (a 15 min exposure once a day to 43ºC for 5 days) and control cells were incubated for 1 h with [3H]20:4 on the 5th day. In the three regions, TAG were faintly labeled, most of the fatty acid being taken up by phospholipids. Caput-derived cells exhibited the highest rate of lipid labeling. Due to heat exposures, caput and corpus cells showed a significant increase in the label from [3H]20:4 in diacylglycerols (DAG), concomitant with a decrease in the label in phosphatidylinositol (PI), phospatidylethanolamine phosphatidylglycerol (PE-PG), and little change in phosphatidylcholine (PC). Quite the opposite occurred in cells from cauda: the label in DAG decreased, while that in PI, PE-PG and PC increased. Our results suggest that epididymal cells are differentially susceptible to short but periodical exposures to mild hyperthermia

LI-P22
SPHINGOMYELINS WITH VERY LONG CHAIN PUFA ARE EXCLUDED FROM RAFT MEMBRANE DOMAINS IN MALE GERM CELLS

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The synthesis of sphingomyelins (SM) and ceramides (Cer) with nonhydroxy and 2-hydroxy very long chain PUFA (n-V and h-V) is a trait of differentiation in male rat germ cells. Here we evaluated how these lipids distribute among cell membranes as differentiation proceeds from pachytene spermatocytes to round and late spermatids, in relation to glycerophospholipids (GPL) and cholesterol. A small light (L) and a larger heavy (H) fraction, both derived from the plasma membrane, and a bulky fraction containing intracellular membranes were obtained. In the three cell stages, the L fraction concentrated Flotillin-1, indicating the existence of rafts membrane domains. This fraction had relatively more SM and more cholesterol than the H fraction in spermatocytes, and also more SM, but less cholesterol, in the two spermatids. In the three cases, the SM of L was rich in saturated fatty acids (SFA), whereas that of H contained virtually all the n-V and h-V of the plasma membrane. The GPL of L and H contained SFA and PUFA, respectively. The intracellular membrane fraction of the three cells contained most of the n-V and h-V Cer species of the cells, likely precursors of the SMs that in due course are incorporated in their plasma membranes. Thus, the global lipid composition and the distribution of lipids among cell membranes are significantly transformed during male germ cell differentiation.

LI-P23
GPAT2 SILENCING ALTERS ARACHIDONATE DISTRIBUTION IN MDA-MB-231 CELLS

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Glycerol-3-phosphate acyltransferases (GPATs) catalyze the first step in the de novo glycerolipid biosynthesis. Although mouse GPAT2 gene was first cloned by sequence homology to GPAT1, we have demonstrated that GPAT2 is notably different to the other GPATs. In order to elucidate its role in lipid metabolism, we explored the arachidonate (AA) distribution among membrane phospholipids in MDA-MB-231 control (scr-MDA) versus GPAT2 stably knocked down (sh-MDA) cells. Fatty acid composition of phospholipids (PL) and triacylglycerols (TG) was evaluated by GLC after a 3-day- incubation with 50 μM AA; AA content in PL and TG of sh-MDA cells was increased (13.6% vs. 6.8% and 13% vs. 3 % respectively). Endogenous AA-containing PL species were measured by LC/MS/MS. sh-MDA cells showed higher amounts of most AA-containing PC and PE species and lower amounts of (18:1/20:4)PI and (18:2/20:4)PI compared to scr-MDA cells. 2[H]-AA incubation for 30 min showed that sh-MDA cells incorporated higher amounts of AA in PC species and lower amounts in (18:0/ 2H 20:4)-PI and (18:1/2H20:4)-PI than scr-MDA cells. Phospholipase A specific activity was also measured and it was 50% lower in sh-MDA than in scr-MDA cells. In conclusion, GPAT2 expression in MDA-MB-231 cells diminished AA content in PC and PE and incorporation of exogenous AA in PC. Results presented here suggest a novel enzymatic activity for GPAT2.

Microbiology

MI-P01
A YEAST VPS SCREENING TO IDENTIFY *Brucella* PROTEINS THAT INTERFERE WITH MEMBRANE TRAFFICKING

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Brucella deliver effector proteins into host cells to interfere with cellular processes and promote infection. Identification and characterization of such proteins has been difficult, often limited by the lack of detectable signal sequences and functional redundancy. In this work, a pathogen effector protein screening in yeast was carried out identifying three *B. abortus* DNA fragments that produced vacuole protein sorting (VPS) defects in yeast. Cloning of the complete genes codified by these fragments independently demonstrated that *htpX* was responsible for the VPS defects in yeast. HtpX is a zinc metalloprotease homologue to *S. cerevisiae* STE24 involved in NH2- and COOH-terminal CAAX processing events of a-factor maturation. An *htpX* deletion mutant in *B. abortus* was constructed, showing a reduce virulence in mice in the chronic infection phase. Overexpression of HtpX in HeLa cells demonstrated that HtpX target the endoplasmic reticulum. Additionally, expression of a Myc-tagged HtpX protein in HEK293T cells led to a significant decrease in the secretion of the reporter protein embryonic alkaline phosphatase indicating that HtpX, as in yeast, also interferes with the host secretory pathway. The use of the VPS yeast screening allowed us to identify HtpX as a potential new Brucella effector protein that interferes with host cell membrane trafficking

MI-P02
VirJ: AN ACCESSORY PROTEIN OF TYPE-IV SECRETION SYSTEM INVOLVED IN PATHOGENESIS OF *Brucella abortus*

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Brucella abortus is a Gram-negative intracellular bacterium, responsible for brucellosis, a worldwide zoonosis of veterinary and human concern. Virulence of this pathogen is associated with the ability to survive and replicate within professional and nonprofessional phagocytes, avoid the fusion of the vacuole that contains it with the lysosomes, and redirect its traffic in order to generate a replicative niche where it will exponentially multiply. Many of these activities are completely dependent on the VirB system, a Type-IV secretion system that secretes and translocates into the host cell effector proteins that reprogram the fate of the *Brucella*-containing vacuole and allow the establishment of the replicative niche. Analysis of the genome of *Brucella abortus* allowed us to identify a homolog of the *virJ* gene of *Agrobacterium tumefaciens* that encodes a periplasmic protein necessary for the secretion of substrates of the *A. tumefaciens* VirB system. Our results show that the absence of this protein in *B. abortus* produces a negative effect in the intracellular cycle of the bacteria and negatively affects virulence in mice. We show that VirJ interacts in a direct or indirect manner with effector proteins, stimulating the VirB dependent secretion process

MI-P03
IDENTIFICATION OF HUMAN ALPHA–ENOLASE AS AN INTERACTION PARTNER OF A *Brucella abortus* VirB SUBSTRATE

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Brucella abortus, the causative agent of bovine brucellosis, invades and replicates within cells inside a membrane–bound compartment known as the *Brucella* Containing Vacuole (BCV). *Brucella* Type IV Secretion System (VirB) translocates effector proteins across the BCV thought to modulate host cell signaling pathways to favor intracellular survival and replication. BPE123 is a VirB–translocated effector protein recently identified. It is a hypothetical protein whose function remains unknown. In an attempt to identify host cell proteins interacting with BPE123, a pull–down assay was performed and human alpha–enolase (ENO1) was identified by LC/MS-MS as a potential interaction partner of BPE123. These results were confirmed by immunoprecipitation. Microscopy studies revealed ENO1

relocalization upon ectopic expression of BPE123 in HeLa cells, where both proteins localized to the ER. Furthermore, ENO1 is recruited to the vicinities of BPE123 positive BCVs in infected macrophages, indicating that interaction with translocated BPE123 might also be occurring during the intracellular phase of *B. abortus*. These preliminary results suggest a direct interaction between BPE123 and ENO1, a multifunctional protein that is required for *Brucella* replication in host cells. Further experiments are underway to determine how BPE123–ENO1 interaction modulates the outcome of the infection process.

MI-P04
MAPA, A PROTEIN INVOLVED IN THE ENVELOPE BIOGENESIS OF *Brucella suis*

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Brucellae have a cell surface with distinctive properties, which make them more resistant to polycationic peptides and more permeable to hydrophobic compounds. It has been proposed that this would reflect an adaptation to intracellular parasitism. We have identified a gene of *B. suis* encoding a protein (MapA) predicted to localize in the cell envelope. The Δ*mapA* mutant did not show differences compared to the parental strain regarding: i) outer membrane protein profiles, ii) growth curves in rich medium or under saline stress and iii) tolerance to acidic pH and complement present in porcine serum. Instead, the mutant showed an enhanced sensitivity to Triton X-100, Polymyxin B (a cationic lipopeptide) and Lysozyme. Observation of the phenotype of Δ*mapA* with acriflavine and LPS analysis by SDS-PAGE and silver staining suggest that the O-antigen portion of the LPS is not altered in the mutant. Interestingly, the mutant colonies in the presence of crystal violet showed a differential staining compared with the wild type. In addition, the Δ*mapA* mutant was impaired in the binding to HeLa cells probably due to an altered cell surface. These results suggest that MapA plays a role in the integrity of the *Brucella* cell envelope. Since it is clear that a defective envelope could be linked to a reduced virulence, we are currently evaluating the virulence of the mutant using the murine model

MI-P05
ROLE OF *Brucella suis* ADHESINS (BmaA AND BmaB) IN THE INITIAL INTERACTION WITH THE HOST CELL

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Brucella spp. is an intracellular pathogen responsible for brucellosis, a zoonotic disease. Adhesion of brucellae to host surfaces was proposed to be a critical step in the infection process. *Brucella* genomes are very similar between species; however, it seems that much of the variability is associated to surface proteins, suggesting that they could contribute to host preference and tissue tropism. Previously, we have identified by phage display a fibronectin binding protein (BmaC) of *B. suis* that belongs to the type I monomeric autotransporter (ATI) family. BmaC was required for the binding of *Brucella* to epithelial cells. Through phylogenetic analysis we identified in the genome of *B. suis* 1330 two additional members of the ATI, BmaA and BmaB, which showed putative adhesion motifs. The aim of this work is to explore the role of BmaA and BmaB in the interaction with the host as well as the presence and variability of their orthologs in different species/strains. Our results show that *bmaA* and *bmaB* became pseudogenes in some strains of *B. melitensis* and *B. abortus*. An heterologous approach revealed that they confer adhesive properties to a non-adherent *E.coli* strain. Besides, mutants in the ATIs showed a significant reduction in the adherence to different model cells, suggesting that they may contribute to an efficient interaction with host cells before internalization.

MI-P06
LACTIC ACID BACTERIA VIRULENCE ASSESSMENT: IN SILICO PREDICTIONS VS *G. mellonella* IN VIVO ASSAYS

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Identification of virulence proteins in sequenced genomes is useful to estimate their pathogenic potential. Nevertheless, mechanisms underlying pathogenesis are rather complex. To check the performance of existing prediction systems on lactic acid bacteria, we analyzed available genomes of representative strains found in

fermented foods, including our own sequenced strains and compared bioinformatic findings with in vivo results using *G. mellonella*. Prediction systems mvirDB and MP3 indicated that all the studied strains would have a large number of virulence factors (VF), whereas Virulence Finder only reported VF in *E. faecalis*. On the other hand Pathogen Finder predicted *E. faecalis* and *E. mundtii* as human pathogens. Infection of *G. mellonella* with *E. faecalis* led to a high mortality rate, *E. mundtii* caused an intermediate mortality, whereas *L. lactis* and *Lb. casei* hardly appeared to be lethal. Furthermore, we determined the contribution of citrate cluster to *E. faecalis* pathogenesis. Infection of larvae with citrate lyase or oxaloacetate decarboxylase deficient strains caused none or low mortality, suggesting that WT induced mortality could be dependent on the presence of an active citrate metabolism. In conclusion, diversity of results thrown by the available prediction software makes the use of experimental in vivo systems a convenient tool to reinforce or discard such results.

MI-P07
THE PHOP/PHOQ SYSTEM AND *MGTES* GENES ROLE IN *Serratia marcescens* PATHOGENESIS

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The *S. marcescens* PhoP/PhoQ system is involved in the adaptation of this bacterium to growth on scarce environmental Mg^{2+}, at acidic pH, and in the presence of polymyxin B. As we previously reported, there are two *mgtE* orthologs in *Serratia* that present a conserved PhoP-binding motif. *mgtE1* expression had been shown to be PhoP dependent and is required for optimal growth under Mg*2+* limiting conditions. To asses *mgtE2* transcriptional regulation we constructed *lacZ*- and *gfp*-containing reporter plasmids. Regarding to their role in pathogenesis, we determined that a *phoP* mutant strain is defective in survival inside epithelial cells and that the PhoP/PhoQ system is involved in prevention of the delivery to degradative/acidic compartments. In *D. melanogaster*, a *phoP* mutant strain was unable to kill A5001 and *Eater Drosophila* mutants in a septic injury model, but regained full virulence in a *Key* mutant background. However, the *phoP* mutant was capable of damaging the insect gut in the early phase of the oral infection model. In CHO cells, the *mgtEs* mutants were unable to survive at late times p.i. and co-localized with acidic compartments. Based on these findings, we propose that a) the PhoP/PhoQ system is involved in the resistance to AMPs in *D. melanogaster*, and b) the *mgtEs* genes could be involved in preventing the delivery to degradative/acidic compartments.

MI-P08
Serratia marcescens INVASION IN EPITHELIAL CELLS: MORE THAN ONE BACTERIUM PER CHO CELL?

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More than one *Serratia marcescens* (*S.m.*) are often seen inside CHO cells at short times p.i. (60 min) suggesting that two or more bacteria could simultaneously enter one CHO cell. Thus, we conducted invasion experiments combining two strains of wild type (wt) *S.m.* expressing GFP or mCherry. We compared invasion rates of these strains separately and in a 1:1 mix by fluorescence microscopy and measuring C.F.U.s. We observed the presence of both *S.m.*/GFP and *S.m.*/mCherry inside one CHO cell indicating that more than one bacterium is able to invade the same epithelial cell either in a cooperative or an independent uptake event. Moreover, percentages of invasion were similar for both strains alone ($p›0.05$) as well as in co-infection experiments where they resulted half of the total for each strain ($p›0.05$). Using this attribute, and having previously established that the flagellum is required for an initial bacterial attachment to the epithelial cell, we attempted to rescue *S.m. flhD* mutant defective in flagella expression and to infect CHO cells. The rates of invasion of *S.m. flhD* and wt were not modified by the presence of the other strain suggesting that the simultaneous entry of bacteria is not cooperative and the flagellar structure attached to the envelope of each invading bacterium is required to exert an anchoring function preceding the internalization process to the host cell.

MI-P09
STABILITY OF THE POINT MUTATION OF THE *Salmonella thyphimurium rcsC11* ATTENUATED STRAIN

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The foodborne diseases are usually caused by *Escherichia coli*, *Shigella*, *Listeria* and *Salmonella*, which is the most prevalent bacterium in Argentina. The *Salmonella* infection involves many steps where the modulation of genes located in different pathogenesis islands are coordinately controlled. The RcsCDB system of *Salmonella* controls the colanic acid, flagellar and biofilm synthesis, as well as others virulence processes. Previously, we have studied the *rcsC11* mutant harboring a point mutation in the histidine kinase domain of RcsC sensor. This change produces the RcsCDB system constitutive activation, resulting in a mutant with mucoid phenotype and attenuation of virulence. Here, we evaluated the ability the *rcsC11* mutant to infect non-phagocytic and phagocytes eukaryotic cells. We also investigated the reversion degree of the *rcsC11* mutant to wild-type state by loss of the mucoid phenotype, by means of temperature changes, crop aging and chemical mutagenic agent challenges. We found that the *rcsC11* mutant produced strong immunogenic response and it was unable to replicate in macrophages. Moreover, no revertant strains were isolated in any of the treatment analyzed, strongly suggesting that the *rcsC11* mutation would be fairly stable. The results of this study represent a significant contribution that supports the use of the *rcsC11* mutant as candidate for vaccines development.

MI-P10
Lactobacillus reuteri CRL1098 SOLUBLE FACTORS EXERT *IN VITRO* AND *IN VIVO* ANTI–INFLAMMATORY EFFECTS

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Soluble factors produced by probiotic bacteria such as lactobacilli can modulate immune system responses. The aims of this study were to determine whether *Lactobacillus reuteri* CRL1098 soluble factors (LrS) were able to modulate *in vitro* the inflammatory response triggered by LPS in murine macrophages (RAW 264.7) and to evaluate *in vivo* their capacity to exert anti–inflammatory actions in acute lung injury (ALI) induced by LPS in mice. *In vitro* assays demonstrated that LrS significantly reduced the production of pro–inflammatory mediators (NO,COX–2,Hsp70) and pro–inflammatory cytokines (TNF–α, IL–6) caused by the stimulation of macrophages with LPS. Results from flow cytometry assays revealed that LrS reduced apoptosis of LPS–challenged RAW cells. Bax expression was reduced while no significant difference was observed in Bcl–2 expression levels. In addition, the effect of LrS on NF–κB pathway was evaluated by immunofluorescence microscopy: inhibition of NF–κB translocation to the nucleus in LrS–treated macrophages exposed to LPS was detected, as well as a reduction of Akt and increase of ERK phosphorylation. *In vivo* assays proved that the LPS–induced secretion of the pro–inflammatory cytokines, inflammatory cells recruitment to the airways and inflammatory lung tissue damage were reduced in LrS treated mice, providing a new way to reduce strong pulmonary inflammation.

MI-P11
CHOLINE CONTRIBUTES TO INCREASE SOME PATHOGENIC FACTORS IN DIFFERENT *Pseudomonas syringae* STRAINS

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P. syringae infects a wide variety of plants and causes necrotic symptoms in leaves, stems, and fruit. It is considered a hemibiotrophic pathogen because it is able to obtain nutrients from living host cells in order to multiply in the apoplast and infect close tissues. Choline (Cho), an alkylammoniun compound, is a normal constituent of plant tissues and in the apoplast is found as a component of phosphorylcholine (Pcho). *P. syringae pv. tomato*, *P. syringae pv. tabaci*, and a local strain that we isolated and named S5, use Cho as nitrogen source. These strains produce the enzyme phosphorycholine phosphatase (PchP), capable to generate free Cho from Pcho. In S5, Cho increases at least two virulence factors: tabtoxin and swarming mobility. S5 was isolated from oat leaves from a south field of Córdoba, and the strain was characterized in our laboratory by bioinformatic, microbiology, biochemical and molecular approaches. By the analysis of *ARN16S*, S5 was classified as *P. syringae pv. atropurpurea*. The BOX fingerprint of S5 resulted similar to those of *P. syringae* isolated from different host plants of our area. We conclude that S5 is a potentially dangerous bacterium, able to obtain nutrients from host and enhance the production of

virulence factors; it is widely spread in nature, and is capable to cause infection in different plants of economic importance as peanut and soybean.

MI-P12
IDENTIFICATION OF *Chlamydia trachomatis* MUTANTS DEFECTIVE FOR PERSISTENCE

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Chlamydia trachomatis (CT) is a leading cause of genital and ocular diseases worldwide. Upon exposure to antimicrobial compounds such as interferon-gamma (IFNg) or beta-lactams, CT transitions into a latent state called persistence. In this state, CT can evade immune detection for long periods of time and transition back into an infectious form once antimicrobial compounds have been removed. Chlamydial persistence has been linked to the occurrence of recurrent and chronic infections in infected individuals. Due to a limited array of molecular genetic tools for routine genetic manipulations in *Chlamydia*, bacterial factors regulating chlamydial persistence remain poorly elucidated. Taking advantage of a collection of ~800 chemically mutagenized CT mutant strains, we carried out a high throughput screen and identified 8 mutants defective in recovering from IFNg- and/or penicillin-induced persistence. By whole genome sequencing, we find that 4 of these strains harbor mutations (including truncations) in genes coding for polymorphic membrane proteins (PMPs). PMPs are a family of Chlamydia-specific autotransporter-like proteins linked to adhesion and antigenic variation. We are currently backcrossing these mutant strains into a wild type background and utilizing recombinant strains to assess whether PMP gene products are linked to persistence.

MI-P13
FIRST STUDY OF *scpB* GENE OF *Streptococcus agalactiae* IN MISIONES, ARGENTINA

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Streptococcus agalactiae (GBS) is the leading cause of neonatal infections. The colonization of the genital tract of pregnant women at term gestational age is significantly associated with these infections. Infants acquire GBS in uterus via ascending through intact membrane rupture or during the birth process. GBS presents different surface proteins of antigenic characteristics, such as α, β, Rib, HylB, Lmb and C5a peptidase. These proteins are encoded by several genes associated with virulence and host interaction acting on bacteria involved in invasiveness. The study of these surface protein antigens is important for the understanding of the pathogenesis and epidemiology of infection. Several of these antigens have been proposed as components of multivalent conjugate vaccines. In this study we investigated the presence of *cspB* gene encoding the C5a peptidase. Recent studies indicate that C5a peptidase vaccine formulations cause a long-term immune response and prevent GBS infection. In this work, two hundred strains of GBS were studied. All strains were recovered from vaginal and rectal swabs from pregnant women with 35-37 weeks of gestation. The search *scpB* gene was performed by conventional PCR. The presence of the *cspB* gene was detected in 100% of GBS strains studied. Our findings highlight the importance of including C5a peptidase in the design of a regional vaccine.

MI-P14
SYNERGISTIC MECHANISM BETWEEN *INFLUENZAA* VIRUS AND *Streptococcus pneumoniae*

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InfluenzaA virus (IAV) and *Streptococcus pneumoniae* (*S. pneumoniae*) are considered as two of the most important human pathogens, and co-infections with both agents usually lead to severe respiratory disease and occasionally, death. We previously described that *S. pneumoniae* remains alive intracellularly for several hours in an endothelial cell model. Here, we have established a co-infection model to analyze the synergism between IAV and *S. pneumoniae* during the first hours of viral infection. We found that IAV favors *S. pneumoniae* uptake in a dose dependent manner. In addition, prior viral infection significantly increases bacterial survival. We focused our study in signaling mechanisms that would allow pneumococcus to sense environmental changes produced by IAV that favors its intracellular survival. We found that the histidine kinase VisR, which belong to the uncharacterized two-

component system VisR (named Vis by viral infection sensing), is essential for increased pneumococcal survival in IAV-infected endothelial cells, since its absence abolished viral effect over bacteria, in the same way as if the cells were not virus infected. This is the first report of a two-component system that sense environmental changes in the virus-infected cells, and this model could be useful to understand the synergistic mechanism between both pathogens.

MI-P15
JUNIN VIRUS ENTRY IN CELLS THAT EXPRESS HDC-SIGN

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Virus entry and disemination can be mediated by c-type lectines as DC-SIGN, which is a member of a family of receptor that recognizes carbohidrates structures. The main goal of this study is to provide more evidence of the interaction between hDC-SIGN and Junin virus (JUNV), which has higly glicosilated proteins, and determine the role of cytoskeleton in the entry pathway. NHI3T3 and NIH3T3-hDC-SIGN cells were infected in presence or absence of EGTA or the carbohydrate binding agent Griffithsin (GRFT). Both compounds inhibited nucleoprotein expression and viral yield in cultures that express the human lectin. We showed in previous work that JUNV DC-SIGN-mediated entry depends on clathrin and cholesterol. As many entry pathways are dependant of cell cytoskeleton, we studied the role of microfilaments and microtubules during early virus infection. Inhibition of nucleoproteins expression and viral yield were observed when cells were pretreated and infected in presence of latrunculinA which disrupts cortical and cytoplasmic microfilament. In contrast, cytochalasin D, that affects cytoplasmic microfilaments or drugs that depolymerize microtubules did not show effect on JUNV multiplication or nucleoprotein expression. Taken together our results demonstrate that JUNV interacts with hDC-SIGN and that JUNV .

MI-P16
TACARIBE ARENAVIRUS USES MACROPINOCYTOSIS AS AN ALTERNATIVE ENTRY PATHWAY

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Tacaribe virus (TCRV) is a nonpathogenic member of the New World (NW) arenavirus family where pathogenic viruses such as Junin virus are found. NW arenaviruses utilize the transferrin receptor 1 (TfR1) from their natural host but pathogenic virus also use the human one. The main goal of this work is to characterize the alternative TCRV entry pathway and the role of the hTfR in it. We use CHO, TRVb (which luck endogenous TfR1) and TRVb1 cells (which stably express hTfR1) and treated with different compounds: cytochalasin D (cyt D) and latrunculin A (lat A), which collapse the actin filament; wortmannin (wort), a PI3K inhibitor and amiloride (amil), an inhibitor of the Na+/H+-exchanger. Wild type (wt) and dominant negative (dn) construction of Rab5 and Rab7 (early and late endosome marker respectably) were also used. After treatment with the drugs or transfections, cells were infected with TCRV. We quantify viral production and percentage of infected cells. In CHO, TRVb and TRVb1 cells, cyt D and lat A inhibit both parameters (70-90%) but wort and amil only reduce these parameters in TRVb1 cells (50-60%). In regard to Rab 5 and Rab7 dn, a reduction on TCRV infection was found only in TRVb and TRVb1 cells (50-70%). These results allow us to postulate that TCRV may use the macropinocytosis pathway to enter in TRVb1 cells and this could be a secondary pathway use in CHO and TRVb cells.

MI-P17
COUPLED EUKARYOTIC EXPRESSION AND BIOTINYLATION OF FUNCTIONAL PESTIVIRUS E2 ENVELOPE GLYCOPROTEIN

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Bovine viral diarrhea virus (BVDV) is a cattle pathogen belonging to the genus pestivirus within the family *Flaviviridae*, which causes a significant impact on the livestock industry worldwide. The virion has an outer lipid envelope bearing glycoproteins Erns, E1 and E2. Entry of BVDV into the host cell requires the interaction of the virus with a set of yet unidentified receptors and co-receptors. E2 mediates virus-receptor interactions and is the major antigen inducing neutralizing antibodies in an infected host. Thus, E2 is considered as an ideal target to be used in subunit vaccines. We developed an eukaryotic expression system that allows for the production of a soluble version of biotinylated-E2. Recombinant E2 is biotinylated in eukaryotic cells by co-expressing E2 fused to the 15 amino acid biotin acceptor peptide (BAP) and the bacterial biotin-protein ligase BirA, which specifically recognizes and attaches a biotin moiety to the single lysine residue of BAP. We demonstrate that soluble biotinylated-E2 is able

to inhibit BVDV-induced cytopathic effect in cell culture, indicating that the recombinant protein is functional. We will discuss biotechnological applications of biotinylated-E2 in the design of subunit vaccines to differentiate infected from vaccinated animals, and in the identification of cell surface receptors for BVDV entry in immunoprecipitation assays.

MI-P18
A DIGUANILATE CYCLASE PROTEIN FROM *Bordetella bronchiseptica* HAS A ROLE IN BIOFILM FORMATION ON LUNG

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Bordetella bronchiseptica is a pathogenic bacterium that causes respiratory infections in a wide variety of host. In a previous work we described c-di-GMP second messenger role in motility and biofilm formation through overexpression of a diguanilate cyclase (BB3576). In this work, we are reporting phenotypic analysis of a BB3576 deletion mutant. C-di-GMP quantification confirmed DGC functionality (0,1977± 0,09 nM/mg in BbΔBB3576 vs 1,1706 ± 0,16 nM/mg in parental strain). As found in other organisms that contain low c-di-GMP levels, we observed an enhanced motility in BbΔBB3576, that was restored to normal levels in the complemented mutant strain (BbΔBB3576::pBBRMCS5BB3576). Surprisingly, biofilm formation on abiotic surfaces was indistinguishable from parental strain in the tested conditions. However biofilm formation on Cystic Fibrosis Bronchial Epithelial Cell Line (CFBE) showed a diminished biofilm formation by BbΔBB3576, but shows no differences in cell invasion. Further research will be focus to understand how motility affects bacterial ability to adhere to the epithelium, as described in other pathogens. Strategies are already designed to analyze protein interaction between BB3576 and the flagellar system.

MI-P19
PHENOTYPIC DIVERSIFICACION AND HYPERMUTABILITY IN *Pseudomonas aeruginosa* BIOFILMS

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Biofilm (BF) growth is one of the adaptive strategies through which *Pseudomonas aeruginosa* (PA) can adhere to inert or host surfaces. During BF growth PA undergoes phenotypic diversification that leads to the emergence of different niche specialists. We have previously shown that BF formed by a *mutS* mutator strain displays a higher diversification than wild type (WT). Competition experiments showed that the *mutS* strain has a higher fitness than WT. Here, we focused on Small Colony Variants (SVC), one of the BF-adapted morphotypes. We observed that when SCV were grown in solid media, the WT-like morphotype emerged from the colony edges. The relative fitness of SCV derived either from WT or *mutS* was higher than the respective ancestor strain. Similarly, the SCV outcompete the reverted morphotypes. These results give further evidences that SCV had acquired adaptive traits to BF growth. Moreover, to confirm if the increased fitness of the *mutS* strain was related to a higher diversification we performed a complementation experiment with a plasmid harboring a functional *mutS* gene. Diversification in the complemented strain was similar to WT and no significant differences were found in relative fitness between them. In conclusion, our results indicate that the advantage of *mutS* strain in BF is due to a higher probability of generating adaptive morphotypes in this environment.

MI-P20
BIOFILM FORMATION INHIBITION BY PhoB IS REGULATED BY POLYPHOSPHATE LEVELS IN *Escherichia coli*

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PhoB, the regulator of the two-component system *phoBR* that responds to media phosphate (Pi) limitation, was described as an inhibitor of biofilm formation. PhoB negatively regulates c-di-GMP, a second-messenger involved in the molecular decision between planktonic motile and biofilm-associated bacterial lifestyles. Polyphosphate (polyP) in *Escherichia coli* stationary phase are modulated by media Pi concentration and its degradation triggers biofilm formation via LuxS quorum sensing system. The aim of this work was to investigate if polyP levels in stationary

phase could regulate *phoB*, influencing biofilm formation capacity. *E. coli* cells were statically cultured in minimal medium varying Pi concentration at different time of growth at 30ºC. For biofilms assays the Cristal violet technique was carried out. Results show that *phoBR* mutant forms biofilm independently of the media Pi concentration, conversely to diguanilates cyclases deficient strains that never form biofilm. When polyP level was present in stationary phase, a high alkaline phosphatase activity was observed as an indicator of *phoB* activation. Varying media Pi concentration, we demonstrate that fluctuation of polyP levels in stationary phase could be involved in *phoB* activation/repression with the consequent regulation of biofilm formation mediated by c-di-GMP and AI-2.

MI-P21
CONTROL OF BIOFILM FORMATION BY *Salmonella*-SPECIFIC TRANSCRIPTIONAL REGULATORS

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Bacterial biofilms are a group of cells physically attached to each other and embedded in a self-produced extracellular matrix. Biofilm-formation by pathogenic bacteria has become a major concern in modern medicine. In particular, this multicellular behavior allows *Salmonella* to survive hostile environmental conditions and confers resistance to both host defenses and antimicrobial agents, contributing to the prevalence of this food-borne pathogen. The transition between planktonic and sessile lifestyles in *Salmonella* is controlled at the transcriptional level by CsgD, the master regulator that activates the synthesis of the major components of the extracellular matrix, cellulose and curli fimbriae. *csgD* expression is in turn finely controlled by several transcription factors that integrate different environmental signals. Based on *in silico* analysis we identified new *Salmonella*-specific transcription factors that affect biofilm-formation. Deletions, as well as overexpression of the genes coding for these factors in different genetic backgrounds provoked marked differences in motility, resistance to antibiotics and the development of characteristic biofilm morphotypes of this species, as well as on *csgD* expression. Our results demonstrate that under specific conditions these *Salmonella*-specific transcription factors are responsible for the establishment of a community lifestyle.

MI-P22
LAP PROTEINS ARE A C-DI-GMP EFFECTOR SYSTEM THAT CONTROLS BIOFILM IN *Bordetella bronchiseptica*

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Bordetella bronchiseptica is a respiratory pathogenic bacterium that forms biofilm-like structures. Previously, we determined that c-di-GMP levels modified ability to form biofilm. However, the bacterial factors involved in biofilm formation has not been yet determined. In *Pseudomonas fluorescens* a c-di-GMP effectors system controls biofilm formation. LapA is a surface adhesin and its binding to the cell surface is controlled by LapD, in response to c-di-GMP. LapD promotes biofilm formation through LapA accumulation. LapG cleaves LapA, freeing the adhesin and preventing biofilm. Accordingly, when LapG is overexpressed or LapD is absent biofilm is prevented. Homologous to Lap proteins were identified in *B. bronchiseptica*. When $LapG_{Bb}$ was overexpressed less biofilm was formed on abiotic surfaces. Moreover, biofilm structures observed by fluorescent microscopy over glass surface and epithelial cells were significantly different. Protruding structures typically observed in wild type biofilms were rarely observed in Bb-LapGBb. To evaluate if LapDBb is involved in biofilm formation, *lapDBb* gene was deleted. Surprisingly, LapDBb deletion enhanced biofilm formation in *B. Bronchiseptica.*

MI-P23
INFLUENCE OF QS MOLECULES IN THE INTERACTION BETWEEN *Arachis hypogaea* AND *Bradyrhizobium*

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Many bacterial species use small molecules signaling to communicate with each other and to coordinate their growth activities, a process commonly referred to quorum sensing (QS). HSLs are a class of QS signal important for cell to cell communication. In recent years it has been shown that plants are capable to detect bacterial signal molecules and are also able to produce substances mimic and inhibitors of QS. In this work, the effect of root exudates of *A. hypogaea* (peanut) on production of HSLs in *Bradyrhizobium* native strains and the presence of signal mimic of QS

was determinate. The utilization of the biosensor *A. tumefaciens* NTL4 (pZLR4) *in vivo* showed an intense production of QS signals in the zone of contact between root-bacteria in comparison to plants control. The result obtained demonstrate that root exudates would determinate a pattern of bacterial colonization and production of HSLs in the rhizosphere penaut. Moreover, it was possible to determine that the plant is capable of synthesize mimic signals which were detected in their root exudates by using an *in vitro* plate assay with *A. tumefaciens* NTL4 (pZLR4). However, the structure and biological activity of these plants compounds are still unknown. Our results reveal that an intense process of communication occurs between peanut plants and *Bradyrhizobium* bacteria in the rhizospheric microenvironment.

MI-P24
THE ABSENCE OF PROTEIN Y4YS AFFECTS THE ABUNDANCE OF T3SS *Mesorhizobium loti* SECRETIN IN MEMBRANES

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Type III secretion systems (T3SSs) are present in several pathogenic bacteria. These systems are multiprotein complexes through which effector proteins are delivered into the host cell and participate in virulence determination. T3SSs are also present in some rhizobia species. *Mesorhizobium loti* MAFF303099 has a functional type III secretion system that is involved in the determination of nodulation competitiveness on Lotus. The *M. loti* MAFF303099 T3SS cluster contains the *y4yS* gene that codifies for a protein (Y4yS) to which no function was yet demonstrated. A mutation in the *y4yS* gene affects the secretion of proteins through T3SS and presents the same nodulation phenotype as a mutant affected in the T3SS functionality. Translational fusion to a reporter peptide allowed determining the bacterial membrane localization of Y4yS. *In silico* analysis indicated that this protein presents a tetratricopeptide domain and has a signal peptide and a canonical lipobox. These features shared with proteins required for the formation of the secretin complex in type IV secretion systems and in the Tad system, together with its localization, suggest that the protein Y4yS is required for the formation of the *M. loti* T3SS secretin (RhcC2) complex. Analysis of RhcC2 in the wt and *y4yS* mutant strains indicated that the absence of Y4yS affects the accumulation of normal levels of RhcC2 in membranes.

MI-P25
THE ROLE OF *Xanthomonas citri* subsp. *citri* ADHESIN FHAB IN ITS INTERACTION WITH PLANTS

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The filamentous hemagglutinin-like protein adhesin, FhaB, of *Xanthomonas citri* subsp. *citri* (*X. citri*) is involved in virulence, adherence, leaf surface attachment and biofilm formation of this citrus pathogen. With the aim to study if FhaB protein is able to induce some changes in plant responses, we expressed different FhaB regions (AR1, AR2 and AR3) because FhaB is a 4,753 amino acids protein. The purified recombinant FhaB regions were infiltrated into pepper, tomato and citrus leaves and the appearance of chlorotic and necrotic lesions was observed. Cell death and callose deposition monitored by DAB and aniline blue staining, respectively showed significant increases in tissues treated with AR1, AR2 and AR3 compared to the control, demonstrating that plants can recognize these FhaB regions. To analyze genes involved in the innate immunity, oxidative stress and defense responses, citrus and tomato leaves were infiltrated with these regions. Quantitative RT-PCR assays showed that the three regions were able to induce the expression of genes involved in defense or resistance responses in plants. Particularly, in tomato plants genes involved in plant basal immune responses were significantly induced. Altogether these results indicate that *X. citri* FhaB can be recognized by plants and this triggers the activation of plant defense responses.

MI-P26
STUDY OF NOVEL FUNCTIONS OF *Xanthomonas citri* subsp. *citri HRP* CLUSTER

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Xanthomonas citri subsp. *citri* (X. citri), the bacterium responsible for citrus canker possesses a *hrp* gene cluster involved in hypersensitive response and pathogenicity. This *hrp* cluster encodes the type three protein secretion system (T3SS). We have demonstrated that *X. citri* T3SS, besides its participation in the secretion and translocation

of effector proteins into the host cell, is also required for biofilm formation, motility and survival on leaf tissue revealing novel functions for this system. A proteomic analysis was performed to identify differentially expressed proteins between *X. citri* and a mutant in the *hrp* cluster (*hrpB*$^-$) grown statically. Most of the identified proteins belong to different classes of outer membrane proteins, suggesting that the lack of the T3SS impairs membrane stability. To test this, in this work we evaluated the survival capacity of *X. citri*, the *hrpB*$^-$ mutant and the *hrpB*$^-$c complemented strain under the presence of membrane destabilizing agents, such as SDS. The resistance to salt and oxidative stress of these strains was also assayed. *X. citri* and *hrpB*$^-$c showed a higher tolerance to these treatments than the *hrpB*$^-$ mutant. Moreover, qRT-PCR assays showed that the expression of the *hrp* cluster is induced upon stress conditions. Our results reinforce the hypothesis that T3SS stabilized *X. citri* membranes.

MI-P27
REGULATION OF LIPID BIOSYNTHESIS IN GRAM-POSITIVE BACTERIA

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A crucial but often overlooked aspect of cell biogenesis and survival is the need for precise coordination between the biosynthesis of its macromolecular constituents. In the case of bacterial cells, this means producing appropriate amounts of four classes of macromolecules: nucleic acids, proteins, lipids and the polysaccharides of the cell wall. The molecular basis of coordination is well known in some cases. In others it is still rather misterious. In this work we combine fisiological and biochemical strategies to study, in the model Gram-positive bacteria *Bacillus subtilis*: i) the role of the signaling nucleotide ppGpp in the two-way coordination between lipid synthesis and cell growth and ii) the transcriptional and activity regulation of acetyl-CoA carboxylase (ACC), a key enzyme of the lipid biosynthetic pathway. Our results pave the way to understanding how Gram-positive bacteria coordinate lipid and membrane synthesis with its other biosynthetic activities and in response to environmental conditions.

MI-P28
STUDY OF THE INTERACTION OF CELL-WALL POLYMERS OF *Lactobacillus casei* WITH THEIR BACTERIOPHAGES

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Understanding of the molecular mechanisms of bacteriophage interaction with their host bacteria can be used to develop strategies to avoid infections during dairy fermentation processes. We characterized the sub-cellular fractions involved in *Lactobacillus casei* phages J-1 and PL-1 recognition and adsorption. In other lactic acid bacteria non-proteinaceous components of the cell wall have been described as receptors. Gram-positive bacteria surface may contain: (i) capsular polysaccharides (CPS) like rhamnose containing glycans (RWPS) often covalently bound to peptidoglycan (PG); (ii) wall polysaccharides (WPS) like Lipoteichoic acid (LTA) or wall teichoic acid (WTA) that may be or not covalently attached and (iii) extra-cellular polysaccharides (EPS) which are released into the cell environment. Each cell wall fraction was purified by different treatments to preserve their integrity and architecture and tested for phage adsorption inhibition. Total Cell Wall fraction (CW) contains all components; TCA soluble material retains WTA and/or RWPS. CW treated by boiling in 4% (w/v) SDS remove lipoteichoic acids and proteins living purified PG. D-alanine esters and O-acetylation were removed from CW by alkali. EPS was obtained from the culture supernatant. TCA soluble material efficiently blocks phage adsorption probably indicating the presence of phage receptors.

MI-P29
CHARACTERIZATION OF ENZYMATIC ACTIVITY OF A *Paenibacillus* sp. XYLANOLYTIC STRAIN ISOLATED FROM SOIL

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Improving xylan deconstruction is of high interest for increasing yields of fermentable sugars from lignocellulosic biomass, which is important in the second generation bioethanol production as well as for pulp and paper industry. Biodegradation of xylan requires the action of two main enzymes: xylanases and beta xylosidases. With the objective of prospecting hemicellulolytic environmental bacteria, a xylanolytic strain has been isolated from a forest soil sample and identified by 16S rRNA sequencing as *Paenibacillus* sp. Major cellular fatty acids were identified as:

anteiso-C15:0 (38.53%), anteiso-C17:0 (38.53%), iso-C16:00 (17.8%) and C16:0 (3.67%), in accordance to the profile found for this genus. The effect of cultivation of the microorganism under different cellulosic substrates was evaluated and the maximum xylanase activity was obtained when growing on minimal medium supplied with xylan from birchwood, for 72 hours. Also, the best condition of reaction was achieved at pH 7 and 50 °C. HPLC analysis of hydrolysis products released from xylan by crude extracts showed four main peaks corresponding to xylotetraose, xylotriose, xylobiose and xylose, indicating that this microorganism has the set of enzymes to obtain a complete degradation of the xylan. Therefore, this strain results of high interest for potential industrial applications and to study its xylanolytic system.

MI-P30
INACTIVATION OF THE GLUTAMATE DEHYDROGENASE ACTIVITY IN *Lactobacillus casei* AND ITS EFFECT ON GROWTH AND FLAVOR

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Lactobacillus casei is one of the strains commonly used in adjunct cultures for the cheese ripening contributing to the final organoleptic characteristics. The amino acids (AA) catabolism contributes to the flavor production being the glutamate a key compound in the recycling and conversion of α-ketoacids. From these compounds, the AA are sources of organic acids, alcohols, aldehydes, ketones and esters which give food flavor. The α-ketoglutarate regeneration by glutamate dehydrogenase (GDH) has been reported be a desired activity of adjunct cultures. We evaluated the role of GDH in the flavor generation from AA metabolism, and the survival and growth on different carbon and nitrogen sources. For that, we made a mutant strain of *Lb. casei* with the *gdh* gene disrupted and its phenotype was compared with that of the wild type (WT) strain. The growth of the mutant and WT strain was evaluated in MRS medium using different carbon and nitrogen sources. The aroma production was assessed in resting cells through the solid-phase microextraction (SPME) system followed by gas chromatographic separation coupled to a mass spectrometer as detector (GC-MS). The relative AT activities of the cell-free extracts was also evaluated. This study provides further evidence of the role and importance of GDH for AA catabolism and survival of *Lb casei* under different carbon and nitrogen sources.

MI-P31
CONJUGATED BIOLIPID PRODUCTION BY BIFIDOBACTERIUM STRAINS WITH POTENTIAL HEALTH PROMOTING PROPERTIES

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Conjugated linoleic (CLA) and linolenic (CLNA) acids are, respectively isomers of linoleic and linolenic acid that exhibit health–promoting properties such as anticarcinogenic, antiatherogenic, anti–inflammatory and antidiabetic activities. Bacteria able to synthetize them are considered suitable for the development of dairy products or as probiotic strains for in situ bioproduction. The aim of this work was to investigate CLA/CLNA production of 8 bifidobacteria strains cultured in MRS–cys and skim milk with LA and LNA. After 48 h–37ºC under anaerobic conditions, lipid were extracted and analyzed by UV method followed by GC. Results showed that all strains were able to form CLA and/or CLNA in both medium with percentages of conversion from 1–14% for CLA and 1–65% for CLNA in MRS–cys broth. In skim milk percentages were from 1–12% for CLA and 1–96% for CLNA. *B. animalis ssp. lactis*INL2 showed the highest production of *c9,t11,c12*–CLNA (MRS–cys) and *B. breve*ZL1228 resulted the best producer of *t9,t11*–CLA (skim milk). Growth compatibility assays between these two selected strains were performed. The relatedness between INL2 and commercial probiotic strains was determined by pulse field gel electrophoresis. These results suggest that INL2 and ZL1228 strains are suitable candidates for the design of new fermented dairy products naturally containing high concentrations of bioactive lipids.

MI-P32
GROWTH PARAMETERS AND BACTERIOCIN EXPRESSION ANALYSIS OF *E. mundtii* CRL35 IN A MEAT MODEL SYSTEM

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Enterococcus mundtii CRL35 is a bacteriocinogenic non-starter strain with biotechnological potential in biopreservation and food safety. Food model systems constitute a useful and necessary instance to study the behavior

of an organism before its application in a real matrix. The aim of this work was to study the growth, acidifying capacity, antimicrobial activity and the expression of bacteriocin genes in a food model based on meat extract at 25°C, in comparison with the laboratory culture media LAPTg. Thus, cell growth (OD_{600}), pH, antimicrobial activity against the food-borne pathogen *Listeria monocytogenes*, as well as the expression of structural (*mun*A) and ABC transporter (*mun*B) genes of Enterocin CRL35 by qRT-PCR, were determined. *E. mundtii* CRL35 was able to grow in the food model system reaching an OD_{600} of 3.12 with a final pH of 4.52 after a 25 h incubation. The same growth and acidification capability were observed in LAPTg (pH=4.6 and OD_{600} =3.06). Regarding the antimicrobial activity, an optimal Enterocin CRL35 production was recorded after 10 h, being highly superior in LAPTg (3.6 x 10^3 UA/ml vs 2.2 x10^2 UA/ml). Furthemore, expression analysis by qRT-PCR evidenced that *mun*A and *mun*B are expressed in both media and at the same level. These results suggest that *E. mundtii* CRL35 could be a suitable bioprotective non-starter culture for meat products.

MI-P33
RELATIONSHIP BETWEEN AIR-LIQUID BIOFILM AND SPORE FORMATION BY *Bacillus subtilis* subsp. *spizizenii*

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Bacillus subtilis is a plant-beneficial, spore forming bacteria. 60% of the known functions that induce biofilm genes are also sporulation genes. *Bacillus subtilis* subsp. *spizizenii* can form exopolysaccharides (EPS) biofilm in the interfase liquid-air. We studied the effect of biofilm formation under different conditions on sessile bacterial enzyme activity and spore formation. *B subtilis* was grown in a minimal salt medium with glucose or glycerol and 55mM L-glutamic acid (in static and agitation). EPS composition was determined by HPLC. Proteolytic enzyme by casein-zymogram. Sporulation frequencies by the ratio of chloroform-resistant colony. In static condition with glycerol, we obtained 1.1 mg biofilm/ml with 80% of EPS and 3.3 10^7 and 2.5 10^4 UFC/mg biofilm, vegetative and spore, respectively. At 80 rpm, more spores were formed compared with 150 rpm and biofilm was not formed. Similar results were found with glucose, both media had high proteolytic activity and biofilms had different EPS composition. The optimum temperature for biofilm formation (1.3 mg/ml) was 37°C, for vegetative cells 45°C (1.2 10^{10} UFC), while optimum spore formation in the biofilm was 25°C (1.2 10^6 UFC). In this bacterium the relation between EPS formation and vegetative cellular density was not lineal. Glucose did not repress spore formation. Biofilm formation did not induce *spo* genes expression.

MI-P34
OPTIMIZATION OF MACROLIDE GLYCOSYLATION IN *Escherichia coli* AND *Streptomyces lividans*

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Glycosylation patterns of macrolides are important determinants of the bioactivities of the molecule. Previously we have demonstrated that substrate flexibility of the UTP-dependent glycosyltransferase pair MegDI-MegDVI from megalomycin gene cluster allowed production of two new antimalarial megosaminylated-macrolides in *E. coli*. However, the efficiency of the system only tolerates bioconversion of 20mg/L of macrolide per fermentation. Analysis showed that the high G+C content *megDI-megDVI* genes from *M. megalomycea* were poorly expressed in *E. coli*. In order to optimize the production of megosaminylated-macrolides new TDP-megosamine operons were constructed using codon-optimazed megDI/DVI genes for *E. coli*. The new operons were introduced in *E. coli* and the protein expression will be analyzed by Western Blot and bioconversion experiment. In addition, we have developed a new glycosylation system using *S. lividans*. In this case we carried out metabolic engineering of endogenous pathways that consume the common glucose-1P intermediate in *S. lividans*. We perform single and double mutation in *pgm, manB, glgC, glgA* and *galU* genes. Here we expected that higher intracellular levels of glucose-1P, and hence of NTP-sugars, increase the biosynthesis of TDP-L-megosamine, which should improve glycosylation of macrolides. This will be tested by analyzing NDP-sugar pool and bioconversion experiment.

MI-P35
ENTEROBACTIN ROLE IN *Escherichia coli* OXIDATIVE STRESS RESPONSE

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Alternative roles for siderophores might explain the potential adaptive advantages of microorganisms having multiple siderophore systems. *Escherichia coli* synthesizes the catechol siderophore enterobactin and has up to nine iron transport systems, most of them involving heterologous siderophore-iron complexes. We previously reported the requirement of enterobactin for *E. coli* colony development in minimal media (M9) and we associate this phenotype with an increment in oxidative stress. Now, we analyzed the role of enterobactin in reducing cell oxidative stress. We showed that *entE* expression increased in cells growing in M9 and in presence of oxidative agents but decline when ascorbic acid was added. Also, we observed that the lack of enterobactin increased H_20_2 and Paraquat cell sensitivity. The addition of enterobactin to the media protected cells and different levels of iron availability did not impact on the stress-sensitivity. Furthermore, we found that total catalase activity decreased in *entE* cells growing in M9 and addition of catalase to the media restored colony growth of an *entE* mutant. Finally, we demonstrated that enterobactin exhibited *in vitro*, high radical scavenging activity against DPPH. These results suggest that enterobactin could be an important piece in the oxidative stress response of *E. coli*, particularly required in the context of colony development in M9.

MI-P36
CHARACTERIZATION OF *Escherichia coli* DAM MUTATIONS

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Escherichia coli strains deficient in DNA adenine methyltransferase (Dam) are mutators, and this deficiency also results in transcriptional defects of several genes. We have isolated some *E. coli* mutants that have partially affected Dam functions. These strains display some phenotypes similar to a Dam deficient strain but have a normal level of DNA methylation in stationary phase. Complementation assays showed that expression of the wild type *dam* gene reverts the phenotype of these strains. DNA sequencing of the endogenous *dam* gene revealed the presence of several mutations in the promoters and the coding sequence. Chimeric constructs between the wild type and a mutant *dam* gene allowed us to identify at least two different mutations able to generate this phenotype. *E. coli* Dam mutants carrying the chimeric constructs containing at least one of these mutations behave similar to the Dam deficient strain in the presence of a mutagen in liquid media. However, these strains behave like the wild type strain when the presence of the same mutagen was analyzed on solid culture media. In addition, after exposure to UV light, while *dam* strains are more sensitive to this agent than the wild type strain, those carrying the chimeric constructs were even more resistant. These phenotypes suggest that these kinds of Dam mutations could result advantageous for *E. coli* adaptation and/or evolution.

MI-P37
THE *SOD* GENE FROM *Rhodobacter capsulatus*. NEGATIVE MODULATION BY REGA

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Rhodobacter capsulatus is a phototrophic α-proteobacteria capable of heterotrophic (aerobic) or photosynthetic (autotrophic) growth. A single *sod* gene encodes a cambialistic superoxide dismutase (RcSOD) that exchanges its cofactor, Fe or Mn, depending on oxygen tension. A *sod* insertional mutant was isolated on minimal medium plates under light in anaerobiosis. The *Rhodobacter* SOD^- strain displayed a 2-times slower duplication time than the wild-type and undergoes intracellular superoxide increase, as deduced by lucigenin promoted luminescence. Lowering of aconitase activity reveals disintegration of 4Fe-4S clusters while DNA oxidative damage was visualized as a 4-fold increase of spontaneous mutation frequency in the *sod* mutant. The conserved RegA/RegB regulatory system provides an overlying layer of redox control of metabolism in bacteria. Three putative RegA binding sites (-10, -53 and -129) upstream the sod gene were identified by means of reported consensus. The expression of *sod* gene was analyzed by western blot, *in-situ* SOD-activity PAGE and galactosidase activity measuring from a *sod::lacZ* translational fusions. Although the amount of SOD increases with the oxygen tension, the *regA* mutant displayed always higher levels of dismutase than wild-type, suggesting a repressor role for RegA in the expression of *sod* in *Rhodobacter capsulatus.*

MI-P38
TRANSCRIPCIONAL REGULATION OF GbdR, THE REGULATOR OF CHOLINE METABOLISM IN *Pseudomonas aeruginosa*

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P. aeruginosa is an opportunistic pathogen widely spread in the environment that causes 8-16% of nosocomial infections around the world. This microorganism uses choline (Cho) as carbon and nitrogen source and, under osmostress, as the metabolic precursor of the osmoprotectant glycine-betaine (GB). The Cho metabolism and enzymes related with its acquisition are regulated by GbdR, an AraC/XylS family transcription factor. In a complex medium, a microorganism must "decide" what metabolic pathway will turn on in order to survival. Thus, in hyperosmolarity, if *P. aeruginosa* uses choline as a nutrient, it also must control which part of the choline pool will be used for osmoprotection. In this work we investigate: i) if the *gbdR* gene is regulated at the transcriptional level; ii) the influence of the environment in its regulation, iii) the identity of global regulators possibly involved in its control, and iv) if the regulation of this gene would determine what metabolic pathway must follow choline in different environmental conditions. By bioinformatics tools, transcriptional fusion and mutants strains we conclude that GbdR expression is under the control of two overlapped σ54-dependent promoters, IHF, two response regulators (NtrBC and CbrAB) and BetI, a transcriptional repressor associated with the transport and conversion of choline to GB in hyperosmolarity.

MI-P39
A COMPLEX MECHANISM MODULATES THE *Pseudomonas aeruginosa choE* GENE EXPRESSION

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The *P. aeruginosa choE* gene (PA4921) encodes an acetylcholinesterase activity (ChoE), which expression depends on the use of choline (Cho) as a nutrient. The aim of this work is to know how this protein is regulated at transcriptional level. Trough different approaches we demonstrate here, the complexity of its transcriptional regulation. The *in silico* analyses of the upstream region revealed: i) putative consensus of the binding sites of regulators: GbdR (-214/ -244 pb and -338/-368 pb), ArgR (-52/ -96 pb), and NtrC (-287/ -304 pb and -445/ -462 pb); and ii) two overlapped promoters dependent on σ^{54} and σ^{70} factors, respectively. The measurement of β-galactosidase activity of several strains constructed with DNA fragments of different lenght (582, 301 or 299 bp) fused to *lacZ* promoter demonstrated the relevance of each region on *choE* expression under various nutritional conditions (succinate/NH4, /Cho, /arginine). The results also indicate that not only Cho is the inductor of ChoE, but also arginine when acting as N source. The mutant strains, Δ*rpoN*, Δ*ntrC*, Δ*gbdR* and Δ*argR*, allowed us to corroborate that Cho/NtrC (by σ^{54}) and Cho/GbdR (by σ^{70}) regulate positively the *choE* expression. Arginine, mediated by ArgR as enhancer of σ^{70}-RNAP, also modulates the *choE* expression. Finally, we postulate a theoretic regulatory model for *choE* expression under different nutritional conditions.

MI-P40
ROLE OF *Staphylococcus aureus* SAOUHSC01313-SAOUHSC01314 TCS IN THE SUSCEPTIBILITY TO STRESS FACTORS

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Staphylococcus aureus (SA) is a pathogen that causes a multitude of diseases. Stress factors act as host-specific signals for the pathogen, inducing the expression of virulence-related genes. Since two component systems (TCSs) are the principal mechanism involved in stress response, we studied two contiguous genes located in SA NCTC 8325, SAOUHSC01313-01314 coding for a histidine kinase (HKsa) and a response regulator (RRsa), respectively. These genes showed homology with *Bacillus subtilis* DesKR, which regulates *des* transcription at low temperature. The SA TCS is located upstream of genes SAOUHSC01311-01312, encoding a putative ABC transporter that carries in its promoter a regulatory box similar to that of *des*. This TCS/ABC link resembles the prototype detoxification module found in *Firmicutes*. In this work we investigated the SA TCS role in exposure to stressors. We constructed a SA mutant lacking the TCS and found that this strain became susceptible to ATB and low temperature. Additionally, we study transcriptional activation of the ABC transporter by HKsa-RRsa by performing β-galactosidase assays of strains carrying a transcriptional fusion of SAOUHSC01311 promoter to *lac*Z. Transcription of P*sa01311-lac*Z is

activated at 25°C in the presence of milk components. These results suggest that SA TCS is important for bacterial fitness when they are exposed to membrane stressors.

MI-P41
NOVEL REGULATION LEVELS IN MYCOLIC ACIDS BIOSYNTHESIS IN MYCOBACTERIA

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Elucidation of mechanisms modulating mycolic acid biosynthesis would shed light on the capacity of *M. tuberculosis* to adapt and survive within the infected host. Phosphorylation of proteins by Ser/Thr protein kinases (STPKs) has recently emerged as a major physiological mechanism of regulation in mycobacteria. FasR, is a key regulatory protein for maintaining lipid homeostasis in mycobacteria, as it modulates fatty acid availability by regulating the transcription of the *fas* gene. In this study, we investigated if phosphorylation of FasR might represent a strategy employed by *M. tuberculosis* to regulate fatty acid biosynthesis. We show that FasR is efficiently phosphorylated in vitro by several mycobacterial STPKs, particularly by PknB. We propose to analyze how phosphorylation modulates its biological activity. On the other hand, the identification of an sRNA in mycobacterium that may be involved in lipid biosynthesis was recently published. Mcr16 was found inside *fabD* gene, which encodes a crucial enzyme in the FasII system. Therefore we pretend to determine if *fabD* expression is regulated by Mcr16. We will construct *M. bovis* BCG overexpression strains for the sRNA Mcr16 to analyze it´s effect over mycolic acid synthesis. We believe that understanding the regulatory network involved in maintaining lipid homeostasis in *M. tuberculosis* will provide new tools to combat this disease.

MI-P42
AN INSIGHT INTO THE PHYSIOLOGICAL ROLE OF MabR IN MYCOLIC ACID BIOSYNTHESIS

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Mycobacteria have two fatty acid synthases (FAS-I and FAS-II) that work in a concerted manner for the biosynthesis of mycolic acids. Our research group has identified MabR as a new transcriptional regulator that controls the expression of *fasII* operon genes by binding specifically to the *fasII* promoter region. Our aim is to unequivocally decipher the physiological role of MabR, an important step towards understanding the complex regulatory network involved in the maintenance of lipid homeostasis in mycobacteria. Using a two-step homologous recombination strategy we constructed *mabR* conditional mutants in *M. smegmatis*, where expression of *mabR* is under the control of P*ami*. These strains were used to analyze the expression of *fasII* genes by real time PCR and also to study the role of MabR on lipid biosynthesis. We have found that MabR modulates the expression of *fasII* genes *in vivo* and that long chain acyl-CoAs and acyl-AcpM modulate the affinity of MabR for its DNA binding site, suggesting that these metabolites are sensed *in vivo* by MabR. Our studies on the regulation of the *fasII* operon has opened now a new area of research and raises several important questions that need to be answered around the mechanism used by these microorganisms to maintain lipid homeostasis.

MI-P43
ACYLCOAS AS EFFECTOR MOLECULES: UNDERSTANDING THE TRANSCRIPTIONAL REGULATORY NETWORK IN MYCOBACTERIA

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Mycobacteria have two fatty acid synthases which work in concert to synthesize fatty acids and mycolic acids. We identified two transcriptional regulators essential for mycobacterial viability: MabR, which controls the expression of *fasII* operon genes, and FasR, which specifically binds to *fas* promoter region and controls the *de novo* fatty acid biosynthesis. The main purpose of our studies is to understand how mycobacteria exert a fine control over the biosynthesis of their membrane. The characterization of the effectors molecules that modulates the affinity of FasR and MabR for its DNA target was studied using EMSA, *lacZ* transcriptional fusions, SPR and *in vitro* transcription. In order to deeply characterize the molecular bases of MabR and FasR interaction with their corresponding DNA

targets and effector molecules, we performed crystallographic experiments. In this work, we show that long-chain Acyl-CoAs are key effector molecules that coordinate the expression of the two FAS systems, by directly binding to FasR and MabR. Furthermore, rod-like crystals of MabR native protein where found in the presence of the DNA interacting probe and thin needles crystals of FasR were obtained in presence of C_{20}-CoA. A better understanding of this complex process of regulation of lipid homeostasis in mycobacteria will greatly contribute to the development of new strategies to control this disease.

MI-P44
SUCROSE METABOLISM BY *Lactobacillus curvatus* CRL705

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Lactobacillus curvatus CRL705 is a meat starter culture that ferments sucrose and produces a potent bacteriocin (lactocin Lac705); these two important phenotypes are associated respectively, with plasmids pRC18 (18,664 bp) and pRC12 (12,342 bp). In this work, the capacity to utilize sucrose by *L. curvatus* CRL705 (pRC12 and pRC18) and two CRL705-derivatives, strains Sac7 (pRC18) and 28B (pRC12), was studied. Sucrose fermentation was observed in CRL705 and 28B, but not in Sac7, and only when cells were incubated under anaerobic conditions during 48 h at 30 °C. Genes related to the sucrose metabolism, such as the PTS system, fructokinase, permease and a sucrose 6-phosphate hydrolase, are present in the genome of strain CRL705, but the presence of an invertase function that could explain the sucrose positive phenotype was not detected. Nucleotide sequence analysis of plasmid pRC12 indicates that this plasmid has fourteen putative ORF. Two of these genes are potential candidates related with sucrose fermentation: *hyd*, which encodes a protein of 934 aa with a hydrolase-like domain, and *ca-atp*, which encodes a protein of 434 aa that has a COG02726 domain related with carbohydrate transport and metabolism. The involvement of genes *hyd* and *ca-atp* in the capacity of sucrose fermentation by *L. curvatus* CRL705 is currently being investigated.

MI-P45
SIGNAL DETECTION AND TARGET GENE INDUCTION BY THE *Bacillus subtilis* TWO COMPONENT SYSTEM YvfT-YvfU

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Two component systems (TCS) play a major role in signal transduction in prokaryotes for cellular adaptation to environmental conditions and stresses. In *Bacillus subtilis*, the DesKR TCS can detect changes in membrane fluidity upon a temperature downshift and induce the expression of a fatty acid desaturase, coded by the *des* gene, allowing cell adaptation to cold shock.*B. subtilis* possess an additional TCS, YvfTU, with unknown function, which present homology with DesKR. In this work, we investigated whether this TCS is able to induce *des* expression after a temperature downshift. We performed β-galactosidase assays of *B. subtilis desKR* null mutants carrying a transcriptional fusion of P*des* to *lacZ*. We observed that YvfTU expression in *trans* recovers the ability of the mutant to express *des* at low temperatures. However, in contrast with DesK properties, YvfT is unable to detect isothermal fluidity changes of the plasma membrane. Besides it cannot interact with DesR. We also found that YvfTU mediates the expression of a putative ABC transporter encoded by the genes *yvfR* and *yvfS*. Surprisingly, expression of such transporter occurs at 37°C and not after a temperature downshift. Despite of the high sequence identity found between kinases, regulators and operator regions of the target genes, DesKR and YvfTU do not show crosstalk *in vivo* and respond to temperature by different mechanisms.

MI-P46
REGULATION OF THE ACID-RESISTANCE *asr* GENE BY RcsCDB AND RstBA SYSTEMS IN *Salmonella*

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Salmonella Typhimurium uses specialized mechanisms to control the gene regulation, like the two component systems, to survive at changes in the environmental. As the RcsCDB system is induced in acidic medium, our goal was to identify genes whose expression depends on this system activation. Our results showed that the RcsCDB

system controls negatively the *asr* transcription, a small acid shock RNA required to establish the *Salmonella* infection. As was reported that the RstBA system upregulated the *asr* expression, we also investigated if exist an RstBA/RcsCDB systems interaction to control this gene transcription, when the bacteria is growing in acid medium. The bioinformatic and gel shift analysis showed that the *asr* gene promoter region contains a potential binding site for RcsB and RstA, and that both regulators exert their effect by directly binding to these boxes. In addition, using the *asr::lacZY* chromosomal transcriptional fusions, we demonstrated that RcsB and RstA act independently to control the *asr* expression, indicating that no interaction between these regulators occurs. However, more advanced studies showed that the RcsB activation in an acid medium, represses expression of *rstA*. This observation suggests that RcsB and RstA indirectly would interact to down or upregulate the *asr* transcription, depending on the medium in which the bacteria is growing.

MI-P47
DISCOVERING NEW Zur TARGET GENES IN THE *Salmonella* GENOME

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Zinc (Zn) is required as a cofactor in many crucial biological processes, but is also toxic. In consequence, maintaining intracellular Zn levels is critical for survival. Restriction to Zn access is one of the mechanisms used by mammals to prevent infections caused by bacterial pathogens, a process termed nutritional immunity. *Escherichia coli* uses two conserved transcription factors to tightly control Zn homeostasis, the Zur repressor, that controls Zn uptake in conditions of deprivation, and ZntR that induced resistance at high Zn concentrations. Both regulatory mechanisms are conserved in the enterobacterial pathogen *Salmonella enterica*. A small number of genes were described under the control of Zur in this pathogen, including *znuABC*, coding for a high-affinity zinc uptake system, and *zinT*, coding for a periplasmic Zn chaperone. These genes were shown to affect *Salmonella's* virulence. To better understand the mechanisms used by *Salmonella* to control Zn homeostasis, especially during Zn deprivation, we underwent a series of *in silico* and genetic screenings to identify other members of the *Salmonella* Zur regulon. We detect new genes under the control of this repressor and identify a hierarchical induction mechanism that allows *Salmonella* to cope with Zn deficiency.

MI-P48
TRANSCRIPTIONAL CONTROL OF A *Salmonella* COPPER-RESISTANCE LOCUS CODING FOR THIOREDOXIN-LIKE PROTEINS

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Oxidoreductases of the thioredoxin superfamily contribute to *Salmonella enterica* serovar Typhimurium invasiveness and the virulence. The thioredoxin (TrxA) and the disulfide bond (Dsb) systems maintain proper periplasmic protein disulphide bond formation. Unlike *Escherichia coli*, *S.* Typhimurium has species-specific thioredoxin-like proteins encoded in the suppressor of copper sensitivity (*scs*) operon. We observed that expression of the *scsABCD* operon is induced in the presence of copper (Cu), an essential micronutrient, that can induce oxidative stress at high concentrations. In fact, a mutant deleted of the whole *scsABCD* operon is more sensitive to Cu than the wild-type strain, but not to other metal ions or oxidative stress inducers. Using bioinformatics as well as genetic approaches, we found that *scsABCD* transcription is regulated by major regulatory systems controlling envelope stress. *In vitro* and *in vivo* analyses indicated that at least one of these transcriptional factors directly interacts with the *scs* promoter. These analyses also allowed us to define a region that negatively controls *scsABCD* transcription. We proposed that this complex control of *scsABCD* transcription is required to increase *Salmonella* survival under severe Cu intoxication, both within host's tissues and in the environment.

MI-P49
TRANSCRIPTIONAL REGULATION ANALYSIS OF PrtA METALLOPROTEASE AND LipBCD TRANSPORTER IN *Serratia marcescens*

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Serratia marcescens is an enteric bacterium that can function as an opportunistic pathogen within immunocompromised hosts. Despite reiterative reported *S. marcescens* infections, its virulence mechanisms are

poorly understood. Part of *Serratia*'s pathogenic potential may be attributed to the large number of secreted exoenzymes, including chitinases, phospholipase, hemolysin, nuclease and proteases, including the metalloprotease PrtA which is produced in our clinical strain. Recent studies showed that PrtA is secreted by the LipBCD transporter, and point to this enzyme as an important virulence factor in different models of infections, including cell line cultures, insects and mouse. However, little is known about environmental signals and regulatory factors that modulate its production. In this work, we have assessed the regulation of *prtA* and *lipBCD* using *lacZ*- and *gfp*-containing reporter plasmids. Results showed that PrtA expression is induced during the stationary growth phase, and is also temperature dependent being levels of *prtA-lacZ* fusion five times higher at 30°C than at 37°C. Moreover, poor aireation of the medium impaired transcription from the reporter systems, at either temperature. Bioinformatic analyses revealed consensus sequence for the recognition of the the response regulator of the Rcs phosphorelay, RcsB, in the *prtA* and *lipBCD* promoter regions.

MI-P50
C-TERMINAL PROTEIN TAGS AFFECTS THE ACTIVITY OF *Trypanosoma cruzi* HEME A BIOSYNTHETIC ENZYMES

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Trypanosoma cruzi, the etiological agent of Chagas disease, cannot synthesize heme. This parasite must acquire it from different hosts and distribute it to several hemeproteins. We have identified the enzymes responsible for the conversion of heme into heme A in *T. cruzi*: TcCox10 (heme O synthase) and TcCox15 (heme A synthase) enzymes. We verified their function in the yeast *S. cerevisiae* and demonstrated that these enzymes are essential in *T. cruzi* epimastigote. Heme A is only present in cytochrome c oxidase complex (CcO). In the present work, we designed and constructed the GFP fusion of these genes and expressed them in *T. cruzi* and *S. cerevisiae*. We demonstrated that the mentioned proteins localized in *T. cruzi* mitochondrion and we observed an altered activity in yeast compared to the untagged versions: TcCox15-GFP was weakly active in yeast *cox15*Δ cells whereas TcCox10-GFP was more active in yeast *cox10*Δ cells. The heme O synthase and heme A synthase genes of other parasites -*Leishmania mexicana* and *Plasmodium falciparum*- were also expressed in yeast but the results were not conclusive as were with *T. cruzi* genes. These results indicate that despite the conservation of the proteins along the species, not all of them complemented the function in yeast and the C-terminal tags affected their interaction or stability affecting in their enzymatic activity.

MI-P51
FUNCTIONAL CHARACTERIZATION OF THE CDF TRANSPORTER SmYIIP IN *Sinorhizobium meliloti*

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In bacteria, membrane transporters of the cation diffusion facilitator (CDF) family participate in Zn^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} homeostasis. The functional role during infection processes for several members has been shown to be linked to the specificity of transport. *Sinorhizobium meliloti* has two homologous CDF genes with unknown transport specificity. Here we evaluate the role played by the CDF SMc02724 (SmYiiP). The deletion mutant strain of SmYiiP (Δ*smyiiP*) showed reduced in vitro growth fitness only in the presence of Mn^{2+}. Incubation of Δ*smyiiP* and WT cells with sub-lethal Mn^{2+} concentrations resulted in a 2-fold increase of the metal only in the mutant strain. Normal levels of resistance to Mn^{2+} were attained by complementation with the gene SMc02724 under regulation of its endogenous promoter. Liposomes with incorporated heterologously expressed pure protein accumulated several transition metals. However, only the transport rate of Mn^{2+} was increased by imposing a transmembrane H^+ gradient. Nodulation assays in alfalfa showed that the strain Δ*smyiiP* induced lower number of nodules than in plants infected with the WT strain. Our results indicate that Mn^{2+} homeostasis in *S. meliloti* is required for full infection capacity, or nodule function, and that the specificity of transport in vivo of SmYiiP is narrowed down to Mn^{2+} by a mechanism involving the proton motive force.

MI-P52
MUTATIONAL ANALYSIS OF A SMALL REGULATORY RNA PROMOTER IN *Sinorhizobium meliloti*

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Small bacterial regulatory non-coding RNAs (sRNAs) are key players in post-transcriptional regulation. Hundreds have been identified in many species, but the biological function remains unknown for most of them. Studying their expression may provide clues about their function. A *S. meliloti* strain bearing a reporter chromosomal *gfp* fusion to the promoter of the conserved sRNA Sm8 showed that *sm8* expression is controlled by the complexity and/or availability of the N source, and that Sm8 might be involved in the regulation of nitrogen (N) metabolism in strain 2011. Overall, the *sm8* promoter sequence resembles the consensus promoter "Motif 1" recently identified by RNAseq (Schlüter et al 2013), although the *sm8* promoter contains a strongly conserved 7-mer downstream the -35 element that is not conserved for the rest of promoters of this kind. Interestingly, this 7-mer has a similarity of 6/7 bases with the DNA recognition sequence of NtrC (Nitrogen regulatory protein C). We hypothesized that this conserved stretch may be associated with its regulation. Replacement of the conserved 7-mer impaired activation of *sm8* expression under inducing conditions. On the other hand, the *sm8* promoter lost its activity upon a point mutation at the single strongly conserved base (A) at the -10 region. We identified critical nucleotides for *sm8* promoter expression and induction by low N in *S. meliloti*.

MI-P53
THE SMALL RNA SM8 MODULATES GROWTH BEHAVIOR AT THE TRANSITION INTO STATIONARY PHASE IN *Sinorhizobium meliloti*

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Riboregulation in bacteria stands for mechanisms controlling prokaryotic gene expression that are orchestrated by RNA molecules. The nitrogen-fixing legume symbiont *Sinorhizobium meliloti* expresses ca. 450 small trans-acting non-coding RNAs with potential regulatory functions. sRNA-mediated processes may play a distinctive role in the fine-tuning of gene expression in *S. meliloti*, both in free-living conditions and during the symbiotic association with the plant host alfalfa. The *S. meliloti* small non-coding RNA Sm8 has been of our particular interest because it has a remote evolutionary origin and shows a high conservation among α-proteobacteria. In *S. meliloti*, Sm8 expression is strongly induced at the transition into the stationary phase of growth. An *sm8* mutant strain shows an altered growth behavior at the phase transition (higher OD_{600}), only when growth is arrested as a consequence of imbalanced nutrient availability. On the other hand, Sm8 overexpression promotes the opposite phenotype. These results suggest that Sm8 likely acts as a sensor of nutrient limitation. Comparative transcriptomic and proteomic approaches between the wild-type and *sm8* strains using cDNA microarrays and differential ^{15}N proteome labeling, respectively, were performed towards to a comprehensive understanding at the molecular level of Sm8 biological role in *S. meliloti*.

MI-P54
A pSYM PLASMID-BORNE LOCUS OF *Rhizobium* IS INVOLVED IN THE SECRETION OF A RTX PROTEIN AND SYMBIOSIS

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The establishment of an effective symbiosis between *Rhizobium leguminosarum* and the legume depends on the expression of genes encoded in the symbiotic plasmid (pSym). The profiles of secreted proteins by *R. l.* 8401 pSym (A34) and the pSym cured strain (A31) were compared by SDS-PAGE-Coomassie staining. The difference between both patterns was the presence of a 49 kDa-protein (EP-49) in A34, indicating that secretion of this protein is pSym dependent. A cosmid library from pSym was screened using a polyclonal antiserum against EP49. One cosmid (pIJ1552) was able to restore EP49 secretion in A31 strain. By Tn*5* mutagenesis of pIJ1552 we isolated a clone that lost the ability to complement A31. Sequencing analysis revealed that the Tn*5* was inserted in the gene of a putative ABC (ATP binding cassette) component of a type I secretion system (TISS). Contiguous and in the same orientation, genes encoding a MFP (membrane fusion protein) component of a TISS and EP49 (based on the amino-terminal sequence) were identified. A mutant in A34 (Z18) was obtained by recombination of Tn*5*-pIJ1552. By Western blot we confirmed that Z18 is impaired in EP49 secretion. Nodulation assays showed that this mutant develops white

nodules in pea after 30 days of inoculation. These results suggest that the pSym harbors a novel ABC-MFP-EP49 locus of *R. leguminosarum* involved in the symbiotic process.

MI-P55
NORMAL MUTATION RATE VARIANTS ARISE IN MUTATOR *Pseudomonas aeruginosa* CHRONIC INFECTION POPULATIONS

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Hypermutability due to DNA mismatch repair system (MRS) deficiency is a common trait evolved by *Pseudomonas aeruginosa* (PA) strains in cystic fibrosis (CF) chronic infections. We have previously investigated the long-term evolution of mutators in CF airways by sequencing 27 genomes of PA from two CF patients (CFA and CFD) infected by single clones. Comparison of the genomes of 11 isolates obtained from single-time point samples showed extensive within-patient genomic diversification; the populations were composed of different sub-lineages that had coexisted for many years since the initial colonization of the patient. Despite of harboring MRS loss-of-function mutations, a small percentage (6%) of the CFD population showed a reduced mutation frequency similar to that of normo-mutable strains. Our sequencing data suggested that the most feasible explanation is the emergence of secondary mutations, in no MRS genes, that compensate for hypermutability, since neither reversion of the original mutation nor duplication of MRS genes were observed in the normo-mutable genomes. Within 112 analyzed genes, which are involved in replication, recombination, DNA repair adaptation, oxidative stress, and SOS response, only 8 were differentially mutated in the normo-mutable isolates. There are no previous studies about a possible compensation for MRS deficiency due to the observed mutations.

MI-P56
IDENTIFICATION OF MANGANESE OXIDIZING BACTERIA ISOLATED FROM BIOLOGICAL FILTRATION WATER PLANTS

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Despite the prevalence of Mn oxidizing microbes and their important role in biogeochemical cycling, the physiological function of bacterial Mn(II) oxidation remains enigmatic. Therefore, the specific objectives of this study were to isolate and identify Mn-oxidizing bacteria; and study, in a future, the physiological function of the bacterial Mn oxidation process. Samples were collected from biofiltration systems that remove the Mn present in underground waters by the biological oxidation of this metal. The samples were dissolved in PBS and different physicochemical parameters were measured. Then they were plated on different solid selective media (Mn-medium, PC-medium and Lept-medium) designed to isolate different species of Mn oxidizers. Mn-oxidizing bacteria were identified through color changes of colonies. In the absence of Mn, the colonies on the agar were whitish while in Mn containing medium they shifted to brownish, due to Mn oxides. 150 putative Mn-oxidizing strains were obtained and subsequently identified by microscopic observations and Gram-staining. Also, amplification by PCR of specific regions of the 16S RNAs allowed the molecular identification of the strains by phylogenetic analysis. Futures studies using these isolates will contribute to the understanding of the physiological role of bacterial Mn(II) oxidation.

MI-P57
ISOLATION OF BACTERIAL STRAINS WITH LIPOLITIC ACTIVITY FROM DAIRY INDUSTRY WASTEWATERS

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Dairy industries generate up to 10 L of wastewater per liter of processed milk causing a significant environmental problem. Since these wastewaters are rich in biodegradable organic molecules, their treatment usually involves bacteria addition. The aim of this work was to isolate bacterial strains for their use in the degradation of dairy wastewater fats. With this attempt, lipase producing strains were screened from a wastewater treatment lagoon of a local dairy industry. Exoenzyme profiles were performed for 58 isolates. Then, phylogenetic clustering of 49 strains was performed by RAPD analysis. 10 strains were evaluated for their capability to adapt to wastewater specific environmental conditions. For this, selected isolates were grown individually in sterile wastewater medium supplemented with glucose or oil as carbon source. Lipolytic activities in supernatants and bacterial growth rate (μ) were monitored. Four strains showed a μ_{max} higher than the mean μ_{max} in both media. Finally, 16S rRNA and/or

housekeeping gene sequences of selected strains were determined. We identified strains with highest shared identity with *Bacillus, Desemzia, Aeromonas,* and *Acinetobacter* species. In summary, in this work we selected strains based on their true-lipase activity against low degradability rate triglycerides and their suitable growth in wastewater media.

MI-P58
GENOTYPING OF *Staphylococcus* spp. ISOLATES BY PULSED FIELD GEL ELECTROPHORESIS

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Mastitis is the most prevalent infectious disease among dairy cattle. The aim of this study was to investigate the clonal relationship among *Staphylococcus spp.* by the technique of PFGE using a DNA extraction method without lysostaphin. The data obtained enable the knowledge of the genetic profiles allowing the understanding of diversity of clones in order to improve control programs. Eight dairy farms located in the South State of Rio de Janeiro were selected for the study. A total of 36 strains of *Staphylococcus spp*. were typed by PFGE. The strains were collected from bovine milk and from the milk production line. Nine representative profiles were generated among the bovine milk isolates, while only three profiles were generated among the isolates collected from the milk production line. Results showed a genetic diversity among the isolates and the absence of a predominant profile. Additionally, it was observed that several isolates from bovine milk production line presented a high degree of similarity. The analysis showed a greater genetic distance among the *Staphylococcus spp.* isolated from bovine milk compared with the isolates collected from the milk production line. These results indicate that the study and monitoring of pathogens isolates from bovine milk as the milk production line is essential for the development of new measures to control bovine mastitis.

MI-P59
IDENTIFICATION OF A FUNGAL STRAIN WITH CELLULOLYTIC CAPACITY, NATIVE OF THE PROVINCE OF MISIONES

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Since several years, many species of white rot fungi with interesting characteristics for use in biotechnological applications are being described. Applications of cellulases from new microorganism in industrial and environmental technologies require large amounts of enzymes at low cost. So it is necessary to select and identify new organisms with elevated synthesis of these enzymes. The aim of this study was to identify a fungal strain with cellulolytic capacity belonging to Phylum *Basidiomycota*, with the help of molecular and bioinformatic tools. From morphological identification in the correct phyllum of a strain with cellulolytic capacity, DNA was extracted in sufficient quantity and quality and ITS1-5,8S-ITS2 regions were amplified and sequenced using universal primers ITS 1 and ITS 4. To compare the obtained information with existing databases, the BLASTn (Basic Local Alignment Search Tool) tool of NCBI (National Center for Biotechnology Information) was applied, and contig consensus with the CAP 3 program was performed. 39 sequences were selected to be analyzed phylogenetically with the MEGA 6 program using Maximum Parsimony, Maximum Likelihood and Neighbor-Joining methods. Consequently, by constructing monophyletic clades, it was possible to identify the fungal strain with cellulolytic capacity, native of Misiones as *Pycnoporus coccineus* with a very high bootstrap value (>90%).

MI-P60
GEOGRAPHIC DISTRIBUTION AND MOLECULAR CHARACTERIZATION OF *Fusarium* SPECIES INFECTING SOYBEAN ROOTS

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Soybean [*Glycine max* (L.) Merr] production can be largely limited by sudden death syndrome (SDS). SDS is caused by four phylogenetically related *Fusarium* species: *F. tucumaniae* (*Ft*), *F. virguliforme* (*Fv*), *F. crassistipitatum* (*Fc*) and *F. brasiliense* (*Fb*), all of them found in Argentina. This diversity of species and the evidence of sexual reproduction of *Ft* in nature offer a challenge for disease management in our country. We first studied the geographical distribution of *Fusarium* species considering isolates obtained from infected soybean roots from 53

different locations and 38 climatic variables and identified the predictor variables of SDS development using binary logistic regression. SDS development by *Ft* or *Fb* was explained through the intersection and maximum temperature (T_{max}) on January; by *Ft* or *Fc* through the intersection and T_{max} on May; by *Ft* and *Fv* through the intersection and July rainfall; by *Fv* or *Fb* through the intersection and October precipitation and by *Fv* or *Fc* only through July rainfall. No explanatory variables were identified if the SDS was developed by *Fb* or *Fc*. Then, the genetic variability within and among these species from different locations was analyzed using sequence-related amplified polymorphism, random amplified polymorphic DNA and intersimple sequence repeat markers. Correlation between locations and genetic diversity of isolates is under progress.

MI-P61
POPULATION STRUCTURE OF METHICILLIN-SUSCEPTIBLE *Staphylococcus aureus* AND ITS RELATEDNESS WITH MRSA IN ARGENTINA

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Since 1990s, methicillin resistant *S. aureus* (MRSA) emerged as a community associated pathogen (CA-MRSA) worldwide. In Argentina, two major Panton-Valentine leukocidin (PVL)(+) CA-MRSA clones were reported with significant differences by geographical regions: CC5-ST5-IV-t311 mainly in South and CC30-ST30-IV-t019 in North of this country. We aimed to describe the population structure of MSSA and to shed light on the origin of these CA-MRSA clones. A total of 132 MSSA clinical isolates collected during Nov-2009 from 66 hospitals (20 provinces and Bs. As. City) were analyzed by *spa*-typing, PVL, PFGE and MLST. A total of 11 clonal complex (CC, MLST) and 65 spa-types (t) were identified. The proportion of isolates belonging to CC5 (t311 and t002) and CC30: (t012, t021 and t018) differed significantly between North and South of Argentina: 36% and 6% vs. 12% and 32 %, respectively. BURP analysis showed that MRSA (previously analyzed) and MSSA isolates belonging to CC5 shared related t (t311 and t002), suggesting that these were closely related. Contrary, for MRSA (t019) and MSSA (mainly t012) isolates from ST30 no common t was found. These results suggest that: 1) CA-MRSA CC5-ST5-IV-t311-PVL(+) clone have emerged from MSSA CC5-t311-PVL(+) ancestor, already established in this country and 2) CA-MRSA CC30-ST30-IV-t019-PVL(+) clone was probably imported from neighboring countries.

MI-P62
GENOME DRAFT SEQUENCE OF A CELLULOLYTIC AND XYLANOLYTIC ISOLATE OF *Cellulomonas flavigena*

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Lignocellulose degrading bacteria have high biotechnological potential in biofuels, feed and paper industries. We have isolated a novel *Cellulomonas flavigena* strain, named B6, from a soil consortium. A draft version of the genome was obtained using the Illumina MiSeq sequencing platform at the UGB laboratory of INTA. The data comprised a number of 1.532.556 paired-end reads, with a length of 500 bp. It was assembled in 512 contigs and annotated by the IGS Annotation Pipeline (http://ae.igs.umaryland.edu). Different bioinformatics analysis showed a high and even coverage of the reference genome *C. flavigena* type strain (134T). Moreover, the ordering of these contigs with the reference genome suggests we have approximately 75% of the de novo genome in the correct ordering. *C. flavigena* B6 has a 74.7% GC content, which is consistent with the previous published data for *C. flavigena* reference strain. Also, interestingly, the initiation codon GTG is present at a high frequency, approximately 62% of start codons. The isolate secretes free cellulases and xylanases and presents mainly high xylanase activity. Coincidently a great number of beta-1,4- endoxylanases coding sequences have been identified in the genome. In summary, we have isolated a *Cellulomonas flavigena* strain with high potential as source of enzymes for biomass deconstruction.

MI-P63
STUDY OF THE TOPOLOGY OF THE β-LACTAM SENSORS/TRANSDUCERS BLAR1 AND MECR1 FROM *Staphylococcus aureus*

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The PC1 β-lactamase and the PBP2a transpeptidase represent the main line of defense of Methicillin-Resistant *Staphylococcus aureus* (MRSA) against β-lactam antibiotics. The level of expression of these two proteins is regulated by the sensor/transducer membrane proteins BlaR1 and MecR1, respectively. These two proteins are embedded in the plasma membrane and are thought to sense the presence of the antibiotic and to subsequently deliver a signal that triggers both systems, by mechanisms still not fully understood. The topology of BlaR1 and MecR1 is a key to unveiling the signal transduction pathway. We studied the topology of BlaR1 and MecR1 in *E. coli* by constructing C-terminal fusions to EGFP. When the reporter protein EGFP is translocated across the cytoplasmic membrane unfolded through the Sec system, it does not recover its fluorescence. The characterization of the different fusion proteins by fluorescence spectroscopy in whole cells and by fluorescence microscopy allowed us to determine the topology of the proteins. Using mutants in the Sec and Tat translocation systems we verified that the insertion of these proteins in the membrane depends on the Sec translocon. We also tested the ability of full length proteins to bind the fluorescent penicillin Bocillin-FL in intact cells, and we hence corroborated the periplasmic localization of the C-terminal sensor domain in *E. coli*.

MI-P64
NOVEL DESCRIPTION OF *Enterococcus faecium* CITRATE GENE CLUSTER

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Enterorococcus faecium is one of the most controversial microorganisms used in food industry due to its role as causal agent of bacteremia and other human infections. Nevertheless it is used as probiotic, and its presence in cheese microbiota as non-starter lactic acid bacteria is truly beneficial for the flavor developing process as a consequence of citrate metabolism. Although citrate metabolism was studied in *E. faecium* no data about genes involved in this pathway has been reported. We have previously stated a classification of *E. faecium* according to *cit* genes present in its genome (*cit-* and *cit+* type I and II). Sequencing of *E. faecium* GM75 (type I) *cit* cluster allowed us to identify the insertion sequence IS256 in regulatory regions, which is not present in *E. faecium* IQ23 (type I). We have analyzed gene function through transport and metabolism and 14C-citrate transport experiments demonstrating that citrate intake and degradation is functional in both strains, with no significant differences. Aroma compound diacetyl was detected, proving the contribution to flavor development of these bacteria, although similar production amounts were obtained for both strains also. In this work we reach a first approach to the description of genetic organization and biochemistry of *E. faecium cit* cluster and give evidence to the great diversity it is subject to.

MI-P65
DRAFT GENOME SEQUENCE OF *Lactobacillus mucosae* CRL 573, A MANNITOL PRODUCER BACTERIUM

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Mannitol is a polyol that has multiple industrial applications due to its interesting physicochemical properties, being mainly used as natural sweetener in the food industry. It could be applied as a food additive or be produced *in situ* by food–grade microorganisms leading to the elaboration of naturally mannitol-enriched foods. *Lactobacillus mucosae* CRL 573 was selected for its ability to efficiently produce mannitol from fructose. Here we present the draft genome of CRL 573 sequenced using the whole-genome shotgun strategy (Ion Torrent personal genome machine). Initial assemblies resulted in a 2.26 Mbp draft genome with 38 contigs and overall GC content of 46.6%. This size and the GC content are comparable with those of the lactic acid bacteria (LAB) group. Genome annotation was done using the Rapid Annotations using Subsystems Technology server and a total of 2,715 coding sequences and 157 of RNAs were predicted. CRL 573 was found to contain a putative mannitol–dehydrogenase (*mdh*) gene, with a genomic context similar to the one present in *Lb. reuteri* CRL1101, that confirms its ability to produce mannitol. Furthermore, the analysis allowed us to identify a threonine–DH–like domain typical of the NAD(H)–dependent alcohol dehydrogenase family (MDR).

MI-P66
METAGENOMICS- BASED APPROACHES FOR EXPLORING BACTERIOCIN GENES IN ARTISANAL CHEESES

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The growing volume of metagenomic sequence data from different environments is a promising source for the *in silico* exploring the presence of bacteriocins genes. Cheeses made from unpasteurized milk and following traditional manufacturing procedures are an important source of strains harboring genetic diversity. Metagenomic approaches were used to study the bacteriocinogenic potential of the microbial community of artisanal cheeses. Metagenomic DNA from two cheese samples (A and B) was shot-gun sequenced by an IonTorrent PGM system. Data analysis was performed by MG-RAST web server and Bagel3 software. Dataset of sample A contained 2,069,638 sequences with an average length of 205 bps. Dataset of sample B contained 1,248,386 sequences with an average length of 219 bps. After quality control pipeline 69.1% of sequences produced a total of 542,929 predicted protein coding regions in sample A and 82.3% of sequences produced a total of 433,688 in sample B. Phylogenetic analysis indicated that cheese microbiome was dominated by *Streptococcus* and *Enterococcus*. This result was correlated with the identification of ORFs encoding for bacteriocins produced by members of those genera, BlpM, BlpN, BlpK, BlpU, termophilin A, Bac32 and enterocins SE-K4, P, B and A, demonstrating the prevalence of bacteriocin-producing and genetically versatile bacterial genera in artisanal cheeses.

MI-P67
COMPARATIVE GENOME ANALYSIS OF LACTIC ACID BACTERIA ISOLATED FROM CHEESE

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Genomic comparative analysis was performed between starter and nonstarter LAB isolated from regional cheeses. We determined the chromosomal sequence of three LAB, *Lactococcus lactis* CRL264, and two strains of *Enterococcus faecium* IQ110 and GM75. Illumina technology was used for sequence and SeqMan NGen software, Advanced Pipmaker and Mauve Genome Alignment for assembly. Genome annotation was carried out using RAST and function prediction was analyzed with Island viewer, Plasmid, Virulence, and pathogen finder. Our results show that *L. lactis* CRL264 possesses specific genetic rearrangements with respect to the European strains (such as IL1403, DL61, TIFN2 and 4). Also, our comparative analysis performed in *Lactococcus* strains confirms that the main feature of the biovar is the capability to metabolize citrate whereas all the *L. lactis* subspecies are able to produce diacetyl from pyruvate. *E. faecium* strains differ in the ability to metabolize citrate. GM75 strain could metabolize citrate while IQ110 strain result unable to degrade citrate. In CRL264 and IQ110 strains no virulence factor were detected while in GM75 strain Efa-Afm adhesion and acm, were found. Thus, our results show that genomic variability among Starter and NonStarter-LAB is determinant for the different contributions to the final quality of cheese.

MI-P68
A NOVEL GENETIC PLATFORM INVOLVED IN *BLA*$_{OXA-58}$ DISSEMINATION AMONG *Acinetobacter baumannii* STRAINS

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The emergency of carbapenem resistance in *Acinetobacter baumannii* is most frequently the result of the over-production of carbapenem hydrolyzing OXA-type β-lactamases (CHβL), a process generally mediated by the insertion of different insertion sequences (IS) upstream of *bla*$_{OXA-58}$ genes thus generating stronger promoters. We report the genetic surroundings of *bla*$_{OXA-58}$ in plasmid pAb242 extracted from a local carbapenem resistant clinical *A. baumannii* strain. *bla*$_{OXA-58}$ is embedded in an imperfect composite Tn*3*-like transposon, in which the upstream ISAba3 was targeted by an ISAba825. The ISAba825 insertion site differs from other target sites present in similar arrangements in other *A. baumannii* strains, indicating a different evolutionary origin of this particular arrangement. Analysis of the downstream region of the 3´-located ISAba3 identified *araC1* and *lysE* regulatory genes, the latter disrupted by a novel insertion of a composite Tn*125* transposon bearing an active *aphA6* gene conferring aminoglycoside resistance. The whole arrangement described above was also bracketed by Re27-like short sequences, thus providing potential recombination sites for the mobilization intra- and inter-genomes of this platform. Evolution of these flexible genetic structures bearing *bla*$_{OXA-58}$ may likely contribute to the rapid worldwide spread of CHβL genes among the global *A. baumannii* population.

MI-P69
TWO CATALASE GENES FROM THE ANDEAN ISOLATE *Acinetobacter* VER3: STRUCTURE AND EXPRESSION ANALYSIS

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High altitude Andean wetlands are characterized by extreme environmental conditions such as high UV radiation, elevated heavy metal content and salinity. Bacteria isolated from these environments evolved to survive in the mentioned extreme conditions. *Acinetobacter* strain Ver3 isolated from Verde Lake (4000 meters OS) displayed high UV tolerance and resistence to pro-oxidants as peroxide and superoxide propagators. Interestingly, total catalase activity in Ver3 cell-free extracts was shown to be fifteen times higher than those of control collection strains, being associated to one of two activity bands observed after non-denaturing electrophoresis. After a genome pyrosequencing strategy and annotation (http://rast.nmpdr.org), two genes were identified corresponding to hydroperoxidases (HP) types I and II. Sequence alignment comparisons display subtle structural differences, but the resulting phylogenetic trees showed a unique HPI group present in the *Acinetobacter* genus and three different HPII clusters. Design of adequate primers allow PCR molecular cloning and expression of the HPII recombinant enzyme in transformed *Escherichia coli* cells using pET28 vector. Non-denaturing gel electrophoresis followed by activity staining enable final identification of the gene responsible of the high catalase activity previously observed in the multiresistent *Acinetobacter* Ver3 isolate.

MI-P70
EFFECTOR PREDICTION METHOD BY PHYLOGENETIC PROFILING APPROACH

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Effector proteins are substrates of several specialized secretion systems, once translocated to the extracellular medium or directly into the host, they play a critical role in the establishment and maintenance of the interaction between the eukaryotic organism and the pathogenic or symbiotic bacteria. Two main approaches have been taken in order to identify effectors, the experimental one by direct culture supernatant screening, and the bioinformatic one by sequence composition analysis and promoter evaluation. In this work, we present a novel bioinformatic approach, based not in the composition of each sequence, but in the co-evolutionary dependence that most of the effectors have to their specific secretion system. Using phylogenetic profiling tools we were able to determine the dependence between a possible effector protein and all of its homologues to the type three secretion system within a large collection of complete and high quality incomplete genomes. This method has been applied to *Pseudomonas syringae* pv. tomato and *Escherichia coli* O157:H7 str. EDL933 with similar performance to sequence dependent methods, but with the advantage that it can also be applied to detect signal-less effectors such as the ones exported by the type six secretion system.

MI-P71
MYCOBACTERIOPHAGE 19ES: A NEW SINGLETON WITH UNUSUAL REPLICATION AND/OR PACKAGING STRATEGY?

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The genomic analysis of 700 mycobacteriophages reported to date provided important information on their evolution and helped developing novel tools for the genetic manipulation of mycobacteria. Due to the simplicity of phage isolation and characterization and the information that could be obtained, we started a project aimed at the isolation of phages from environmental samples by replication on the fast-growing *Mycobacterium smegmatis* mc^{2}155. We have isolated more than 40 mycobacteriophages, and sequenced 18 isolates using a GS-FLX 454 pyrosequencer. One of them named 19ES is a member of *Siphoviridae* family, has a particular sequence and unusual propagation conditions. When comparing its genome by dot plot against known mycobacteriophage genomes, it didn´t match groups reported in databases, indicating it is a novel singleton with a particularly high content of GC (70%). Using the genome editor DNAMaster we located 85 ORFs (structural, lysis and DNA packaging genes), one of which displayed homology to a chaperone DnaJ domain not previously described in bacteriophages. In spite of several attempts to sequence the end of the genome of 19ES, we were unable to retrieve sequence information. This trait along with the ability of 19ES of replicating efficiently without extra cations makes it intriguing for further investigation.

MI-P72
GROUP *Pseudomonas putida*: A SILENT ENVIRONMENTAL RESERVOIR OF ANTIMICROBIAL RESISTANCE GENES

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The *Pseudomonas putida* group encompasses at least 12 environmental species, from which *P. putida* and *P. monteilli* represent opportunist pathogens exhibiting antimicrobial resistance. We conducted a taxonomic classification of carbapenem resistant clinical isolates of the *P. putida* group recovered from Argentina, and characterize genetic platforms carrying blaVIM-2. A phylogenetic tree employing housekeeping genes identified 7 species, including *P. putida*, *P. monteilii*, and 5 unreported species from 13 *P. putida* group isolates. Three different genetic platforms containing blaVIM-2 were observed as judged by PCR mapping and sequencing, all including an unusual class 1 integrons. InT7633 (1) was identified on 10 strains including *P. putida*, *P. monteillii* and three undescribed species. Moreover, pLD209-like conjugative plasmids (1) carrying InT7633 were found in 5 strains and mobilized to *P. aeruginosa* and *Escherichia coli*, suggesting that horizontal transfer (HTG) of blaVIM-2 was responsible of resistance dissemination. Overall results suggest that blaVIM-2 is mobilized by transposition, and disseminated by HTG among different members of the group. This work uncovers the ability of species of the *P. putida* group to represent silent environmental reservoirs of resistance genes and, to provide them to opportunist pathogens in the clinical settings. 1. Marchiaro *et al.* 2014. AAC 58:1816.

MI-P73
COMPARATIVE GENOME ANALYSIS OF A HIGHLY PROSPEROUS AND A NON-PROSPEROUS XMDR *Mycobacterium tuberculosis*

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About 4.5% of new tuberculosis cases have Multi-drug-resistant (MDR-TB) tuberculosis, which are resistant to the first-line antibiotics. Some MDR-TB strains have the ability to evade the host human defenses and that may be one of the reasons of the high mortality rates of people infected with MDR-TB. In particular, strain M is highly prosperous in the Argentine and is able to build up further drug resistance without impairing its ability to spread. The 410 strain, a M-highly related MDR Haarlem strain that produced only one case, was used as contrasting strain in order to find the genomic particularities of M that could be involved in its interaction with the human immune system. Strains M and 410 were sequenced up to 99 %. The differences observed in genomic sequence, including non-synonymous SNPs, insertions and deletions, were analyzed. Altogether there were 675 in strain M and 724 in 410 of non-synonymous SNPs found, of which 533 were identical in both strains and 594 genes differed from H37Rv. In these 594 genes, mutations in 369 genes were identical in both strains, while mutations in 136 genes were specific to M and 193 were specific to 410. A comparative analysis of M and 410 with Mtb strains showed that a set of genes were exclusively different in the Argentinean strains.

MI-P74
EZRA, MREC, MRED, RODA AND FTSA INTERACTS WITH PBP2B DURING CELL DIVISION OF *Streptococcus pneumoniae*

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The penicillin-binding proteins (PBPs) are enzymes involved in peptidoglycan synthesis and cell division in bacteria. In *S. pneumoniae*, we have described that the pbp2b28 mutations confer β-lactam resistance and caused bacillary shape, atypical septum formation, asymmetrical divisions, and a PBP2B and FtsZ delocalization, suggesting that PBP2b is involved in the cell shape determination and cell division mechanisms. By bacterial two-hybrid assays, we revealed that PBP2b and PBP2b28 interacted with proteins such as EzrA, MreC, MreD, RodA and FtsA, which belong to divisome. We constructed insertion mutants in the genes that encode these proteins, and these ones displayed morphological and cell-wall biosynthesis alterations detected by vancomycin-fluorescein staining, which label peptidoglycan biosynthesis de novo. We also obtained the *mreC*, *mreD*, *rodA*, *ftsA* and *ezrA* mutants transformed with vectors that expressed PBP2b-GFP and PBP2b28-GFP. The expression of PBP2b28-GFP produced terminal thickens, shape changes and PBP2b28-GFP aggregates in all mutants. However, morphological defects such as “twisted-towel” were observed only in the *mreC*, *mreD* and *rodA* mutants. These results suggested that MreC, MreD, RodA, FtsA and EzrA interact in different manner with PBP2b, probably coupled to other divisome proteins, to determine cell shape and cell division mechanism of *S. pneumoniae.*

MI-P75
PROTOPLASTS FROM *Listeria monocytogenes* 7 RESISTANT MUTANT CANNOT INTERACT WITH ENTEROCIN CRL35

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Enterocin CRL35 is a pediocin-like bacteriocin produced by *Enterococcus mundtii* CRL35. It is active against the food-borne pathogen *Listeria monocytogenes*. To further study its mechanism of action, two resistant *Listeria* variants were isolated from sensitive cells. Once confirmed the identity of the mutant strains by rep-PCR and 16S ribosomal RNA gene sequencing, its phenotype was compared with the sensitive parental strain, *Listeria monocytogenes* 7. The growth of the resistant mutants was slower as compared to the parental strain with lower glucose consumption and a lower medium acidification, showing differences in their metabolisms. On the other hand, even though enterocin CRL35 was able to bind to both sensitive and resistant cells with similar affinity, it was unable to interact with the protoplasts of one of the resistant mutant, as it was revealed by fluorescence polarization. The lack of membrane association in protoplasts strongly suggests that major changes in the plasma membrane of that mutant may take place. Therefore, a complete analysis of the membrane was carried out in order to fully describe the enterocin interaction. The present work shows novel information about the enterocin CRL35 requirements for membrane binding, which is believed to be the key step in the mechanism of action.

MI-P76
ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES β–β

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Staphylococcus aureus is a leading cause of community and hospital acquired infections. The emergence of resistance to β–lactams, followed by the development and spread of strains resistant to multiple antibiotics has made therapy of staphylococcal disease a global challenge. Bacteriophages (phages) have been proposed as an interesting alternative, or addition, to antibiotics to combat this versatile pathogen. One hundred ten strains isolated from animal, human and environmental samples were analyzed. Typification of the isolated *Staphylococcus* strains was performed by conventional biochemical tests as well as by molecular biology techniques (Loop-Mediated Isothermal Amplification). The latter technique was compared to Polymerase Chain Reaction, in order to validate its use as a routine low cost, easy to perform diagnostic technique. Phages were isolated both directly from samples and also from staphylococcal clones present in them by inducing the lytic cycle with mitomycin C or UV light. Currently we have isolated 85 phages of which 30% are lytic. Host range analysis showed that this new phages were highly active against many of our available *Staphylococcus* strains. Pulsed-field Gel Electrophoresis analysis allowed for the estimation of genome size of the phages; genomic sequence now in progress will allow us to find genes encoding useful activities such as antibacterial endolysins.

MI-P77
DESIGN AND CONSTRUCTION OF *Streptomyces* MODULAR VECTORS

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Streptomyces species are widely known by their capacity to produce a vast array of bioactive compounds, which include many antibiotics. Although the genetics and molecular biology of *Streptomyces* sp. have made considerable advances in the last years, the tools that are available to engineer these cells still represent a major limitation for ambitious metabolic engineering applications. In this sense, we are currently working in developing a series of modular vectors using BioBricks^{TM} assembly approach. The goal is to combine, in a customizable way, antibiotic resistance cassettes, conjugation and genome integration functions, promoters and genes, in order to generate plasmids according to the user need. The flexibility and combinatorial properties of this system will provide a myriad of new genetic tools to better understand *Streptomyces* physiology and to continue the development of *Streptomyces* metabolic engineering tools.

MI-P78
CYTOTOXIC ACTIVITY OF AN ASPARTIC PROTEINASE ISOLATED FROM *Salpichroa origanifolia* FRUITS

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Salpichroa origanifolia is a native plant from Brazil, Uruguay and Argentina used in folk medicine for its pharmacological properties. We have isolated an aspartic proteinase (salpichroin) from its mature fruits. The majority of plant aspartic proteinases have a domain named "plant specific insert" (PSI) with high structural homology to saposin like proteins (SAPLIPs). This family of proteins has cytotoxic activity based in the membrane interaction. The aim of this work was to evaluate salpichroin cytotoxic activity in order to compare with other plant aspartic proteinases previously reported. Suspensions of cysts of *Phytophthora capsici* or conidia of *Fusarium solani* were incubated with different amounts of purified salpichroin. Inhibition of germination was determined after 20 h incubation. To check whether the proteinase has an effect on membranes, an assay based on the uptake of the fluorogenic dye SYTOX Green was used. The results obtained show that salpichroin inhibits germination of *P. capsici* and *F. solani* in a dose dependent manner without changing its morphology. Moreover, the increase in proteinase concentration also causes an increase in fluorescence due to the uptake of SYTOX Green. This result suggests that salpichroin produces a cytotoxic effect on *P. capsici* and *F. solani* mediated by a direct interaction with its surface structures.

MI-P79
RIBULOSE 5-PHOSPHATE EPIMERASE FROM *Trypanosoma cruzi* PRESENTS TWO ISOFORMS

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Ribulose 5-Phophate Epimerase (RPE), an enzyme of the non oxidative branch of the Pentose Phosphate Pathway (PPP), catalyses the interconversion of ribulose 5-phosphate and xylulose 5-phosphate. PPP is implied in the protection of the parasite against the oxidative stress, as a source of the NADPH required to maintain reduced the trypanothione, which plays a crucial role in intracellular thiol-redox balance in *Trypanosoma cruzi*. The genome of the CL Brener clone of *T. cruzi* contains two genes encoding RPEs with an identity of 52%; one of them (RPE2) predicts a PTS1 C-terminal glycosomal targeting signal (SHL). We have cloned, expressed, and purified both RPEs as active enzymes, and determined their kinetic parameters. Both isoforms showed Michaelian behaviour, but the catalytic efficiency of RPE1 was two orders of magnitude higher than that of RPE2. For both the optimal pH with Ribulose-5P as the substrate was 7.5. SDS-PAGE analysis gave apparent molecular masses of 27 kDa for RPE1 and 28 kDa for RPE2. Superdex 75 gel filtration showed that both are oligomeric, with two active protein peaks for RPE1 and one for RPE2; in both cases there were only traces of the monomer. Preliminary subcellular localization experiments of immunofluorescence and digitonin extraction suggest that RPE1 is cytosolic, while RPE2 is glycosomal, in good agreement with the presence of a PTS1 signal.

MI-P80
UNUSUAL LOCALIZATION OF POLY(ADP-RIBOSE) METABOLISM-RELATED ENZYMES IN TRYPANOSOMATIDS

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Poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase (PARG) are responsible for poly(ADP-ribose) polymer (PAR) metabolism and have been previously reported in trypanosomatids by our group. We investigated the localization of both enzymes in *T.cruzi and T.brucei* and, interestingly, PARP was localized mostly in the cytoplasm, whereas after oxidative stress (OS), PARP translocated to the nucleus. However, PARG was always nuclear. These localizations are opposite to what has been described in other organisms. In accordance, PAR formation was detected in the nuclei of the cells after OS. To date, neither potential nuclear localization signals nor other putative sequences involved in the protein targeting to different subcellular compartments in these parasites have been ascertained. By using parasites overexpressing the full length PARP and different PARP domains tagged to eYFP or HA, we demonstrate that the N-terminal region is crucial for PARP nuclear localization, even in the absence of OS. This data also implies that nuclear PAR metabolism may be regulated by the nucleus-cytoplasm shuttling of

the only PARP. This particular localization of PARP in kynetoplastids could be related to the maintenance of the integrity of kinetoplast DNA (kDNA), essential for these parasites and could be a strategy to alternatively deliver the protein to this organelle or to the nucleus.

MI-P81
IMPACT OF A RHOMBOID PROTEASE KNOCK OUT MUTATION ON THE PROTEOME OF THE ARCHAEON *Haloferax volcanii*

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Rhomboid proteases are polytopic membrane proteins that perform proteolysis within the hydrophobic environment of the lipid bilayer. Their activity displays and/or releases peptides often involved in cell signaling. To date, only a few rhomboid substrates have been identified. We previously constructed a rhomboid homolog gene (*rhoII*) deletion mutant in *Haloferax volcanii*, which evidenced defective protein glycosylation. To better understand the role of RhoII and to identify potential endogenous targets, we used a proteomics approach. Proteome maps of membrane fractions from *H. volcanii* wt and Δ*rhoII* strains were obtained by means of tryptic digestion followed by RP-nanoLC-ESI-MS/MS. We identified 1906 proteins which corresponded to 47% of *H. volcanii* proteome. Of these, at least 29 changed significantly in amount between the parent and mutant strain (1.2 to 28.7 fold). These proteins belonged to various functional categories including protein degradation, oxidative phosphorylation, signal transduction, ion and protein transport. Of the differential proteins, 9 are predicted as integral membrane proteins and may constitute RhoII substrates. Among the differential soluble proteins 2 enzymes involved in protein glycosylation were down-regulated consistent with the phenotype previously observed in the *rhoII* mutant.
Supported by CONICET, UNMDP, EMBO and Boehringer-Ingelheim

Neuroscience

NS-P01
ROLE OF ACYL-COA SYNTHETASE 4 OFASTROCYTES IN CELL FUNCTION.

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The acyl-CoA synthetase 4 (Acsl4) an enzyme involved in the metabolism of arachidonic acid is involved in cell migration in tumor cells, in the axonal transport of synaptic vesicles and required for normal development of the nervous system in Drosophila. Moreover, this enzyme is mutated in families with non-syndromic mental retardation associated with the X chromosome. Previously we demonstrated the expression and regulation of Acsl4 by cAMP on neonatal rat astrocytes. In this study we use neonatal astrocytes and C6 cells, the rat astroglioma cell line, as model of normal and tumor cells respectively, to study the expression of Acsl4 and its role in cell migration. We demonstrate by Western blot and immunofluorescense that Acsl4 is overexpressed in C6 cells, compared with rat astrocytes. Treatment with triacsine C, an inhibitor of Acsl4 activity, produced significant inhibition of cell migration in both cell models analyzed by wound healing assay. These results were confirmed in C6 cells by knocking down Acsl4 expression by siRNA treatment. The knock down of Acsl4 also reduced the levels of phospho Akt, phospho p70 S6 kinase, and phospho S6 ribosomal protein, one of the main signals in mRNA translation. These data suggest that Acsl4 is an important regulator of cell function in glia cells.

NS-P02
NON-VISUAL PHOTOCHEMISTRY IN THE NON-MAMMALIAN INNER RETINA

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Melanopsin (Opn4) is an important non-visual photopigment present in the vertebrate retina that has been shown to be involved in the synchronization of circadian rhythms, pupillary light reflexes and other light-regulated tasks. In non-mammalian vertebrates there are two Opn4 genes, *Opn4m* and *Opn4x*, the mammalian and Xenopus orthologs respectively. Opn4x is only expressed in non-mammalian vertebrates while both Opn4 photopigments are found in

the chicken inner retina (Verra et al. 2011). Although Opn4 has been extensively characterized, there is still an open question regarding the mechanisms involved in its chromophore regeneration. To this end, we have applied in vivo and ex vivo strategies to investigate retinoids in the chicken inner retina. In isolated retinal layers from chickens exposed to light or maintained in the dark we found that levels of 11 cis-retinal (11cRal) decreased in the photoreceptor cell layer after light exposure, however 11cRal levels were maintained constant or elevated in the retinal ganglion cell (RGC) layer under the same light condition. Besides, cultures of immunopurifided RGCs supplied with all trans-retinal (atRal) displayed the capacity to isomerize atRal into 11cRal after light stimulation. These results strongly support the idea of a novel light-dependent mechanism of chromophore regeneration in the chicken inner retina.

NS-P03
BIOLOGICAL EFFECTS OF GDNF/GFRα1 ON NEURAL CORTICAL PROGENITORS.

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Neurons generation from proliferating progenitor cells is a complex process involving an interplay between intrinsic cellular programs and extrinsic cues such as growth factors. One factor that might play a role in regulating progenitor cell biology is the neurotrophic factor GDNF. Reverse transcription analysis shows that GDNF receptor, GFR1, is expressed at early developmental stages in the forebrain suggesting that GDNF/GFRα1 might play a role in cortical precursor development. Here we show that cultured cortical progenitors maintained in proliferating conditions show lower levels of GFRα1 expression than those maintained in differentiating conditions. Furthermore, cell precursors growth in presence of GDNF result in a significant increase in the morphological complexity of the differentiated neurons. Notably, Ki67 positive proliferative progenitors decreased significantly upon GDNF treatment. Addition of GDNF to proliferating progenitors forming neurospheres resulted in the downregulation of cyclin D and E, required for cell cycle progression, and in the upregulation of p21, a potent cyclin-dependent kinase inhibitor, showing that GDNF induces an arrest of cell cycle of cortical precursors. Thus, our results indicate that GDNF/GFRα1 signaling may play an essential role controlling the transition of neuronal progenitors from a proliferative condition towards neuronal differentiation.

NS-P04
ANANDAMIDE HYDROLYSIS IS MODULATED BY CANNABINOID RECEPTORS IN AGED RAT CEREBRAL CORTEX.

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Fatty acid amide hydrolase (FAAH) has been reported as the main enzyme involved in anandamide (AEA) hydrolysis. Among the multiple functions of AEA we can highlight the regulation of synaptic plasticity and its antiinflammatory role. The aim of this study was to analyze AEA hydrolysis in cerebral cortex (CC) subcellular fractions during physiological aging and its regulation by cannabinoid receptors (CBR). CC membrane and synaptosomal fractions from adult (3 mo) and aged (28 mo) rats were isolated by differential centrifugation and the synaptosomal fraction was purified in ficoll gradients. AEA hydrolysis was assayed using [3H]AEA and its product was quantified from the aqueous phase. Aging differently modulated FAAH by increasing and decreasing its activity in membranes and synaptosomes, respectively. In the presence of FAAH specific inhibitor URB-597 AEA hydrolysis activity assay corroborates that AEA is the main enzyme involved in CC AEA degradation. CBR agonists decreased FAAH activity, mainly by CB2R, thus increasing CC AEA availability. Our results show that while aged CC membrane AEA availability decreases, possibly compromising its antiinflammatory functions, the endocannaboid level in synaptosomes increases, protecting against the synaptic dysfunction inflicted by aging. AEA availability could be increased by targeting CB2R, thus improving brain damage caused by aging.

NS-P05
DISSECTING THE TRANSCRIPTIONAL CODE OF THE DOPAMINE D2 RECEPTOR GENE.

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The dopamine D2 receptor (D2R) plays a major role in the central control of locomotor, appetitive, emotional and cognitive functions. Despite its significance, the molecular mechanisms that control the expression of the D2R gene

(Drd2) in different brain areas remain unknown. BAC transgenic mice carrying a genomic fragment of 220 kb containing Drd2 and EGFP coding sequences (BAC BX37), express the reporter protein in most brain areas where the D2R is normally found. Using BAC engineering we shortened this recombinant BAC to 60 kb. Transgenic mice carrying the 60 kb BAC showed an EGFP expression pattern that was indistinguishable from BX37 mice. To isolate regulatory elements capable of directing the expression into diverse subpopulations of neurons expressing Drd2 we further dissected DNA regions based on sequence conservation criteria. Putative regulatory regions were subcloned upstream of a minimal promoter followed by coding sequences of a red fluorescent protein gene. Analysis of transgenic mouse lines carrying different conserved sequences showed expression of the reporter gene in distinct regions including the septum, endopiriform nucleus, striatum, olfactory tubercle and midbrain. Thus, this methodology seems to be effective for the molecular dissection of transcriptional enhancers of a complex gene.

Plant Biochemistry and Molecular Biology (*Plants*)

PL-P01
NNSR1, A CLASS III RIBONUCLEASE INDUCED UNDER Pi STRESS IN *Nicotiana*, IS LOCALIZED IN ER COMPARTMENTS

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Ribonucleases (RNases) T2 are endonucleolytic enzymes that catalyze the cleavage of single-strand RNA, producing mononucleotides with a terminal 3´ phosphate (Pi). In plants, RNases T2 are grouped in three classes: Class I and class II S-like-RNases are induced in a variety of stress scenarios, including Pi starvation. The induction of these RNases contributes to Pi mobilization from nucleic acid sources. Class III includes S-RNases, involved in the self-incompatibility reaction and non S-RNases, whose function is unknown. Here, we compare the expression of several RNases of *Nicotiana alata* under Pi deprivation. We confirmed that class III NnSR1 (Nicotiana non S-RNaseI) and class I NE are, thus far, the only RNases induced for Pi mobilization in this plant. To test its subcellular localization, NnSR1 was transiently expressed in Arabidopsis protoplasts and Nicotiana leaves as a fusion protein, conjugated to a fluorescent protein. In both cases, NnSR1 colocalized with a fluorescent ER marker. A subcellular fractionation of roots exposed to Pi deprivation showed that the induced native NnSR1 was predominantly associated with ER membranes. NnSR1 appeared to be expressed slightly before the extracellular NE, suggesting that intracellular RNA may be the first source of Pi used by the cell under Pi stress.

PL-P02
EXPRESSION AND PURIFICATION OF A NOVEL KAZAL-TYPE PROTEASE INHIBITOR OF *Arabidopsis thaliana*

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Serine protease inhibitors are widely distributed in plant kingdom. The physiological role of these proteins includes regulation of endogenous proteinases, mobilization of storage proteins and protection against several pathogens. To date there are no reported kazal serine protease inhibitors in plants. However, an in silico analysis revealed the presence of two open reading frames which encode for two kazal-like proteins in *Arabidopsis thaliana*. The aim of the present work was the expression and purification of a putative kazal serine protease inhibitor, which was named *A. thaliana* Kazal-type protease Inhibitor 1 (AtKPI-1). We determined the appropriated host for an efficient expression of the rAtKPI-1 in bacteria testing *E. coli* BL21 pLys, BL21 (DE3) and C41 (DE3) strains. After addition of IPTG, a protein with apparent molecular mass of 12 kDa was detected in BL21 (DE3), while no significant expression levels of rAtKPI-1 was found in BL21 pLys and C41 strains. The presence of the rAtKPI-1T eluted from Ni-NTA chromatography column was confirmed by SDS–PAGE and western blot analysis. From 1 L LB of bacterial culture corresponding to ~ 8 g of *E. coli* cells, 22 mg of purified rAtKPI-1T was obtained. We report an efficient method for the production of large amount of highly purified AtKPI-1T required for studies concerning the biological functions of the members of the kazal family.

PL-P03
CHARACTERIZATION OF A NEW DIHYDROFLAVONOL REDUCTASE/FLAVANONE REDUCTASE FROM MAIZE

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Dihydroflavonol reductase (DFR) activity, involved in anthocyanin biosynthesis, is widely distributed in higher plants. Flavanone reductase (FNR) activity, in contrast, has only been described in some species being implicated in 3-deoxyflavonoid biosynthesis. Maize *A1* locus encodes a protein with DFR and FNR activities and is involved in anthocyanin and flavan-4-ol formation, the latter leading to brick-red phlobaphene formation in kernels. *A1* mutants have brown colored kernels and they are not complemented by the *A4* locus, a paralog of *A1* on which information is limited, suggesting differential expression and regulation among these loci. The objective is to characterize A4 activity in maize. We demonstrate that both loci are activated by the transcription factors P*1* and C*1*/R, regulating phlobaphene and anthocyanin accumulation, respectively. Also, both genes are regulated by UV-B radiation, showing higher transcript levels in leaves from high altitude landraces compared to inbred lines, suggesting that anthocyanin accumulation as another protection mechanism against UV-B radiation developed by these landraces. We isolated the *A4* coding region and expressed it in *E .coli* for *in vitro* activity assays. Our results indicate that A4 has both DFR and FNR activities. We are now testing for its ability to complement Arabidopsis *tt3* mutants to further analyze its function *in planta*.

PL-P04
EXPRESSION ANALYSIS OF THE CATALASE ISOFORMS IN TOMATO PLANTS

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Catalases are one of the major systems for the enzymatic removal of hydrogen peroxide in plants. Plant catalases are tetramers of Fe-heme containing polypeptides. Higher plants may simultaneously express homotetrameric and heterotetrameric isoforms. In most plants three catalase genes have been identified. However, in tomato four genes have been found: *cat1, cat2, er60 and cat3*, being the latter identified in our lab. In order to elucidate the specific functions of catalase isoforms in tomato, enzyme activity, isoform profile employing activity gels and westerns, and gene expression in different parts of *Solanum Lycopersicum* plant (cv. Micro-Tom) were investigated, under normal and stress conditions. The catalase activity and the isoform pattern were different in the various parts of the plant. Also it was found that *cat* genes were expressed in different proportion in the different parts of the plant. This analysis allowed the classification of the tomato catalases according the classification based on the expression pattern and functional analyses established by Willekens. A phylogenetic tree including the tomato catalases supported this classification. These results showed that the different isoforms may contribute to alleviate oxidative stress under different conditions, probably displaying non-redundant functions.

PL-P05
CHARACTERIZATION OF THE CLPB/HSP70 THERMOTOLERANCE SYSTEM OF *Arabidopsis thaliana* CHLOROPLASTS

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Cells exposed to heat stress suffer perturbation of their protein homeostasis. Under these conditions, misfolded proteins lose their function and trigger aggregation of other proteins, threatening cell viability. To cope with these potential mishaps, cells have several defense mechanisms which include protein rescuing by molecular chaperones. In plants, an ATP-driven bi-chaperone system, ClpB/Hsp70, is thought to solubilize and reactivate aggregated proteins. The exact mechanism by which ClpB and Hsp70 act upon protein aggregates to promote their dissolution remains elusive and is completely unknown in plant chloroplasts. In this regard, we aim to unravel this mechanism and its role in the plant thermotolerance process. The chloroplastic *Arabidopsis thaliana* genes CLPB3, CPHSP70-1 and CPHSP70-2 were cloned and heterologously expressed as fusion proteins in suitable strains of *Escherichia coli*. All three proteins were successfully expressed and purified by IMAC. They were properly folded and displayed ATPase activity. Under our experimental conditions cpHsp70s exist in monomeric and dimeric forms as judged by gel filtration chromatography. cpHsp70-1 and -2 displayed different substrate preferences to *E. coli* extract proteins. Our working model is that they select the substrates that ClpB will subsequently disaggregate.

PL-P06
THIOREDOXINS FROM BUNDLE SHEATH AND MESOPHYLL CELLS: ROLE IN MAIZE C4 MALIC ENZYME REDUCTION

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Thioredoxins are ubiquitous small proteins with a highly conserved active site which is linked to thiol redox exchange processes. In chloroplasts, thioredoxins make a connection between the light-mediated processes and the activity of many photosynthetic enzymes. Recent studies with corn chloroplasts show six different groups of thioredoxins with differential expression patterns in the two cell types: Vascular Bundle sheath (BSC) and Mesophyll Cells (MC). Furthermore, proteomic and transcriptomics studies indicate that most thioredoxins are expressed in the BSC cells, except for one belonging to the m4 group, which is localized in MC. Despite the important role of thioredoxins in the regulation of the photosynthetic process, they have not been characterized in maize yet. In the present work, two putative m4 type thioredoxins (called thioBSC and thioMC) were cloned and successfully expressed as recombinant proteins. ThioBSC and thioMC show particular pattern of expression in BSC and MC from maize leaves. Moreover, both thioredoxins were able to catalyze, although to different extent, the reduction and activation of maize photosynthetic NADP-malic enzyme (ZmC4-NADP-ME), the enzyme responsible of the production of CO2 from malate in BSC chloroplasts. The structural basis for the better performance of thioBSC compared to that of thioMC in regulating ZmC4-NADP-ME activity are proposed.

PL-P07
HETEROLOGOUS EXPRESSION OF ASES, AN ELICITOR SUBTILISIN ISOLATED FROM *Acremonium strictum*

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AsES protein, a subtilisin-like serine protease isolated from the avirulent fungus *Acremonium strictum*, induces plant defence response in strawberry and other species. Sequence analysis reveals that AsES possesses a typical pre-pro-mature organization that consists of a signal sequence, an inhibitor domain (pro-peptide) in the N-terminal region with chaperone function and a mature proteinase domain with catalytic activity. The aim of this work was to obtain AsES protein by heterologous expression in order to assess its elicitor activity. The coding sequence of AsES pro-protein was cloned and expressed in *Escherichia coli* and then purified by affinity chromatography. Proteolytic activity was evaluated in vitro by specific substrate hydrolysis and elicitor activity was assessed through ROS formation and protection against *Colletotrichum acutatum*, the causal agent of anthracnose disease. The results of this study suggest that AsES mature protein of 34 kDa is formed by autolysis of the pro-peptide (45 kDa) after its expression and it is functionally active. The mature protein can protect plants against anthracnose and produces the accumulation of ROS at 4 hpi. Direct application of AsES recombinant protein could be used as a new strategy for diseases biocontrol helping to reduce agrochemicals minimizing environmental impact.

PL-P08
PURIFICATION OF AN ASPARTIC PROTEINASE FROM *Solanum elaeagnifolium* FRUITS (SEAP-1)

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Solanum elaeagnifolium is an endemic plant from the northeast of Mexico. This plant in some places of Mexico has been used for decades in the manufacture of artisanal filata-type asadero cheese. The milk-clotting activity of *S. elaeagnifolium* has been attributed to aspartic proteinases. The aim of this work was to purify aspartic proteinases from fruits of *S. elaeagnifolium*. Purification was performed from lyophilized fruits of *S. eleagnifolium* by ammonium sulfate precipitation, ion exchange chromatography and Pepstatin A affinity chromatography. SDS- PAGE analysis of proteins eluted from the affinity column shown only one protein band corresponding to 59 kDa, approximately. Identity of this protein band as aspartic proteinase was demonstrated by Western blot analysis, using as primary antibody IgG-anti *St*AP1 (*Solanum tuberosum* aspartic proteinase 1). Results obtained from gel filtration chromatography (Superose 12) demonstrates that, isolated SeAP1 is a monomeric enzyme, with an estimated molecular weight of 52 kDa., approximately. Additionally, we determined that, like almost monomeric plant APs, *Se*AP-*1* exerts cytotoxic activity towards plant pathogens in a dose- dependent manner. These results suggest the

presence of saposin- like domain into the sequence of the mature *Se*AP-*1* and therefore, new biotechnological applications for *Se*AP-1.

PL-P09
RECOMBINANT EXPRESSION OF *Arabidopsis* SNRK1 KINASE IN DIFFERENT OLIGOMERIC FORMS

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Sucrose non-fermenting-1 related protein kinases 1 (SnRK1s) are heterotrimeric proteins composed of α (catalytic) and β plus γ (regulatory) subunits. Members of this kinase family act as central regulators of metabolism. They are generally activated by starvation conditions, generating the trigger of changes in carbon and nitrogen metabolism to maintain energy homeostasis. Kinase action modifies gene expression and enzyme activity through phosphorylation. *Arabidopsis* genome has two genes codifying for SnRK1 α subunit (At3g01090 and At3g29160), three for the β subunit (At5g21170, At4g16360 and At2g28060) and two for the γ subunit (At3g48530 and At1g09020). The latter implies the probable existence of different SnRK1 heteroligomeric forms with distinctive kinetic and/or regulatory properties. Herein we report a protocol to recombinantly express different SnRK1 forms in Escherichia coli after cloning the α, β, and γ genes into pDUET vectors. All the combinations (single gene or co-expression of two or three genes) of the SnRK1 proteins were produced and analyzed for activity. We also expressed the kinase (At3g45240) that phoshorylates (to activate) the SnRK1 catalytic subunit, as well as the α mutant mimicking a phosphorylated state. These molecular tools are critical to understand the kinetic, regulatory and target specificity properties of the different SnRK1 assemblies.

PL-P10
OVEREXPRESSION OF GLYCINE RICH RNA BINDING PROTEINS IN TOMATO FRUITS

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The genome of *Solanum lycopersicum* presents three genes coding for glycine rich RNA-binding proteins (GRPs) named *LeGRP1a-c*. For each gene, three transcriptional products are identified, the un-spliced pre-RNA, the mature mRNA and the alternative spliced mRNA (*preLeGRP1a-c*, *mLeGRP1a-c* and *asLeGRP1a-c*, respectively). In this study, *S. lycopersicum* cv. Micro-Tom overexpressing the premature transcript of *LeGRP1a* gene (*preLeGRP1a*) in tomatoes was analyzed. The expression pattern of *LeGRP1a-c* were evaluated in transgenic fruits and compared with wild type in three stages of fruit development, immature green, mature green and red ripe. Particular circadian profiles of expression of all transcriptional forms of *LeGRP1a-c* and the corresponding total immunoreactive LeGRP1 indicates that the overexpressed transcript (*preGRP1a*) is processed and also regulates the expression of the other family transcripts. LeGRP proteins show the classical N-terminal end RNA recognition motif and the C-terminal end glycine-rich region, suggesting conservation of functional properties. Recombinant LeGRP1a protein binds single strand RNA but not double strand DNA. Future experiments in order to elucidate the candidates of this protein will explain its importance in response to various stresses.

PL-P11
PROTEOLYTIC FRACTION ISOLATED FROM *Phytolacca dioica* L. BERRIES

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Phytolacca dioica L., commonly known as “ombú”, is a massive tree native to the Pampa of South America and member of the Phytolaccaceae family. The partially purified extract of berries has been characterized in our previous studies. This extract contains proteolytic activity using casein and hemoglobin as substrates, and it is completely inhibited by E-64. The electrophoretic patterns show one major band of 25.7 kDa (characteristic of cisteinproteases) and two less intense bands of 29 and 44 kDa, approximately. Isoelectrofocusing analysis displays multiples bands in pI range 5.20 - 6.55 and a few with lower pI. In the present study we have isolated the proteolytic fraction of the extract by means of ion exchange chromatography. The extract (previously ultrafiltrated using 10 kDa membrane) is passed through an anion exchange column obtaining thereby three retained peaks, one of which with proteolytic activity. This fraction corresponds to the proteins of 25.7 kDa and pI between 5.20 and 6.55. We also proved that the

proteins of 29 and 44 kDa have not proteolytic activity and present acidic pI. The final purpose of our study is to isolate the protease responsible of the proteolytic activity.

PL-P12
RELEASE OF β-AMYLASE IS MEDIATED BY THIOREDOXIN H AND DEPENDS ON PROTEIN CONTENT IN BARLEY GRAIN

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β-amylase is one of the most important starch degrading enzymes in cereals. It is found in two forms in mature barley seeds, soluble and insoluble. The insoluble form is linked to storage proteins by disulfide bonds and it is released during germination. It has been found that thioredoxins belonging to the h family (Trx h) are very active during seed germination, where they modulate the activity of various enzymes by thiol-disulphide redox transformation. Two isoforms, h1 and h2, were described in barley but their specific functional roles have not been completely clarified. The aim of this study was to evaluate the ability of Trx h1 and h2 to release β-amylase molecules through the cleavage of their disulphide bonds with storage proteins to make them active. Both h1 and h2 isoforms were heterologously expressed in *Escherichia coli* and purified. Their activities were tested on samples of ungerminated seeds with different protein concentration. It was found that both isoforms were able to release β-amylase. Moreover, they showed differential activity depending on seed composition. In our knowledge, this is the first study that assigns to seed thioredoxins this kind of function during germination, different from their previously described activity over α-amylase inhibitors.

PL-P13
ACTIVITIES OF α-AMYLASE AND ITS INHIBITORS IS AFFECTED BY NITROGEN AND SULFUR CONTENT IN BARLEY SEED

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Plant seeds have different proteinaceous α-amylase inhibitors that participate in both the control of the endogenous α-amylase and the protection of seeds from pests. Barley α-amylase/subtilisin inhibitor (BASI) is synthesized during grain filling and inhibits the major α-amylase isozyme synthesized during germination (AMY*2*). It is known that mineral composition of the grain may affect the content of various proteins, whether they are structural, storage or metabolic proteins. The aim of this work was to evaluate the effect of nitrogen (N) and sulfur (S) grain content on the activities of α-amylase and its inhibitors. Seeds with different chemical composition were placed under germination conditions and α-amylase activity during germination and its enzymatic inhibition were measured up to 48 h after imbibition. The enzyme activity increased in the 48 hours following imbibition and was highest in seeds with greater content of N. Higher nutrient concentration (either N or S) resulted in lower inhibitor activity. These results indicate that chemical composition of the seeds could affect germination by regulating both α-amylase activity and that of its inhibitors.

PL-P14
DC1-DOMAIN-CONTAINING-PROTEINS: FUNCTIONAL CHARACTERIZATION AND THEIR ROLE IN PLANT DEVELOPMENT

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A comprehensive study of genes involved in plant defense responses led us to identify two DC1 domain genes (*At2g17740* and *At2g44370*) whose expression is strongly induced by pathogens and elicitors in *Arabidopsis thaliana*. The analysis of insertional mutants revealed that these genes are required for normal development of the female gametophyte. In order to functionally characterize these genes we made different expression reporter lines. The analysis of promoter:GUS reporter lines showed low expression levels under normal or biotic stress conditions. Overexpressing lines that were constructed as translational fusions with GFP, allowed us to identify the subcellular localization of these proteins in the plasmatic membrane. Through Bimolecular Fluorescence Complementation (BiFC) assays we determined that these proteins interact with two different transcription factors that participate in the regulation of the defense response to biotic stress. Supported by Conicet, ANPCyT y UNMdP

PL-P15
PHENOTYPIC ANALYSIS OF *Arabidopsis thaliana* LINES WITH MODIFIED LEVELS OF NADP-MALIC ENZYMES

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Throughout recent years multiple roles have been proposed for the NADP-malic enzymes (ME) of C_3 plants at different stages of plant development. In order to reveal physiological processes most influenced by the activity of these enzymes in *Arabidopsis thaliana*'s metabolism, eight transgenic lines with constitutive alterations in the content of malic enzyme were characterized phenotypically along the whole plant development. Each line showed specific pattern of variations, however, the Arabidopsis line knockout in the cytosolic constitutive isoform ME*2* was the most affected. This line presented altered features such as germination, root growth, leaf production, shoot growth and seed production. On the other hand, considering the different stages of the plant life, the reproductive stage was the most affected by changes in ME levels.

PL-P16
DIFFERENTIAL EXPRESSION OF NEUTRAL/ALKALINE INVERTASE ISOFORMS IN WHEAT TISSUES

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Alkaline/Neutral invertases (A/N-Invs) are sucrose hydrolyzing enzymes with optimum pH ranged from 6.8 to 8.0. They have been identified in plants and cyanobacteria. In plants, from being minor proteins, scarcely mentioned in comprehensive descriptions of carbon flux, they recently became leading actors in sucrose metabolism. A/N-Invs have significant roles in root morphogenesis, photosynthetic apparatus biosynthesis, and abiotic stress adaptation. In the present work we analyze by Western blot and RT-PCR the occurrence of different isoforms in leaf and root extracts from wheat seedlings after a cold treatment. The results showed that in leaves, at least, a novel A/N-Inv isoform is induced in response to low temperature. Also, the expression of wheat A/N-Inv putative genes is differentially regulated in roots and leaves in response to adverse environmental conditions. Our data support the role of A/N-Invs in relation to abiotic stresses and that they may be involved in responses to cope with them (supported by PIP 134, UNMdP (EXA645), PICT 1288, and FIBA).

PL-P17
PHYSIOLOGIC AND METABOLIC CHANGES ASSOCIATED TO ALTERED EXPRESSION OF UKCCT IN *Arabidopsis*

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UKCCT belongs to a large family of uncharacterized genes which possess just a single CCT domain. CCT domain-containing proteins are considered to function as transcriptional regulators implicated in diverse processes such as photoperiodic flowering, regulation of circadian rhythms, light signalling and gene expression in response to sugars. During the photoperiod, fine control of redox homeostasis is needed to prevent ROS overload due to excess light. Failure to maintain redox balance results in growth defects or initiation of cell death. Arabidopsis plants over-expressing UKCCT (OE-UKCCT) show a deregulation of O2- and H2O2 levels, chlorotic and hyponastic leaves resembling the phenotype observed in cat2 mutants. The analysis of the mechanisms probably involved in the detoxification of ROS revealed that the high production of H2O2 overwhelms its clearance even total catalase activity is increased. Moreover, these plants accumulated differentially metabolites related to stress such as proline, raffinose and galactinol. Interestingly, OE-UKCCT lines develop spontaneous lesions where intensity of damage seems to be photoperiod dependent. A detailed phenotypic analysis of mutant lines of this gene revealed a higher photosynthetic performance and increased seed set. Our working hypothesis is that UKCCT could act in the regulation of ROS homeostasis during photoperiod.

PL-P18
CLONING AND PRODUCTION OF RECOMBINANT MAIZE CYCLIN D6 PROTEIN

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Cyclins are primary regulators of the activity of cyclin-dependent kinases and play crucial roles in cell cycle progression in eukaryotes. In plants, D-type cyclins (G*1* cyclins) regulate cell cycle reentry during meristem activation to promote successful germination and early seedling growth. Moreover, the larger number of cyclin D (CYCD) genes in plants compared with animals is indicative of the importance of these proteins being involved in the linkage between environmental conditions and the plant cell cycle. As part of a project that studies posttranslational modifications (MPT) of proteins related to cell cycle in plants, herein we present the cloning and expression of one of the Zea mays L (maize) cyclin D -Zeama, CycD6,1- characterized by lacking the pRBR-binding sequence. The ADN fragment corresponding to CycD6 was isolated using specific primers and cloned in a pGEX 4T-2. Initially BL21-DE3 E. coli strain was chosen as the expression system. Since the level of recombinant protein produced by this system was low and mainly located in inclusion bodies, the plasmid was transferred to Rosetta gami pLysS strain. This system resulted in a significant improvement regarding the levels of recombinant protein production. Through analysis using informatics tools an approximation of the protein sites susceptible to suffer MPT will be presented.

PL-P19
PPZAT12 EXPRESSION IN PEACH FRUIT: A TRANSCRIPTION FACTOR WITH A ROLE AGAINST CHILLING INJURY

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Harvested peaches [*Prunus persica* L. Batsch] deteriorate quickly at ambient temperature. Refrigeration is therefore used to prevent the rapid fruit decay and to extend fruit shelf life. However, cold storage can generate physiological disorders collectively termed ‘chilling injury’ (CI). Heat treatment (HT) prior to cold storage has been proved to be an efficient post-harvest technology given that it prevents or alleviates CI symptoms while maintaining organoleptic quality. To address the molecular mechanisms associated to CI, our group has characterized the differential transcriptome and proteome of peaches cv. Dixiland subjected to HT. One of the HT-induced transcripts was PpZAT12 which encodes a C2H2 zinc finger protein. RT-qPCR analysis confirmed that PpZAT12 transcriptional levels are induced by HT and short-term refrigeration. Moreover, 21-days-refrigerated samples from different cultivars with contrasting susceptibility to CI showed distinct transcript accumulation patterns. PpZAT12 promoter analysis reveals the presence of classic hormone- and stress-response cis-acting elements. We also aimed to define PpZAT12 targets in peaches based on transcriptomic information from ZAT12-overexpressing Arabidopsis plants which had augmented freezing tolerance. These results point that PpZAT12 could have a function against CI and may contribute to HT effectiveness in countering CI symptoms.

PL-P20
COX19: A NEW PLAYER IN METAL HOMEOSTASIS IN PLANTS

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Cytochrome c oxidase (COX) biogenesis is a complex process that involves the action of many proteins. The metallochaperone COX19 participates in copper (Cu) delivery for COX assembly. To understand the role of COX19 in plants, we silenced the expression of both A. thaliana COX19 genes using artificial microRNAs. Silencing of AtCOX19 caused a decrease in Fe levels, the induction of genes encoding Fe transporters and other Fe-deficiency responsive genes and a decrease in the activity of the low-copper responsive MIR398a promoter. The roots of plants with decreased AtCOX19 levels were longer than those of wild-type plants when grown under Fe-deficient conditions and were less affected by an increase in Cu concentration. The results suggest that a decrease in AtCOX19 expression originates a condition of copper sufficiency and iron deficiency in the plant. Cu and Fe are essential nutrients but also toxic when present in excess. Our results indicate that, in addition to its role in COX assembly, COX19 participates in the regulation of metal homeostasis in plants.

PL-P21
FUNCTIONAL STUDIES OF THE *Arabidopsis thaliana* TCP8 TRANSCRIPTION FACTOR

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Proteins of the TCP family are transcription factors that can be found only in plants and regulate many structural and developmental traits. Initially, they were thought to play a role in cell growth and proliferation but now it is known that they are also involved in the regulation of hormone homeostasis, the circadian cycle, germination, and senescence, thus regulating plant growth and development in response to both internal and external stimuli. In this work, we studied the function of TCP8, a member of Arabidopsis class I TCP subfamily. We found that plants that express TCP8 from the 35SCaMV promoter flower earlier than wild-type and have changes in the number and shape of leaves and in plant architecture. In agreement with these results, FT and SOC[1]. two genes involved in the promotion of flowering, showed increased expression in these plants. Expression studies using RT-qPCR indicated that TCP8 is expressed in roots, rosette and cauline leaves, and flowers along the entire plant life cycle. Plants transformed with a promoter:GUS fusion showed reporter activity mainly in meristems and vascular tissues. Our results suggest that this transcription factor regulates several aspects of plant development and architecture in Arabidopsis.

PL-P22
ATTCP15 PARTICIPATES IN THE ELONGATION OF STAMEN FILAMENTS IN *Arabidopsis thaliana*

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The transcription factor family TCP is specific of plants. This family is characterized by the presence of the TCP domain, which binds specific DNA sequences. A. thaliana has 24 TCP proteins divided into two classes, I and II. In this work, we characterized the role of TCP15 in stamen development. Expression of TCP15 in anther filaments was observed with a GUS reporter. Plants that express a fusion of TCP15 to the EAR repressor motif developed shorter stamen filaments, while plants that express TCP15 under the control of the 35S promoter showed longer filaments compared to wild-type plants. We then measured transcript levels of SAUR63, an auxin response gene implicated in filament elongation. We found reduced and increased levels of SAUR63 in TCP15-EAR and 35S:TCP15 plants, respectively. Given that SAUR63 has in its promoter the specific sequence recognized by TCP transcription factors, these results suggest that TCP15 may activate directly its expression. The elongation of the filament is promoted by gibberellins (GA). Then, we also tested the possibility that GA regulates TCP15 expression by measuring its transcript levels in flowers of a mutant in DELLA proteins. Both, TCP15 and SAUR63 were induced in the mutant, suggesting a coordination between GA and TCP15 for the normal elongation of the stamen filament.

PL-P23
ATTCP15 IS INVOLVED IN GYNOECIUM DEVELOPMENT THROUGH THE REGULATION OF AUXIN AND CYTOKININ RESPONSES

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The TCP transcription factor family belongs exclusively to plants and is involved mainly in plant development. We studied the function of a TCP protein from A. thaliana, TCP15. We transformed plants with the coding region of TCP15 under the control of the 35SCaMV promoter. These plants present defects in the apical portion of gynoecia, where lack of fusion between the two carpels was observed. In contrast, the expression of TCP15 fused to the EAR repressor domain originates the development of outgrowths topped with stigmatic papillae from the replum. In Arabidopsis, the correct development of the gynoecium is regulated by an auxin gradient, with a maximum in the apical portion. Two auxin biosynthesis genes, YUC1 and YUC4, presented lower expression levels in 35S:AtTCP15 plants, and higher in TCP15-EAR plants. In addition, 35S:AtTCP15 plants showed lower expression of the auxin reporter DR5:GUS in gynoecia. The effects of overexpressing TCP15 or its repressor form on gynoecium development are enhanced after cytokinin treatment. In addition, treatment of a loss-of-function tcp14 tcp15 double mutant with cytokinin originates an increased development of outgrowths from the replum and the induction of the auxin biosynthesis gene YUC4. These results suggest that AtTCP15 participates in the proper development of the gynoecium by influencing the cross-talk between auxin and cytokinin.

PL-P24
ATERF019 IS INVOLVED IN THE CROSSTALK BETWEEN LEAF SENESCENCE AND OXIDATIVE STRESS IN ADULT PLANTS

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AtERF019 transcription factor, which is a member of the APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR (ERF) gene family, was induced under oxidative stress originated in chloroplast. Plants overexpressing *AtERF019* (ERF019 lines) showed not only a delay in the onset of leaf senescence but also an enhanced drought tolerance compared to Col-0 plants that was observed in 36-day-old plants but not in 15-day-old plants. The delay in natural senescence was corroborated by measuring the different senescence associated physiological markers, such as chlorophyll and protein contents as well as RuBisCO large subunit and glutamine synthetase levels. Added to this, dark induced senescence was observed in adult plants but not in ERF019 seedlings. In line with these results, ERF019 and Col-0 leaves from adult plants were subjected to treatments that induce senescence such as salt, ethylene and ABA. In all these experiments it was observed that chlorophyll degradation occurred to a lesser extent in ERF019 lines than Col-0 plants. Taking into account these results, we suggest that AtERF019 transcription factor may play a role in the crosstalk between stress resistance response and senescing process in adult plants. Furthermore, AtERF019 may require coordination with developmentally regulated components in order to activate certain genes related with senescence and stress tolerance.

PL-P25
THE ARABIDOPSIS HETEROCHROMATIN REMODELING PROTEIN MOM1 REGULATES DEFENSES AGAINST PATHOGENS

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In Arabidopsis, transposable elements (TEs) are concentrated at centromeric heterochromatin but also present at the proximity of genes. Different mechanisms repress TE expression by recruiting epigenetic marks. Among them, MOM1 negatively affects a subset of pericentromeric TEs from the *Athila* superfamily allowing methylation of Lysine 9 from histone H3. Consequently, *mom1* mutant shows activation of these elements. To study how this protein affects defenses against hemibiotrophic pathogens, we used the *mom1* mutant in infection studies. *mom1* was more resistant to *Pseudomonas syringae* pv *tomato*, but did not up-regulate defense genes at basal conditions. RT-PCR analysis and available *mom1* transcriptomes suggested that this plant was prone to induce pathogen defense genes. In fact, infection assays confirmed that the mutant exhibited priming. Some TEs modulate the expression of neighboring genes by affecting local chromatin structure. After evaluating this possibility, we propose a model for MOM1-mediated defense activation.

PL-P26
GENERATION OF EPIGENETIC VARIABILITY IN THE POTATO CULTIVAR SPUNTA USING A DEMETHYLATING AGENT

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Changes in DNA sequences are not the only responsible for the observed variability, epigenetic changes as DNA methylation alters chromatin structure and gene expression. Established varieties of potato (*Solanum tuberosum*) are genotypes that have a narrow genetic base, making progress in potato improving slow and often accompanied by deterioration of characters. Modify the methylation pattern of potato cultivars commercially important as Spunta, is an alternative to generate epigenetic variability useful for potato breeding. The aim of this study was to evaluate the effects of demethylating AzaC (azacitidine) on the generation of new epigenetic variation in cultivated potato clones. From potato clones of the variety Spunta (in vitro) uninodal stakes were obtained which were differentially treated with the demethylating agent AzaC. From these stakes plants were regenerated, overall methylation status were analyzed by MSAP (Methylation Sensitive Amplified Polymorphism) technique. Preliminary results show changes in methylation patterns of clones treated with AzaC compared to controls. This will be an important contribution to the development of new tools for crop improvement.

PL-P27
EPIGENETIC REGULATION ON EPIDERMAL CELL DIFFERENTIATION AND ROOT HAIR DEVELOPMENT IN *Arabidopsis*

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Our goal is to elucidate the mechanisms of epigenetic regulation on gene expression related to cell differentiation and root hair formation in the root epidermis of *Arabidopsis thaliana*. To achieve this, we tuned up a protocol developed by Deal & Henikoff to isolate purified nuclei from both trichoblasts (cells that form root hairs) and atricoblasts (cells that do not form root hairs). To determine the methylation marks on cytosines in specific loci known to be involved in the biological processes mentioned above, we will perform the bisulfite technique on total DNA from such purified nuclei. In addition, to identify the accompanying chromatin modifications, we will follow the Chromatin Immunoprecipitation procedure on the same purified nuclei. This course of action will be taken on both wild type and mutant lines knocked out in the genes encoding transcription factors involved in either root epidermal cell differentiation or development of root hairs. In this context, to study the influence of epigenetic marks on hair development, we will also investigate them in plants mutant for different DNA- or histone-methyltransferases displaying an overt phenotype regarding length, density or distribution of root hairs. So far, we found that plants mutants for VIM1 (cofactor of DNA methyltransferase) and KYP (a histone methyltransferase) exhibit root hairs shorter than those of WT plants

PL-P28
LIGHT, CHROMATIN AND ALTERNATIVE SPLICING

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Light is not only a source of energy but also a key regulator of plant physiological adaptations. We have previously shown that light/dark conditions affect alternative splicing of the pre-mRNA for At-RS31, a Ser-Arg-rich splicing factor. This led us to investigate whether chromatin modifications play a role in this light regulation of alternative splicing. Increasing concentrations of trichostatin A (TSA), a drug that suppresses histone deacetylase activity and therefore increases histone acetylation, mimic the effect of light on At-RS31 alternative splicing in a dose-dependent manner. The light/dark effect and the TSA effect on alternative splicing are not observed in the pre-mRNA for At-RS2Z33, another SR-splicing factor used as a control. Global histone acetylation does not seem to change between light and dark treated plants. Using Arabidopsis mutants defective in different histone deacetylases we found that the light/dark effect is strongly reduced in hd1 mutants. In contrast, the light/dark effect is still observed in hda15 mutants, suggesting a mechanism that involves specific histone modification enzymes. Further experiments such as a chromatin inmunoprecipitation assay of chromatin marks will be carried out in the future to determine the link between chromatin modifications and alternative splicing regulation by light.

PL-P29
THE ROLE OF ISOPRENOIDS IN THE PROTECTION AGAINST UV-B RADIATION

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Isoprenoids are a group of biologically active molecules present in all living organisms, and some of them have general functions in growth and development. There are two unrelated routes for the synthesis of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), which are the precursors for the biosynthesis of all types of isoprenoids in plants: the acetate/mevalonate (MVA) pathway occurs in the cytoplasm while the MEP pathway (named for the first committed molecule in the pathway, 2Cmethyl-D-erythritol 4-phosphate), occurs in the plastids. In order to analyze the role of isoprenoids in protecting plants against UV-B radiation, different Arabidopsis thaliana transgenic lines with increased or decreased levels of enzymes of the MEP pathway, which catalyze the first and the penultimate steps in the biosynthesis of IPP and DMAPP, were UV-B-irradiated and we evaluated DNA damage accumulation by dot blot assays. Physiological parameters were also assessed immediately after the UV-B treatment, such as the maximum efficiency of photosystem II, chlorophyll and flavonoid content. Together, our data demonstrate a possible protective role of isoprenoids against UV-B radiation.

PL-P30
EPIGENETIC REGULATION OF P1 IN LEAVES OF HIGH ALTITUDE MAIZE LANDRACES: EFFECT OF UV-B RADIATION

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P1 is a R2R3-MYB transcription factor (TF) that regulates the accumulation of a specific group of flavonoids in maize floral tissues, among them the flavones and phlobaphenes. Maize landraces adapted to high altitude also express P1 in leaves. In this work, we have analyzed the epigenetic regulation of the P1 gene in leaf tissues of different landraces at the level of DNA methylation and histone modifications, and also investigated if different P1 targets, which were identified in pericarps, are also regulated by this TF in response to UV-B. Our results show a decrease in the percentage of DNA methylation of three different P1 regions by UV-B (proximal promoter, intron1 and intron2). This DNA modification is accompanied by a decrease/increment in the level of histone methylation/acetylation of histones, respectively. We also observed that the basal DNA methylation levels are lower in the landraces respect to B73, a low altitude line. The transcript levels of some P1 targets in leaves show an induction in the analyzed landraces in response to UV-B, and also higher basal expression levels in comparison to B73. In conclusion, P1 expression is regulated epigenetically promoting the expression of this TF, which in turn regulates the expression of different target genes, some of them involved in the synthesis of flavonoids in response to UV-B in maize leaves.

PL-P31
ROLE OF ATMSH7 IN UV-B-INDUCED DNA DAMAGE RECOGNITION AND RECOMBINATION

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Highly conserved DNA mismatch repair (MMR) systems promote genomic stability by correcting DNA replication errors, antagonizing homeologous recombination and responding to various DNA lesions. The first step of the pathway involves recognition of the DNA lesion by MutS proteins. Higher plants contain an extra MutS protein named MutSγ (MSH2-MSH7). To further understand the in vivo role of MSH7, we present data from this protein in *Arabidopsis thaliana*. Histochemical staining of transgenic plants that express the β-glucuronidase (GUS) under the control of the MSH7 promoter indicated that *MSH7* is restricted to proliferating tissues. Then, we identified *msh7* T-DNA insertion mutants. Plants deficient in MSH7 show increased levels of UV-B-induced cyclobutane pyrimidine dimers (CPDs) relative to wild-type (WT) plants. We next analyzed the role MSH7 during somatic recombination between homologous or homeologous repeats (divergence level of 1.6%) using a GUS recombination reporter assay. Disruption of *MSH7* has no effect on the rates of somatic recombination under control conditions or after UV-B exposure. However, the rate of meiotic recombination between two genetically linked seed-specific fluorescent markers was 97% higher in *msh7* than in WT plants. Taken together, these results suggest that MSH7 is involved in UV-B-induced DNA damage recognition and in controlling meiotic recombination.

PL-P32
A MODEL SYSTEM FOR ACTIVATION OF COMPLETE AND INCOMPLETE PROLINE CATABOLISM IN *Arabidopsis*

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Proline (Pro) metabolism affects the development of the Hypersensitive Response (HR), a local defense program against biotrophic pathogens. In particular, proline dehydrogenase (ProDH) is necessary for optimal generation of the oxidative burst, a hallmark of this response. ProDH converts Pro into Δ1 pyrroline-5-carboxylate (P5C). In the complete catabolic pathway, P5C is transformed into Glu by P5C dehydrogenase (P5CDH). In the incomplete pathway, P5C can be reconverted to Pro by P5C reductase (P5CR) activating the Pro/P5C cycle. To start dissecting the effect of ProDH on HR, we generated transgenic Arabidopsis plants allowing activation of each of these pathways. These plants carry the *Est:ProDH* trasgene, either in the wild type (WT) or *p5cdh* backgrounds. As expected, estradiol induced *ProDH* in WT plants. Moreover, it also activated *P5CDH* indicating this plant stimulated the complete catabolic pathway. On the *p5cdh* background, estradiol induced *ProDH* as in WT plants confirming stimulation of incomplete Pro catabolism. Both plants were used to analyze ROS-sensitive markers and expression of Pro biosynthetic genes. Different responses were obtained in each of them indicating that ProDH may have diverse cellular impacts depending on the possibility of coupling with the P5CDH activity

PL-P33
EFFECT OF MAGNETITE IRON OXIDE NANOPARTICLES ON WHEAT PLANTS

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The rapid development of nanotechnology will inevitably release nanoparticles into the environment with unknown consequences. Moreover, few studies have focused on phytotoxicity of nanomaterials. For that reason, the aim of this work was to evaluate if Fe_3O_4 nanoparticles are toxic for wheat (Triticum aestivum L.) plants grown under hydroponic conditions by measuring $O_2^{.-}$ and H_2O_2 content, catalase, peroxidase, guaiacol peroxidase and superoxide dismutase activities, electrolyte release, cell death and malondialdehyde content. Visualization of root sections by transmission electron microscopy showed that Fe_3O_4 nanoparticles (nFe_3O_4) probably entered through the apoplastic route and were then detected in the root epidermal cell walls. However, nFe_3O_4 were not found in shoots, implying that nanoparticles could not be transported by vascular tissues in wheat, at least in the experimental conditions of this study. In the concentrations used, nFe_3O_4 did not modify the cell death percentage, the electrolyte leakage, the malondialdehyde content, the accumulation of H_2O_2 or $O_2^{.-}$, but the antioxidant enzyme activities increased considerably in roots and shoots compared to control plants. These preliminary results show that these nanoparticles are not phytotoxic, suggesting that nFe_3O_4 could potentially be useful for the design of new products for agricultural use.

PL-P34
ROLE OF CHLOROPLAST-GENERATED REACTIVE OXYGEN SPECIES IN PLANT-PATHOGEN INTERACTIONS

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Plant production of reactive oxygen species (ROS) in various cellular compartments is a hallmark of pathogen recognition. Chloroplast-generated ROS direct strategic decisions on plant responses and contribution of this organelle to plant immunity is further underscored by the fact that plant defence systems have been linked to light perception mechanisms. The role of chloroplast-borne ROS in plant defence is evaluated using transgenic tobacco lines expressing a plastid-targeted flavodoxin, which showed increased tolerance to multiple abiotic stresses and lower ROS accumulation in chloroplasts. These are employed to study the light requirements for ROS production in chloroplasts during interactions with pathogens. We report herein the phenotypic and biochemical characterization of the interaction between these plants and different pathovars of *Pseudomonas syringae* that display compatible or incompatible interactions, in the presence or absence of light. Qualitative and quantitative ROS assessment revealed that transgenic plants accumulated fewer ROS and suffered less damage than wild-type siblings only in the presence of light. Metabolic profile analyses showed that flavodoxin-expressing lines are able to better cope with any type of pathogen when light is present. The results provide insights into the role of light and ROS on the succession of events that follow pathogen recognition.

PL-P35
ROS ACTIVATED IN STRAWBERRY PLANTS BY SMS IS SUPPRESSED BY SUPERNATANT OF *Colletotrichum acutatum*

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The generation of Reactive Oxygen Species (ROS) is one of the early defense responses activated in plants by biotic and abiotic stresses. Previously, we observed ROS production and up-regulation of *catalase* and *pathogenesis related protein 1* (*FaCAT* and *FaPR1*) genes in strawberry leaves subjected to Soft Mechanical Stimulation (SMS, softly pressing the leaves with the fingertips). The aim of this work was to evaluate the effect of treatment with the supernatant of the virulent isolate M11 of *Colletotrichum acutatum* (SN-M11) on the biochemical and molecular responses of SMS in strawberry. Four treatments were applied to axenic *in vitro* strawberry plants by triplicate: 1) SN-M11, 2) SMS, 3) SN-M11 and 4 hours later SMS and 4) H_2O. Leaves where then removed for histochemical staining (NBT and DAB) and RNA extraction. The expression of FaCAT and FaPR1 genes were analyzed by qPCR. Results showed that leaves treated with the SN-M11 exhibited lesser H_2O_2 and O_2^- production than SMS treated plants, showing also a down-regulation of FaCAT transcripts. Nevertheless, up-regulation of FaPR1 was observed in treatments 2) and 3) as compared to 1) and 4). These results suggest that SN-M11 suppresses the production of ROS but not the defensive response activated by SMS.

PL-P36
MAIZE FRATAXINS: A ROLE IN IRON HANDLING AND ROS PROTECTION?

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Iron is essential for many cellular functions but free iron can be toxic at physiological conditions, thus it must be carefully regulated. The highly conserved protein Frataxin (FH), characterized in bacteria, yeast, humans and Arabidopsis thaliana (AtFH), represents a candidate for iron management. FH has been proposed to participate in Fe-S cluster assembly, iron homeostasis, heme metabolism, ROS and REDOX control and protection against oxidative damage. In eukaryotes, FH is nuclear encoded with mitochondrial localization. We identified in maize two coding sequences -ZmFH42 and ZmFH55- homologous to AtFH. Recently, we have been cloned, purified and characterized the iron binding properties of these isoforms by the attenuation of Fenton´s reaction and their capacity to maintain Fe(II) in solution. We have also determined their subcellular localization by fluorescent confocal microscopy, suggesting a dual localization in mitochondria and chloroplast. In this work, we show the self-assembly properties of both recombinant proteins in the presence of iron by nondenaturing PAGE. Besides, we demonstrate their protective role against oxidative damage in Saccharomyces cerevisiae FH knock out cells complementation assays. These preliminary results suggest that ZmFH42 and ZmFH55 may play important roles in protection against oxidative stress and iron homeostasis and bioavailability.

PL-P37
CHARACTERIZATION OF ARABIDOPSIS SINA-L7, A POTENTIAL MEDIATOR OF A RRM AND ITS INTERACTION WITH GAPC

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Arabidopsis Seven in absentia-like 7 (SINA-L7), belonging to the “seven in absentia” family is a putative Zinc finger (SIAH-like) nuclear-cytosolic protein that is expressed in various growth stages and almost all plant tissues. Its homolog was first identified in Drosophila associated with the development of photoreceptors, cell cycle arrest and protein polyubiquitination. We found SINA-L7 expression was altered in different plant lines with mitochondrial dysfunction as for example in gapc-1 KO plants. The glycolytic enzyme GAPC was also found in the nucleus and associated with the mitochondrial external membrane in plants, thus it may have other important roles. However, little is known about these possible secondary functions in higher plants. In this context, we face the characterization of SINA-L7 and study its interaction with GAPC in vitro. Results show that SINA-L7 is highly expressed in tissues with active cell division and induced after UV exposure. Furthermore, using an ubiquitination assay we found that SINA-L7 has E3-ubiquitin ligase activity and that the K124 residue is involved in such process. In addition, SINA-L7 ubiquitinates and physically interacts with GAPC by pull down analysis and residue K231 in GAPC is responsible of its interaction. Furthermore, residue K76 in GAPC is ubiquitinated by SINA-L7, and this modification regulates GAPC activity.

PL-P38
ROLE OF CHLOROPLAST-GENERATED REACTIVE OXYGEN SPECIES IN THE PLANTS-NECROTROPHIC FUNGI INTERACTION

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Plant pathogenic fungi have evolved two main infection strategies. Biotrophs proliferate within living plant tissues causing minimal damage. Necrotrophic fungi, on the other hand, kill host cells with lytic enzymes and toxins, and induce processes indicative of programmed cell death in host tissues. There is strong evidence of an oxidative burst at the host-fungal interface, localized cell death and redox-related signaling events. The role of chloroplast-generated reactive oxygen species (ROS) in this process was evaluated in transgenic tobacco plants expressing a plastid-targeted flavodoxin (Fld) and exposed to the necrotrophic fungus *Botrytis cinerea*. Fld acts as a general antioxidant specific for chloroplasts. Phenotypic, biochemical and microscopic studies were carried out to evaluate the plant-fungus interaction. ROS were visualized with a specific fluorescent probe by confocal microscopy. Leaf necrosis and ROS accumulation caused by fungal infection were reduced in Fld plants, as compared to the wild type. Estimation of photosynthetic parameters showed that Fld lines were less affected by the pathogen, resulting in reduced sensitivity to *B. cinerea*. Other aspects of the defense response were also analyzed. This study shed light on the role of chloroplast-generated ROS in plant responses to infection by necrotrophic fungi.

PL-P39
STEROID SYNTHESIS IN ARABIDOPSIS: IS THE MITOCHONDRIAL ELECTRON SHUTTLE ADXR-ADX-P450 INVOLVED?

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We have identified and studied Arabidopsis mutants with an insertion in a nuclear gene encoding for a mitochondrial adrenodoxin reductase (ADXR) that has female gametophyte development defects. In mammals and insects mitochondria, adrenodoxin (ADX) mediates electron transfer from NADPH via ADXR to the terminal electron acceptor, a cytochrome P450 (P450), which constitutes the first step in steroid hormones synthesis. In this work, we study the existence of a similar steroid biosynthetic pathway in plants, which would be necessary for female gametophyte development. Arabidopsis has an ADRX homologue and 2 ADXs located in mitochondria. However, no mitochondrial P450s were described in Arabidopsis so far. We performed a two-hybrid screening to detect possible ADX redox partners using ADX1 as bait.We found that the P450 CYP711A1 binds to ADX1. The interaction between ADX1 and CYP711A1 was confirmed by BiFC assays. We also showed that this P450 has a mitochondrial localization by means of GFP translational fusions and transient expression in Nicotiana Benthamiana. Using the same approach we studied the subcellular localization of different P450s with a predicted signal peptide targeting mitochondria. Two additional P450s were found localized to mitochondria and showed interaction with ADX trough BiFC assays. The female gametophyte steroid profile will be analyzed by UL-MS-MS.

PL-P40
THE ELECTRON SHUTTLE ADX-ADXR IS ESSENTIAL FOR GAMETOPHYTE DEVELOPMENT IN *Arabidopsis thaliana*

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In Arabidopsis, the female gametophyte is a polarized structure composed by seven cells the egg cell, the central cell, two synergids and three antipodal cells. We here show the study of Arabidopsis mutants with insertions in ADX or ADXR, which are nuclear genes encoding for mitochondrial adrenodoxin and adrenodoxin reductase respectively. Siliques of ADXR/adxr plants presented around 30% of abortions and reciprocal crossings showed that transmission trough the female gametophyte is severely compromised. DIC studies revealed mutant gametophytes with abnormal cell morphologies that are not able to reach complete maturation. The identity of all cells inside adxr embryo sacs was analyzed by studying the expression of cell specific markers for egg cell, central cell, antipodal and synergid cells. The results of anilin blue staining assays and pollination using the pollen tube marker pLAT52-RFP line as a pollen donor, suggest that attraction of pollen tubes is impaired in mutant embryo sacs. Pollen tubes showed abnormal growing patterns, including invading embryo sacs, which suggest a communication problem between the male and the female gametophytes. The expression pattern of ADXR indicates that its expression is specific for the maternal tissues of the ovules. Furthermore, expression is also detected in leaves and sepals, indicating that ADXR might have sporophytic functions as well

PL-P41
MITOCHONDRIAL PPR-CONTAINING PROTEINS ARE ESSENTIAL IN EMBRYO DEVELOPMENT IN *Arabidopsis thaliana*

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PPRs containing proteins are characterized by a motif of a degenerate 35-amino acid repeat. Most PPRs proteins are specific RNA-binding proteins which are involved in RNA processing, including editing, maturation, stability, and translation. We focused on the role of PPR proteins potentially involved in embryo development. Four T-DNA insertion mutants encoding for three PPR proteins (At2g02150, At1g79490 and At3g29290) were analyzed. Transient transformation with these PPR proteins fused to GFP showed they are localized in mitochondria. Mature siliques of mutants in these genes showed ~25% of shriveled seeds which do not germinate, not even in MS sucrose medium, suggesting embryo lethality. As we were unable to obtain homozygous mutant plants, silencing assays were developed with two independent artificial microRNAs for each gene. Mature siliques of silenced plants in a ~70% were preliminary analyzed. Most siliques were short length, containing aborted embryos. To further investigate this phenotype, we examined female and male gametophyte development. Apparently, embryo sac and pollen grain development proceeded normally. These results suggest that the expression of these mitochondrial PPR genes are

essential to sustain embryo development and the proteins encoded may probably affect processes related to fertilization in *Arabidopsis thaliana*.

PL-P42
THE CARBONIC ANHYDRASE DOMAIN OF RESPIRATORY COMPLEX I IS LINKED TO PHOTORESPIRATION IN *Arabidopsis*

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The NADH-ubiquinone oxidoreductase (Complex I) of the mitochondrial respiratory chain has in a variety of eukaryotic organisms, except animal and fungi (Opisthokonta), an extra domain composed of trimers of putative gamma carbonic anhydrases, named Carbonic Anhydrase (CA) domain. Its physiological role in plants is not fully understood. Here, we present evidences that in plants this domain has acquired a role in photorespiration, thus regulating photosynthesis efficiency and plant biomass. *Arabidopsis thaliana* mutant plants affected in two CA subunits show a weak photorespiratory phenotype. These mutants grown in normal air present growth retardation compared to wild type plants. However, this phenotype is rescued by cultivating plants in a high (2000 ppm) carbon dioxide atmosphere. Moreover, under photorespiratory conditions, carbon assimilation is reduced and glycine accumulates, which suggests a photorespiratory disbalance. Furthermore, transcription of all five CA subunits is repressed in plants grown under non-photorespiratory conditions. These results strongly suggest that the CA domain of plant complex I contributes to sustain an efficient photosynthesis at ambient (photorespiratory) conditions

PL-P43
ANALYSIS OF THE RAFFINOSE FAMILY OF OLIGOSACCHARIDES (RFO) PATHWAY IN PEACH EXPOSED TO COLD STRESS

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Peaches deteriorate quickly at ambient temperature. Cold storage is commonly used to prevent fruit decay, however, it affects fruit quality causing physiological disorders collectively termed 'chilling injury' (CI). In previous studies, relative levels of transcripts encoding enzymes involved in galactinol and raffinose metabolism were studied by qRT-PCR to explain the increase observed in these two metabolites after refrigeration of six different peach cultivars. Results obtained indicated that the transcript levels of PpGolS (galactinol synthase) and PpRS (raffinose synthase), increased dramatically after refrigeration in comparison to fruits of the same post-harvest age kept at 20 °C, although the level of increase was different between them. In the present work, to extend the study of this pathway, transcript levels of genes encoding two α-galactosydases (PpSIP1 and PpSIP2), involved in the hydrolysis of raffinose, were analyzed in the six varieties after cold treatment of fruits. Once again, a variety-dependent increase was found after refrigeration. Furthermore, full-length cDNAs of PpGolS and PpRS were analyzed in the six varieties and no differences were found. We propose that RFO pathway is transcriptionally regulated and that the particular pattern of expression observed in each variety could reflect different strategies to cope with chilling stress in this fruit.

PL-P44
A β-XYLOSIDASE GENE COULD ACT AS GOOD INDICATOR OF TOLERANCE TO CHILLING INJURY IN PEACH FRUIT

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The storage of peaches at low temperatures for prolonged periods can induce a form of chilling injury (CI) called woolliness, characterized by a lack of juiciness and a mealy texture. As this disorder has been associated with abnormal cell wall dismantling, resistant (Springlady), intermediate (Rojo 2) and susceptible (Flordaking) cultivars were selected to study the levels of twelve transcripts encoding proteins involved in cell wall metabolism. Of all cell wall-related genes studied, only PpXyl, which encodes for a putative β-xylosidase, seems to act as good indicator of tolerance to CI, as its expression correlated with the different susceptibility to this disorder after cold storage. PpXyl full-length was cloned and sequenced in the three cultivars in order to look for changes in PpXyl that may alter the capacity of function of the protein. In addition, a fragment of 1637 bp, corresponding to the promoter region of the gene, was isolated from Springlady, Rojo 2 and Flordaking genomic DNA in order to investigate the presence of putative regulatory sequences that may be responsible for the differential patterns of expression. The presence of cis-

acting elements associated with hormone and stress-related responses, along with the results obtained in this work, suggest a role of PpXyl in determining different tolerance to CI in peach fruits.

PL-P45
TRANSCRIPTIONAL ANALYSIS OF POTATO PLANTS WITH IMPROVED TOLERANCE TO DROUGHT

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We have already proven in tobacco a succesfull approach to gain stress tolerance. It is based on the replacement of the stress-sensitive ferrosulfoprotein ferredoxin (Fd), which is the final electron acceptor of the photosynthetic electron chain, by the stress-resistant isofunctional counterpart flavodoxin (Fld), which is present in many cyanobacteria and algae but not in plants. To evaluate this technology in a crop, we generated potato plants accumulating Fld in their plastids and we evaluated their responses to drought. Transgenic lines under drought showed a remarkably less wilted phenotype than WT. To advance our understanding into the mechanism of tolerance we carried out a transcriptome analysis of leaves after 3 days of water deficit and under control conditions. The analysis has shown differential expression (at least 2-fold change) in 4620 genes between transgenic and WT plants under drought. Furthermore, 1675 genes exhibited a differential behavior between both lines under control conditions. Most central metabolic routes were less inhibited in transgenic lines under drought than in WT, including genes coding for components of the photosynthetic apparatus (such as Fd) and Calvin cycle. Furthermore, Fld-expressing plants under drought had a lower number of induced genes involved in stress tolerance or in regulation of gene expression

PL-P46
A NOVEL *Arabidopsis* BHLH TRANSCRIPTION FACTOR REGULATES SEED GERMINATION ANTAGONIZING ABA-SIGNALING

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Seed germination is influenced by a myriad set of abiotic factors. Soil salinity is a potent inhibitor of this process in which the hormone abscicic acid (ABA) plays a central role. Here, we present a characterization of a basic/helix-loop-helix (bHLH) transcription factor related to the ABA signaling pathway during germination. Following a reverse genetic assay, we used a set of bhlhX T-DNA Arabidopsis insertional mutants to test the germination rate in the presence of ABA or salt (NaCl). Among the mutant lines, one showed enhanced inhibition of the germination rate in the presence of increasing concentrations of NaCl compared to WT seeds. Concomitantly, this mutant also exhibited an increased sensitivity to ABA during germination, as well as a higher dormancy rate when germinated on soil. To underscore the physiological mechanisms behind this transcription factor, we performed a phenotype comparison between the bhlh mutant and known ABA signaling mutants. The abi4 mutant, defective in the ABI4 transcription factor with diverse roles in ABA responses, displayed an opposite set of characteristics including viviparism, ABA and salt insensitivity. Interestingly, the bhlh mutant showed increased expression of ABI4. We propose a model in which bHLH and ABI4 regulate each other in an antagonistic manner to modulate the germination rate upon challenging environmental conditions.

PL-P47
EFFECT OF REDOX IMBALANCE ON WHEAT ROOT APICAL MERISTEM: MODIFICATIONS OF THE TRANSITION ZONE

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Adverse environmental conditions affect plant growth. We studied the response of the wheat root apical meristem (RAM) to Cd (a redox inactive metal), Cu (a redox active metal), MV (methyl viologen) and H^2O^2 (two well-known oxidative stressors). Seeds of *Triticum aestivum* L. were floated for 48 h on distillated water (control) or in the presence of the corresponding stressor. The concentrations that altered root length (10 µM Cd^{2+}, 5 µM Cu^{2+}, 0.5 µM MV and 1 mM H2O2) hastened cell displacement from the cell division zone to the elongation/differentiation zone,

resulting in a shortened meristem. Metals increased reactive oxygen species in the root apex as indicated by the fluorogenic probes 2´,7´-dichlorodihydrofluorescein diacetate and dihydroethidium. Treatments increased protein oxidation. Transcription in the RAM of genes related to the cell cycle G1/S transition (rdr, pcna, mcm2) was down regulated by oxidative conditions. This effect might be associated to the alteration of cytokinins (CKs) homeostasis, as suggested by the analysis of transcripts related to CKs metabolism by qRT-PCR. Four from five expansin genes transcripts analyzed (TaEXPA5, TaEXPA6, TaEXPA8, TaEXPB8 and TaEXPB10) were upregulated by the treatments. These results indicate the importance of the transition zone as an oscillatory zone that connects root growth and development with environmental cues.

PL-P48
STUDY OF THE REGULATION AND CONTENT OF FLAVONOIDS DURING SEED DEVELOPMENT IN *Sorghum bicolor*

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For over a century, seed dormancy in cereals has been associated with red colour in different species. Therefore, the intermediate and final metabolites of the phenylpropanoid and flavonoid pathways not only affect seed coat colour, but they could also be associated to the sprouting phenotype in *S. bicolor*. In this work, the accumulation of phenolic compounds was analyzed during the development of the caryopsis in the seed coat of two sorghum lines with contrasting sprouting phenotypes (Redland B2, susceptible, and IS9530, resistant). The analysis of phenolic compounds by HPLC shows differences in the composition and concentration between the susceptible and the resistant lines at different developmental stages. Also, transcripts for flavonoid pathway enzymes (CHI, CHS, LAR, ANS), and a basic-HLH transcription factor were analyzed during grain maturation. Transcripts levels increased gradually in both lines during the development of the caryopsis but following different patterns. For example, the expression of b-HLH was detected between 15-20 DAP in the resistant line, while in the sensitive line it was only detected starting at 25-30 DAP. A regulating role of this b-HLH in the flavonoid pathway during the development of sorghum seed was also analyzed. These data provide insight into pigment accumulation of seed coat colour formation and its regulatory mechanisms in sorghum.

PL-P49
FINDING MASTER MOLECULAR REGULATORS OF INFLORESCENCE ARCHITECTURE IN *Brachypodium distachyon*

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Grasses produce florets on a structure called a spikelet, and variation in the number and arrangement of both branches and spikelets contributes to the great diversity of grass inflorescence architecture. In Brachypodium, the inflorescence is an unbranched spiciform raceme with a terminal spikelet and a limited number of lateral spikelets. Spikelets are indeterminate and give rise to a variable number of florets. To gain insight into the genetic regulation of Brachypodium inflorescence development and architecture, we characterized T-DNA insertional mutants with altered expression of inflorescence genes. Compared to wild type, BdLFY mutants develop more spikelets supported by shorter internodes. Flower and seed development seems to be normal, suggesting that BdLFY regulates the fate of spikelet rather than floral meristem, as it counterpart in Arabidopsis. Similarly, BdPAP2 mutants shows increased branching and a slightly delay in transition from vegetative to inflorescence meristem. A stronger delayed in flowering time was observed in BdRCN mutants, since the transitional state in this mutants correspond to completely developed flowers in wild type plants. The results presented here will be helpful to understand the molecular basis of Brachypodium inflorescence development and even to elucidate master genes responsible for the high diversity of inflorescences observed in grasses.

PL-P50
TRANSCRIPTIONAL PROFILING OF TOMATO FRUITS VARIETIES WITH DISTINCT ORGANOLEPTIC PROPERTIES

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Most of the commercial cultivated tomatoes in Argentina are imported hybrids. Given that these tomatoes have been mainly selected for long shelf life and pest resistance, consumers are complaining about their taste and flavor. Therefore, tomato populations cultivated by inhabitants from Argentine Andean Valleys were analyzed in this work in an attempt to incorporate these varieties with better consumer preferences to the local and regional breeding programs. For this purpose a transcriptomic approach was used to evaluate transcriptional differences in red fruit varieties of contrasting flavor as previously determined by sensory analysis. In a global analysis, we observed ca. 45% of genes with a significantly differential expression between a bad flavor variety and the others tested. Functional classification of the transcripts differentially expressed was performed using the tool MapMan. Interestingly, one of these accessions of salient organoleptic characteristics has an increment in expression of genes associated with cell wall degradation and AA transport. In addition, a global correlation between transcript and metabolite profiles evidencing relevant relationships among them will be presented.

PL-P51
IMPROVING LABORATORY TOOLS TO STUDY STARCH EXCESS 4 (SEX4) ALGAE HOMOLOGOUS ENZYMES

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Phosphoglucan phosphatases are novel enzymes that remove phosphates from complex carbohydrates. In plants, these proteins have a regulatory role in the night time remobilization of leaf starch being SEX4, which primary structure contains a DSP (dual-specificity phosphatase) as well as a CBM (carbohydrate-binding module) domain, its major representative member. To understand the evolution of catalysis and regulation of these enzymes we decided to investigate SEX4 homologous enzymes in eukaryotic green algae. These organisms are an excellent model to investigate the properties of photosynthetic physiology because they present the critical photosynthetic functions as higher plants but a decreased gene redundancy. To this end we first set up laboratory conditions to culture and cryopreservate *Ostreococcus tauri, Nannochloropsis gaditana, Micromonas pusilla and Chlamydomonas reinhardtii* strains. We tested and optimized different total RNA, DNA and protein extraction procedures in order to find the best isolation protocol for each macromolecule in terms of quality and yield. These laboratory tools will permit us to analyze evolutionary aspects related to phosphoglucan phosphatases from *O. tauri*, the smallest free-living eukaryote identified to date.

PL-P52
ISOLATION OF TOTAL RNA FROM ROOTS OF POTTED *Ilex paraguariensis*: ASSESSMENT OF FOUR METHODS

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There are no published studies that indicate appropriate methods for isolation and purification of RNA from roots of yerba mate. The aim of this study was to evaluate the suitability of total RNA extracted from roots of yerba mate grown in soil by 4 commercial methods for downstream molecular biology experiments. Roots were collected from 3 potted plants. Extractions were performed by triplicate with: SpectrumTM Plant Total RNA kit (Sigma), RNeasy® Plant Mini Kit (QIAGEN®), TRI Reagent® (Molecular Research, Inc.) and SV Total RNA Isolation System (Promega). After digestion with DNase I, integrity was checked by agarose gels electrophoresis. Quality was assessed by (260-320)/(280-320) and (230-320)/(260-320) ratios. Samples with (260-320)/(280-320) ratio greater than 1.8 were quantified spectrophotometrically. cDNA obtained by RT was used to amplify a β-tubulin sequence by qPCR. The quality ratio of RNA obtained with TRI Reagent® was not enough to quantify. SV Total Isolation Kit had the lowest yield with acceptable quality. RNeasy® Plant Mini Kit and Plant Total RNA Kit SpectrumTM provided higher quality and quantity of RNA, the second one showed best performance. All them showed good integrity. qPCR was positive for all samples tested. Considering quality, integrity, performance and cost, the commercialized kit by Sigma® is the best method to extract total RNA from studied tissue

PL-P53
A TIME AND SPACE-SAVING PROTOCOL TO IDENTIFY *Arabidopsis* T-DNA NULL MUTANTS

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Arabidopsis is widely used as a model organism in plant biology. Its genome can be easily modified by transformation with *Agrobacterium tumefaciens* and a large number of mutant lines are thus available from Stock Centers. Working with mutant lines of interest requires the identification of homozygous individual plants from seed-pools that might also contain WT and heterozygous plants. Standard protocols use purified nucleic acids from four-week-old plants to confirm T-DNA insertion in both alleles and the absence of gene transcript. The aim of this work was to develop a fast and efficient protocol to identify T-DNA null mutants. In order to achieve this goal we performed an alkali treatment on a small piece of a leaf from a two-week-old plant to use it as template in tissue PCRs to identify homozygous plants for T-DNA insertion. In this way the screening time was shortened in two weeks and less space was required in growing chambers because only those plants carrying the T-DNA in both alleles were selected and continue to grow. Finally, in order to confirm the absence of the transcript of interest, four-week-old plants were analyzed. This novel protocol provides improved management of time, space, and even money, to work with genetically modified Arabidopsis lines. Identification and characterization of the Arabidopsis *polyamineoxidase 3* T-DNA null mutant is presented as an example.

PL-P54
NUTRIENT DEPLETION AND SA RESPONSE DETERMINE PATTERN OF GENE EXPRESSION IN TUMV-INFECTED *Arabidopsis*

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When a plant virus succeeds infecting a host, it typically determines a broad range of physiological and molecular alterations. Besides the symptoms arising from disturbing host RNA silencing machinery, a plethora of symptoms emerge which could have different origins. Our previous work characterized the differential symptomatology caused by two TuMV strains on A. thaliana regarding senescence acceleration, antioxidant accumulation and reactive oxygen production linking these phenotypic and metabolic changes with differential salicylic acid response. Here we provide evidence suggesting a role for nutrient competition between the virus and its host in determining the nature and the extent of symptomatology produced by TuMV. The expression of key genes involved in nutrient uptake and recycling was dramatically changed over the course of the infection compared to uninfected plants. Major changes in genes involved in Pi metabolism were observed at later times of infection when symptoms are more contrasting, pointing out to nutrient as a limited building block disputed by both organisms. When analyzing the expression of genes with roles both in nutrient stress response and senescence or hormone response, complex patterns of accumulation emerged, showing that the final outcome of compatible interactions is determined by opposing forces acting simultaneously triggered by pathogens.

PL-P55
EFFECT OF ARSENIC ON SOYBEAN PLANTS ASSOCIATED TO PATHOGENS AND ARBUSCULAR MYCORRHIZAE

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Arsenic (As) induced oxidative stress in soybean plants was determined by lipid peroxidation, which remained elevated in leaves. The goal of this work was to evaluate the oxidative stress in soybean plants exposed to arbuscular mycorrhizae (AM), a phytopathogenic fungi and Arsenic. To this end, catalase (CAT), superoxide dismutase (SOD), guayacol peroxidase (GPOX), glutathione reductase (GR) activities and glutathione (GSH) levels were determinated. Plants were grown in pots containing soil:vermiculite (3:1). *Rhizophagus intraradices* and *Macrophomina phaseolina* was employed as phytopathogen. Soils were treated with different As concentrations ranging from 0 to 50 mg/ kg substrate. Plant biomass was evaluated and antioxidant response was tested in the leaves. Results here presented demonstrated that an increment in biomass in plants treated with AM and AM + pathogen occurred. Nevertheless, a diminution was observed in plants treated with *M. phaseolina*. In plants subjected to AM, CAT and GPOX activities as well as GSH, levels were enhanced respect to controls. Surprisingly, GR, CAT and SOD activities as well as GSH levels diminished in the presence of phytophogenic fungi. In co-inoculated plants a positive response was observed respect to the result obtained with MA. These results let us assume that the co-inoculation enhances antioxidant defence as well as biomass.

PL-P56
OVEREXPRESSION OF SUNFLOWER GERMIN-LIKE PROTEIN (HAGLP1) ENHANCES RESISTANCE AGAINST FUNGAL PATHOGEN

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Germin-like proteins (GLPs) are a large, diverse and ubiquitous family of plant glycoproteins that belong to the Cupin super family. They have been widely studied due to their diverse roles in important plant processes such as defense. Here, we isolated the HaGLP1 gene from sunflower, which is the first GLP characterized from the family Asteraceae. To analyze whether constitutive in vivo expression of the HaGLP1 gene may lead to disease tolerance we developed transgenic Arabidopsis plants that were molecularly characterized and biologically assessed against *Sclerotina sclerotiorum* and *Rhizoctonia solan*i. Our results demonstrate that the expression of HaGLP1 in adult Arabidopsis plants confers tolerance to destructive *S. sclerotiorum* at first stages of disease and interferes with *R. solani* infection, giving rise to significant resistance against this pathogen. Furthermore, the expression of HaGLP1 elevates the endogenous levels of ROS in Arabidopsis plants. The enhanced tolerance provided by HaGLP1 does not appear to be related to the regulation of the plant defense or ROS-related genes examined here. In conclusion, our data suggest that HaGLP1 is an interesting candidate gene for engineering plants to confer protection against pathogens, and also for the selection of naturally overexpressing genotypes for conventional breeding purposes.

PL-P57
ROLE OF MAIZE SILKS FLAVONOIDS COMPOUNDS IN RESPONSE TO *Fusarium verticilloides* INFECTION

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Fusarium verticillioides is the major causative agent of ear rot in maize crop in Argentina. The fungus penetrates mostly silks or wounded ears, contaminated them with human and animal harmful mycotoxins. Since silk is usually the first tissue with which the pathogen comes in contact, it is reasonable to hypothesize that in resistant materials, the initial barrier to infection would be in these tissues. The purpose of this study was to determine if flavonoids compounds from maize silks influence *F. verticillioides* infection. Two maize inbreed lines of contrasting resistance under field conditions were used. The in vitro effect of silks flavonoids extracts was assayed. Significant differences in *F. verticillioides* growth were observed for extracts that appeared to stimulate or inhibit fungal growth. A HPLC analysis of silks extracts was carried out. A quantitative real time PCR of seven important genes related to the flavonoid synthesis pathways were studied. On the other hand spectral analysis indicates an increment in flavonoids content in the susceptible line after fungal inoculation, indicating a positive influence of these compounds in fungal growth. The results of this study indicate that flavonoids compounds might be involved in *F. verticillioides*-maize interaction

PL-P58
BIPHASIC ACTIVATION OF DEFENSE RESPONSE IN STRAWBERRY PLANTS TREATED WITH ELICITOR PROTEINASES

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AsES is a subtilisin-like protease isolated from the avirulent fungus *Acremonium strictum* which exhibits defense-eliciting activity against virulent *Colletotrichum actatum* M11 in strawberry. In this work we focus on elucidating the effect of AsES on some biochemical and molecular markers in strawberry plants at different times. We studied production of reactive oxygen species (ROS) and the expression of genes related to ROS detoxification (*FaCAT*), salycilic acid (*FaPR1*) and ethylene (*FaACS, FaACO2, FaETR1*) defense pathways at 2, 12, 24, 48, 72, 92, 120 hours post treatment (hpt) with AsES. Our results showed a strong, biphasic, oxidative burst (accumulation of H2O2 and O2-) with peaks at 12 and 120 hpt and downregulation of FaCAT gen at 2, 48 and 120 hpt. A biphasic behavior was also observed in the expression of FaPR1, with a peak at 72 hpt, and a much greater one at *1*20 hpt. In contrast, expression of the gene *FaETR1* (an ET receptor) was downregulated *1*20 hpt suggesting a negative cross-talk between the two pathways. Furthermore, the genes *FaACS* and *FaACO2* (ET synthesis enzymes) were downregulated up to 48 hs and then expression level increases being higher the *FaACO2*, indicating a late activation of ET pathway. These results agree with previous observations of our group that elicitation was higher when inoculation with M11 was carried out 120 hpt with AsES.

PL-P59
PEACH LEAF CURL DISEASE: STUDY OF *Taphrina deformans* - *Prunus persica* INTERACTION

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Taphrina deformans is the causative of Peach Leaf Curl, a disease that affects orchards throughout the world. It is an important threat to the tree, shoots and fruits. Only a few cultivars are considered tolerant. To characterize the plant-fungus interaction a resistant and two susceptible genotypes were inoculated with blastospores (yeast phase) and histological, biochemical and metabolic profiling by GC-MS were conducted during 120 hpi. Confocal microscopy revealed the induction of fungal dimorphism, accounted by hyphae entering stomata at 24 hpi in either resistant or susceptible genotypes. In contrast, metabolic profiling showed important differences between the resistant and the susceptible genotypes, with main differences observed at early times (6-12 hpi) and later at 96 hpi. In susceptible genotypes, most amino acids increased at 6-12 hpi, probably serving as nutrients for fungus reproduction and development, while tryptophan greatly decreased during the first day, in agreement with *T. deformans* active synthesis of auxins. Differences in organic acids, sugars, alcohol sugars, flavonoids and phenolic compounds were also observed among genotypes. Plant defenses were activated earlier in the resistant than in the susceptible genotypes. Together, a picture of early events during T. *deformans* infection is currently being constructed in both tolerant and susceptible genotypes.

PL-P60
CHARACTERIZATION OF PLANTS WITH ALTERED LEVELS OF PNPS IN PLANT DEFENSE

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Xanthomonas axonopodis pv. *citri*, the citrus canker-causing pathogen, contains a gene encoding a plant natriuretic peptide (PNP)-like protein (XacPNP) that shares significant sequence similarity and identical domain organization with PNPs. It has been shown that *XacPNP* expression is induced upon infection of citrus plants, suggesting that this peptide is involved in plant-pathogen interaction. Moreover, XacPNP is able to improve host photosynthesis, rendering a healthier tissue that favors its own survival. We found that AtPNP from *Arabidopsis thaliana* is highly induced upon infection with *Pseudomonas syringae* pv. *tomato*. To characterize the effect of PNPs in plant infection, we obtained *A. thaliana* transgenic lines that overexpress XacPNP and AtPNP as well as knock out plants in its endogenous gene. Using a flooding assay we characterized the response of these modified *A. thaliana* lines to bacterial pathogens that are able to elicit different responses, such as disease and hypersensitive response. Our results indicated that the transgenic lines present altered responses to infection when compared to the parental line. Also, *in planta* pathogen growth experiments and the evaluation of the expression of genes related to plant defense showed differences among the transgenic lines bearing or not XacPNP and AtPNP, putting forward that PNPs have a role in plant pathogenesis and defense.

PL-P61
OXIDATIVE RESPONSE IN SOYBEAN SEEDS AGAINST SOUTHERN STINK BUG HERBIVORY

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Southern stink bugs (*Nezara viridula* L.) (Hemiptera: Pentatomidae) are one of the major pests in many soybean producing areas. They cause a decrease in yield and affect seed quality by reducing viability and vigor. It has been reported that alterations in the activity of antioxidant enzymes occur in this crop due to insect activity. The role of these enzymes is linked to signaling, synthesis of defense compounds and tolerance to oxidative stress. The aim of this work was to compare oxidative responses of two contrasting genotypes of soybean, IAC-100 (resistant) and Davis (susceptible), in response to *Nezara viridula* damage. Guaiacol peroxidase (GPOX), catalase (CAT) and superoxide dismutase (SOD) activities were quantified spectrophotometrically in seed extracts from greenhouse-grown soybean after 72 h of stink bug damage at R6 stage, and compared against undamaged seeds. To analyze H2O2 generation, seeds were excised, immersed in a 1% solution of 3,3-Diaminobenzidine and visually analyzed. Seed fresh weight (FW) was determined in each genotype and treatment. Only Davis showed less FW compared to its control after insect

treatment. Our results show greater GPOX, CAT and SOD activities and a decreased content of peroxide in IAC-100 after stink bug attack, indicating a differential oxidative reaction between soybean genotypes with contrasting response to insect feeding.

PL-P62
EFFECT OF THE SIGNALS EMITTED BY *Artemisia annua* WITH AND WITHOUT HERBIVORY ON SOYBEAN DEFENSE

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The aim is to determine the effect of *A. gemmatalis* herbivory on the production of phytoalexins in soybean growing with *A. annua*. A trial was conducted following a completely randomized factorial design with 3 replications. The factors were: i) 3 density levels: Pure soy, soy + 2, 4 *A. annua* plants/m^2 and ii) 2 levels of herbivory: with and without. The crop was planted at 40 pl/m^2 in 1 m^2 plots. After the emergence of crop, *A. annua* plants were added in the center of each plot. To evaluate the production of phytoalexins in soybean, in R*1*-R5, nine caterpillars were placed in one trifoliate leaf of three plants randomized selected of each plot, within a tulle bag to ensure consumption. At 24 hours the leaves were harvested, weighed and dried, the phytoalexins were extracted with methanol, and analyzed by High Performance Liquid Chromatography. The chromatograms for each treatment show similar profiles although they differ in the relative abundances of some compounds. The results indicate a higher level of flavonoids and isoflavonoids in mixtures with *A. annua*, specifically in treatment 2A. In all cases the presence of rutin was determined. The content of total phenols mean values was similar among treatments, however treatment 2A presents the highest values. The data show a possible positive effect of signals emitted by the "weed" on the pool of defense compounds of soybean crop.

PL-P63
MODULATION OF PUTRESCINE METABOLISM IN *Arabidopsis thaliana* BY SALICYLIC ACID

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Salicylic acid (SA) is a key hormone for defense responses in plants, and plays a critical role in resistance to pathogens. Increments of SA levels activate different signaling cascades in which H2O2 acts as a secondary messenger and in turn activates a large number of mechanisms that contribute to overall plant defense. Polyamines are a group of compounds with multiple physiological roles, which also participate in defense mechanisms against pathogens. The metabolism of the diamine putrescine (Put) is altered by stressful conditions in plants, which generally result in increased Put levels. Previous results showed that exogenous addition of SA induces a high increase in Put levels. In this work, we give evidence that this increase in Put level is mediated by an increase in arginine decarboxylase 2 (ADC2) activity. Moreover, apoplastic Put levels were increased and amine oxidase activity was decreased in plants treated with exogenous SA. Diaminebenzidine (DAB) staining of *adc2* mutants (with contain lower Put levels than WT plants), showed that these plants accumulate higher H2O2 levels than WT plants after SA addition. Put supplementation to *adc2* mutant plants decreased the H2O2 levels. These results indicate that the accumulation of Put, after the exogenous addition of SA, probably contributes to regulate H2O2 levels during plant defense responses.

PL-P64
CHARACTERIZATION OF AN ACYL-COA CARBOXYLASE COMPLEX FROM *Xanthomonas*

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Phytopatogen *Xanthomonas axonopodis pv. citri* (Xac) produce canker, pathology that affects citric. Xac, as an obliged plant parasite, is unable to survive out of its host for long periods of time. Nevertheless, during the epiphytic survival state, over the leaf, many metabolic and nutritional aspects remain unknown. Branched-chain amino acid catabolism prevents their over-accumulation, facilitates branched-chain fatty acids biosynthesis and provides energy for the cell. The enzymatic complex 3-methyl-crotonyl-CoA carboxylase (MCC) is essential for leucine degradation pathway. MCC carboxylates its substrate, 3-methyl-crotonyl, to finally generate acetyl-CoA and acetoacetate which are latter incorporated into different metabolic pathways in case of nutritional stress or amino acid excess. MCC

complexes belong to the biotin-dependent acyl-CoA carboxylases (ACCases) group. We identified by bioinformatics two genes that may encode subunits of an ACCase complex in *Xanthomonas*, and others gram negative bacterial pathogens. The amino acidic sequences of these subunits present a high level of similarity to proteins of previously characterized MCC complexes from human and *Pseudomonas aeruginosa*. In this project we purified the MCC subunits and performed enzymatic activity assays using different acyl-CoAs as substrate. Now we propose to analyze the role of this complex in bacterial virulence.

Structural Biology

SB-P01
MITOCHONDRIAL RESPIRATORY SUPERCOMPLEXES LIMIT REACTIVE OXYGEN SPECIES (ROS) PRODUCTION.

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The mitochondrial respiratory chain is organized as supercomplexes (SC) which confer substrate channeling and scaffold for assembly/stability of Complex I (CI). Some studies suggested that SC may also prevent excessive reactive oxygen species (ROS) production from CI and Complex III (CIII) but failed to show if SC dissociation is a cause or a consequence. In the present study we addressed this issue in proteoliposomes containing CI and CIII from bovine heart mitochondria. When proteoliposomes are at low lipid/protein ratio, BN-PAGE shows that CI and CIII are present as SC. Their treatment with dodecyl maltoside disassembles SC into CI and CIII. Alternatively, SC are not formed when proteoliposomes are at high lipid/protein ratio. NADH-cytochrome c reductase activity (CoQ_{10} channeling) is dramatically decreased when SC are disassembled, while CI is functional active. These results show that CoQ_{10} channeling shifts to a less efficient pool behavior while reconstituted proteoliposomes at high lipid/protein ratio with 20% cardiolipin restores CoQ_{10} channeling. Under disruption or prevention of SC assembly we observe a strong increase of ROS production from CI, detected with dichlorofluorescein. This is the first demonstration that dissociation of SC is a cause of oxidative stress from CI that may perpetuate a vicious cycle of ROS generation and bioenergetics failure.

SB-P02
STRUCTURAL CHARACTERIZATION OF BA41, A TPM SUPERFAMILY MEMBER FROM THE ANTARCTIC *Bizionia argentinensis*.

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Bizionia argentinensis (Ba) is a recently annotated Antarctic psychrophilic flavobacterium. Pursuing a comprehensive structural coverage and accurate functional annotation of the Ba genome, a rigorous bioinformatic protocol was applied and several ORFs have been cloned and expressed. BA41 is a hypothetical protein composed by a predicted transmembrane region, a compact domain belonging to the TPM family and a terminal low complexity region. The central TPM domain (BA41s) was cloned, expressed and purified. SLS and DLS studies showed it behaves as a globular monomer of 16 kDa, as expected for a properly folded 150-residue polypeptide. Far-UV CD spectra indicated a high predominance of β-strand secondary structure. Thermal unfolding experiments demonstrated that BA41s is irreversibly denatured and the treatment with EDTA reduces its thermostability by 6°C. The BA41s crystal structure was determined at 1.7 Å resolution by molecular replacement. BA41s displays a Rossmann fold similar to an acid phosphatase from *A. thaliana* and other two TPM domains belonging to uncharacterized prokaryotic proteins. In accordance with CD experiments, the crystal structure revealed a Zn^{2+} ion selectively stabilizing the active site folding. Further structural and biochemical studies would help elucidate the functional properties of this protein.

SB-P03
EGG DEFENSES OF *Pomacea canaliculata*: IDENTIFICATION OF KUNITZ-TYPE INHIBITOR AND SEQUENCE OF PCOVO.

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Pomacea canaliculata eggs have antipredator defenses including antinutritive, antidigestive and neurotoxic proteins. PcOvo is the most abundant factor, providing eggs with warning coloration and antidigestive properties; several authors have also ascribed a proteinase inhibitor activity to it. Recently, we sequenced three of its subunits. Here we identified the major proteinase inhibitor activity of the egg as a separate protein, and report 3 new PcOvo subunits. Using 2DE and MS identification followed by a search in *P. canaliculata* transcriptome, 3 more subunits of PcOvo were identified. Sequence analysis showed that the 6 subunits share at least 30% identity indicating that they have probably arised by duplications. Remarkably no proteinase inhibitor domain was detected in PcOvo. Analysis of egg fluid fractions identified the proteinase inhibitor activity in a fraction named PcPV3. N-terminal sequencing and transcriptome analysis confirmed that this fraction contains a 22 kDa Kunitz-type inhibitor. Finally, cross linking experiments were assayed to evaluate PcOvo interaction with trypsin. Our results show that the multifunctional PcOvo is a defensive reserve protein with warning signal and antinutritive properties, while the PcPV3 fraction provides antidigestive properties to the eggs, via Kunitz-type protease inhibition. PcOvO subunits would be the product of gene duplication.

SB-P04
STRUCTURAL-FUNCTIONAL STUDIES OF THE INTERMEDIATE DOMAIN OF THE PPKL BSL2 IN *Arabidopsis thaliana*.

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PPKL (Protein phosphatases with Kelch-like domains) belong to a subfamily of the PPP Ser/Thr phosphatases superfamily present only in green organisms and in alveolates. They are composed by three different domains: an N-terminal Kelch-like β-propeller domain, a C-terminal catalytic domain and an intermediate domain of unknown affiliations or functions. We have previously shown that these proteins are able to form homotypic interactions, an unusual feature in the PPP family, and described that both the catalytic and/or the intermediate domains, dependig on the isoforms analyzed, are necessary and sufficient for the interaction to take place. We here report a structural-functional analysis of the 205 residue long intermediate domain of one of the paralogs in *Arabidopsis thaliana*, BSL2, in which this domain is necessary for the interaction. The BSL2 domain could be expressed and purified in soluble form in *E.coli* and behaved as a mostly intrinsically disordered protein in solution, although it displayed a fairly high proclivity to adopt secondary structure elements with changes in the milieu. We suggest that induced folding of (parts of) this domain upon interaction with its partner drives to dimerization and possibly serves other functions. Funded by CONICET (PIP2011-414)

SB-P05
DIMERIZATION AND DOCKING DOMAIN OF BCY1, THE REGULATORY SUBUNIT OF PKA FROM *Saccharomyces cerevisiae*.

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Protein kinases A (PKA) are formed by a dimer of regulatory subunit (R2), which binds cAMP, bound to two catalytic subunits (C). In mammals, the N-terminus of R2 is responsible for dimerization and for binding of PKA to AKAPs. Our model is the R (Bcy1) subunit of PKA from *S. cerevisiae*. Previously, we have reported that: a) a mutant of Bcy1 lacking the first 85 amino acids, no longer binds to specific interactors; b) a bacterial recombinant construct of Bcy1 (1-50) contains the dimerization domain (DD) which has been structurally characterized. Using the following techniques: *in vitro* crosslinking with EGS, size exclusion chromatography, sucrose gradient sedimentation and static light scattering, we demonstrated that the predominant form of the recombinant DD in solution is tetrameric. This result is in accordance with the packing of the DD in the crystal structure. We have expressed the DD construct in a yeast vector fused to a tag of thioredoxin, His tag, and enterokinase cleavage site; studied the overexpression conditions, as well as its purification. We compared the protein profiles of Ni-agarose bound WT versus WT+DD overexpressing extracts and demonstrated that DD binds a differential set of proteins, which are competed by

incubation with a purified preparation of whole Bcy1. These results indicate that in Bcy1 (1-50) resides not only the dimerization domain but an interacting domain.

SB-P06
MECHANISTIC STUDIES OF MYCOBACTERIOPHAGE TM4 LYSA COMBINING BIOINFORMATIC AND EXPERIMENTAL APPROACH.

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Bacteriophages are widely used as biotechnology tools. Phage encoded lysins, proteins that lyse bacterial cell walls, represent desirable alternatives as antimicrobial agents. Mycobacteriophage TM4 hosts a lysin A (LysA; gp29). LysA sequence was analyzed using the BLAST search engine and Pfam database. We were able to identify a peptidoglycan-recognition domain (cd06583) of 125 amino acid length (position 225 to 350) and a second domain belonging to amidase 2 family (pfam01510) harboring the substrate binding and Zn hosting catalytic sites. To unravel the reaction mechanism of LysA, we performed structural bioinformatic calculations and *in vitro* experiments. Sequence analysis revealed two histidines (H226 and H335) and an aspartic acid (D347) as the possible Zn coordination residues. Optimized structures of the LysA active site model shows that Zn could have tetra, penta or hexa coordination states, being the tetra and penta the most commonly found. Also, the glutamic acid E290 is predicted to be a key residue for the catalytic activity, acting as base. The expression profile of the heterologous protein in *E. coli* was evaluated and the enzymatic activity was tested by a chloroform assay and by zymogram using *M. luteus* peptidoglycan as substrate.

Signal Transduction

ST-P01
CROSS-TALK BETWEEN THE PHEROMONE AND HIGH OSMOLARITY PATHWAYS IN THE YEAST *Saccharomyces cerevisiae*

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We study how cells integrate multiple stimuli using S. cerevisiae as a model system. Recently, we discovered that the MAPK pathway activated by pheromone (PR) is able to stimulate the p38- pathway activated by high osmolarity (HOG). PR activates HOG by increasing the efflux of glycerol (the osmolyte used to counteract the external osmolarity), leading to a loss of turgor, followed by HOG activation. As a result of this mechanism yeast have a greatly improved osmoadaptation capacity (shorter volume recovery time after an osmolarity shock). Here, we characterize a mutant Hog1 (the yeast p38) that discriminates between inputs: in yeast with this mutant the PR is unable to induce a HOG reporter, but response to a high osmolarity shock is normal, indicating that the two inputs regulate differently Hog1 transcriptional control, for example by altering its cytoplasmic retention. Surprisingly, despite lack of reporter expression, mutants retain an improved osmoadaptation capacity after exposure to pheromone. This result suggests that PR activation of HOG activation improves osmoadaptation via a cytoplasmic mechanism. Interestingly, two of the four mammalian p38s conserve in the equivalent position the same amino acid than Hog1, while the other two have an amino acid similar to that of our mutant, suggesting an important role of this position in the subfunctionalization of these kinases.

ST-P02
TARGETS OF SHP2 TYROSINE PHOSPHATASE REGULATED BY cAMP IN MA-10 LEYDIG CELLS

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SHP2 is a ubiquitously expressed non-transmembrane protein tyrosine phosphatase, that serves multiple hormone receptors. In steroidogenic tissues, it is involved in the cAMP-dependent and independent acute stimulation of steroid production. However, the targets that relate Ser/Thr phosphorylation with Tyr dephosphorylation are poorly known. Thus, our aim was to analyze the targets of SHP2 in protein modifications triggered by cAMP. For that purpose, we performed a reverse phase protein array (RPPA), a high throughput antibody-based technique developed for functional proteomics studies. MA-10 Leydig cells were transfected with or without specific SHP2 shRNA and incubated 15 min with or without 0.5 mM cAMP, prior to the RPPA. The results indicate that SHP2 up or down regulates cAMP action depending on the target. SHP2 upregulates the changes triggered by cAMP on cMyc, G6PDH, Histone H3, Notch1, eIF4G, CDK1, N-Cadherin levels, while it inhibits cAMP action on GSK3 and AKT phosphorylation. The activation of ERK1/2 is one of the widely described targets of SHP2 in other systems, as well as of cAMP action in steroidogenic cells. However, in the later, we found that cAMP increases ERK1/2 phosphorylation in a SHP2 independent fashion. The results suggest that the participation of SHP2 on cAMP action is dependent on the intracellular location of cAMP/PKA, SHP2 and their respective targets.

ST-P03
SIGNALING EVENTS INVOLVED IN ZEBRAFISH SPERM MOTILITY ACTIVATION

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In teleost fishes with external fertilization, sperm remain immotile in the male genital tract and become activated upon contact with the aqueous medium where fertilization takes place. The mechanism by which sperm motility is triggered, and how external signals such as hypotonicity are integrated into the signalling network involved in the acquisition of fertilizing competence are deficiently understood. With the final goal to improve IVF as well as cryopreservation techniques for commercial and conservational values, this work aims to uncover the signaling events leading to the promotion of sperm motility, using zebrafish (*Danio rerio*) as a model. Our data indicate that the activity of both PKA and PKC are involved in this process. We have standardized Western blot analysis for the examination of the activity of these enzymes by using antibodies that specifically recognize consensus phosphorylation sequences of each kinase. Moreover, by dual staining with SYBR Green and propidium iodide, we have been able to track and follow sperm movement in vivo under a fluorescence microscope. Our data indicate that sperm motility is triggered immediately upon dilution in hypotonic media. A dilution of 50% promotes the longest motility period, followed by sperm death in all cases. These data represent the first analysis of signalling events related to zebrafish sperm motility.

ST-P04
CHARACTERIZATION OF THE UNFOLDED PROTEIN RESPONSE BY FLUORESCENT REPORTERS IN SINGLE CELLS

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The Unfolded Protein Response (UPR) is a cellular stress signaling cascade that can be activated by different signals such as accumulation of misfolded proteins in the lumen of the Endoplasmic Reticulum (ER). This homeostatic response, which involves the activation of three parallel pathways (IRE1, PERK and ATF6), promotes cell survival in the short term but stimulates apoptosis if misfolded protein levels remain high. UPR deregulation plays an important role in malignant neoplasms as myeloma, breast and prostate cancer. In order to characterize UPR dynamics in human single cells, we created a set of fluorescent reporters to monitor the activation of each UPR pathway in real time. These constructs allowed us to measure cell-to-cell variation in the activation of UPR, an analysis that cannot be done by standard technics like western blot or PCR. Moreover, these reporters proved to be useful to analyze the dynamic movement of UPR components along different subcellular compartments. Our goal is to find well-defined patterns that provide us with information about the state of the cell and which can be associated to a specific cell fate.

In the long term, we hope that these patterns can help us to understand why certain cells develop tolerance to stress conditions or escape antitumor drugs.

ST-P05
1α,25(OH)$_2$-VITAMIN D3-MODULATION OF CYCLIN DEPENDENT KINASES 4 AND 6 IN SKELETAL MUSCLE CELLS

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We reported the VDR and p38 MAPK participation in cell proliferation and differentiation triggered by 1,25(OH)2-vitamin D3 [1,25D] in C2C12 muscle cells. Moreover, 1,25D induced the expression of cyclin D3 and cyclin dependent kinases (CDKs) inhibitors in a VDR- and p38 MAPK-dependent manner. We here show that CDKs 4 and 6 also play a role in 1,25D stimulation of myogenesis. C2C12 myoblasts were infected with pLKO.1 plasmid encoding a shRNA against mouse VDR, which was knocked down 80%. p38 MAPK and ERK1/2 were inhibited using SB203580 and UO126 compounds, respectively. Investigation of changes in cellular cycle proteins by immunoblotting and immunocytochemistry showed that the VDR is involved in the 1,25D –induced increase of CDK4 and CDK6 levels during the S-phase peak. Up-regulation of CDKs 4 and 6 by 1,25D (12 h) was abolished in C2C12 cells pretreated with the ERK1/2 inhibitor UO126. However, CDKs 4 and 6 expression induced by the hormone was not dependent on p38 MAPK activation. Confocal images showed that there is no co-localization between VDR and CDKs. Silencing of cyclin D3 only diminished the levels of CDK 4. The results indicate that the VDR and ERK1/2, but not p38 MAPK, are involved in the control of the muscle cellular cycle by 1,25D through CDKs 4 and 6 as part of the mechanism of hormone modulation of myogenesis.

ST-P06
ROLE OF SIGNALING PATHWAYS IN N- ZEB1 PHOSPHORYLATION AND IN EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

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ZEB1 (Zn Finger E-box Binding Homeobox) is a transcription factor that mediates the EMT and invasion.It contains 2 Zn finger domains (ZD1-ZD2) which bind DNA independently. ZEB1 expresses as two phosphorylated (P) forms important for its biological role. We had characterized the role of phosphorylated C-term ZD2. Our goal is to uncover the role of signaling pathway/s on N-term ZD1 (N-ZEB1) biological function on EMT. IP assays followed by WB with antiP-substrates Ab revealed that N-ZEB1 is modified by PI3K(AKT), MAPK and PKC pathways.IGF-1 induced relocation of a nuclear N-ZEB1-GFP clone harboring the first 490aa (eGFPZ1) into the cytosol of CHO-K1 cells; a 2nd clone, eGFPZ2 (included in the former) was unresponsive to IGF-1. PMA/IONO treatment relocated both clones in the cytosol. NMuMG cells were used to investigate the role of N-ZEB1 in EMT. These epithelial cells, that can turn into mesenchymal under various treatments, were stably transfected with eGFPZ2 or empty vector (control). The epithelial markers E-cadherin and actin assessed by WB and confocal microscopy were diminished or absent in eGFPZ2 cells. Also, cell proliferation and cell migration were significantly increased ($P<0.001$ and $P<0.05$, respectively) in eGFPZ2 cells vs. controls. We have identified a small fragment in ZEB1 that induces EMT by itself. The activity of N-ZEB1 could be regulated by IGF-1 and PKC effectors.

ST-P07
SYNTHETIC DAF-12 MODULATORS WITH POTENTIAL USE IN CONTROLLING NEMATODES LIFE CYCLE

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*C.elegans*has the ability to sense environmental quality and nutrient availability and adjusts rates of maturation accordingly. In favourable environments, *C.elegans*develops from embryo through four larval stages into a sexually reproductive adult. In unfavourable environments, animals arrest development at an alternate third larval stage called dauer diapause. Environmental and physiologic cues are transduced through various signal transduction pathways that converge within steroidogenic tissues to promote production of the DAF-12 ligands called the dafachronic acids (DAs). DAF-12 is a ligand dependent transcription factor that acts as a molecular switch mediating the choice

between arrest at diapause or progression to reproductive development. DAs are 3-keto colestenoic acids bearing a carboxylic acid moiety at the end of the steroid side chain. In this study, we evaluated the in vitro and in vivo activity of Δ–4 synthetic analogues with modified side chains using transactivation cell based assays and*daf–9 C.elegans*mutants, which are unable to produce DAs. Our results revealed that introduction of a 24,25 double bond on the cholestenoic acid side chain did not affect DAF-12 agonist activity while shortening the side chain lowered the activity. Most interestingly, the C24 alcohol 24–hydroxy–4–cholen–3–one was an antagonist of the DAF-12 receptor both in vitro and in vivo.

ST-P08
THE PHEROMONE RESPONSE PATHWAY OF YEAST IS ROBUST TO CHANGES IN THE ABUNDANCE OF RECEPTORS

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Receptor theory postulates that cells responds to extracellular ligand concentration through the formation of a receptor-ligand complex, and transduce information of the amount of this complex to downstream effectors. A simple prediction of this model is that modifications in the abundance of receptors should modify the dose response curve. We tested this prediction in the pheromone response pathway of the yeast Saccharomyces cerevisiae, and found no differences in the transcriptional response when the levels of the receptor were varied. We developed a detailed mathematical model of this system, which includes the activation of the heterotrimeric G protein and its coupling to the receptor. Analysis of the model suggested a mechanism by which the response can be robust to changes in the total amount of receptors. Our model is consistent with many results from the literature that are hard to explain by other mechanisms. We are currently working on further experiment to test the predictions of our model.

ST-P09
DEVELOPMENT OF A MICROFLUIDIC-BASED METHOD FOR STUDING GRADIENT SENSING AT SINGLE CELL LEVEL

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Cells are exposed to gradients of regulatory molecules and accurately determine both ligand concentration and gradient direction. We used the chemotropic growth towards mating pheromone (αFactor, αF) of *S. cerevisiae* as model system of gradient sensing. Here we present (1) the development of a reliable microfluidic-based method for analyzing chemotropism and (2) the generation of molecular tools for analyzing the dynamics of cell polarization. The microfluidic device used had open chambers to which two different media are delivered in opposite sides by a strip of microchannels. First, we determined the linearity of gradient, the time of formation and stability in time using fluorescent dyes. Next, we exposed cells with YFP under the control of an αF responsive promoter to different gradients of αF. We found an almost linear relation of position and reporter induction. When we measured the angle of the polarizations, we observed that cells determine direction more efficiently in gradients of 0-50nM. Once the method was established, we tagged Far1, Cdc24 and Bem1, key players in the polarization process, with a triple copy of mNeonGreen, a new bright and photostable fluorescent protein. Mutant strains showed normal αF response and the localization of fusion proteins could be tracked over time. With these method and tools we will pursue the study of gradient sensing in single cells.

ST-P10
SINGLE CELL ANALYSIS OF MEMBRANE RECRUITMENT DYNAMICS OF THE MATING MAPK PATHWAY SCAFFOLD PROTEIN

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In *S. cerevisiae*, pheromone activates a GPCR coupled to a MAPK cascade pathway that, among other effects, arrests cell cycle progression in G1. However, in cells committed to a new round of cell division, Cdk activity blocks pheromone response. Plasma membrane (PM) recruitment of the mating MAPK scaffold, Ste5, is a key step in pheromone signaling. It is this membrane interaction that is inhibited by Cdk activity by phosphorylating residues flanking the Ste5 PM binding domain. Recently we developed a quantitative method to measure protein relocalization over time using microscope cytometry. Here, using this method, we studied the early dynamics of Ste5 recruitment as a function of the cell cycle position in single live cells. In contrast to our expectations, we found that initial

recruitment is similar in G1 and S phase cells, but then it rapidly declines in S phase cells. Remarkably, this decline in S phase is strictly dependent on the activity of the mating MAPK Fus3. Nevertheless, Fus3 activity alone cannot displace Ste5 from the membrane, since Ste5 recruitment persists over time in G1 cells or when it lacks the Cdk sites flanking its PM domain. These findings reveal that Fus3, on top of its classic mating promoting functions, cooperates with the Cdk in a complex negative feedback, bringing about distinct patterns of temporal dynamics at different cell cycle stages.

ST-P11
DIFFERENT WAYS TO DETECT EXTRACELLULAR INFORMATION AND THEIR EFFECT ON THE RESPONSE DYNAMIC RANGE

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An important characteristic of every living cell is its ability to communicate with the surrounding environment. This exchange of information is called cellular signaling and is based on the capacity of the cell to give proper responses to environmental signals. Mathematical/computational modeling based on biological information can be used to improve our understanding of cellular signaling, thereby enhancing predictive accuracy. The first step in sensing extracellular information usually involves the reversible binding between an external ligand and a membrane receptor. We have shown in a recent publication that the dose-response curve of this sensing step (dose=ligand, response=ligand-receptor complex) is shifted to the high doses region when measured before reaching equilibrium, meaning that the utilization of extracellular information before ligand-receptor binding reaches equilibrium expands and shifts the input dynamic range (the dynamic range is the range of input concentrations that elicit distinguishable outputs). In this work we study how the properties of the ligand-receptor dose-response curve that allow PRESS are affected by adding complexity to the description of this sensing mechanism: consumption of ligand, receptor synthesis and internalization, and multiple receptor states.

ST-P12
CORRELATIONS BETWEEN METABOLIC PROFILES AND LIFESPAN IN *Saccharomyces cerevisiae* CELLS

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In all living organisms, cell survival is mediated by metabolic regulation in response to environmental conditions. *S. cerevisiae* is used to study the molecular and genetic mechanisms of aging in two distinct ways.The first, replicative life span (RLS), is a measure of the number of mitotic events an individual mother cell can undergo before senescence; the second, chronological life span (CLS), is a measure of the time a non-dividing cell population remains viable. RLS has been suggested to be a model for the aging of mitotic tissues, whereas CLS has been likened to the aging of post-mitotic tissues. Since it is known that both some amino acids and mutations in some amino acid permeases genes can extend CLS, we measured the levels of Uga4 and Agp1 permeases along the aging process. We found a deregulation of their expression in chronologically aged cells. We also analysed the metabolic fingerprints revealed with Fourier Transformed-Infrared (FT-IR) spectroscopy in cells with different lifespan (i.e. cells deficient in TOR or Sch9 kinases). During the aging process, metabolic profiles significantly changed exhibiting important alterations, particularly in the proteins and lipidic peroxidation spectral windows. Moreover, an excellent metabolomics-based discrimination between cells with different lifespan was established.

ST-P13
ROLE OF THE DAL80 TRANSCRIPTION FACTOR IN THE CONTROL OF ALL LEUCINE PERMEASES IN *Saccharomyces cerevisiae*

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Yeast can use a wide variety of nitrogen compounds. However, the ability to synthesize enzymes and permeases for catabolism of poor nitrogen sources is limited in the presence of a rich one. This general mechanism of transcriptional control is called nitrogen catabolite repression. There are different permeases that can transport all naturally occurring amino acids. In this work we studied the regulation of Agp1 and Gap1, two amino acid permeases with broad substrate range, and Bap2 and Bap3, both involved in uptake of leucine, isoleucine and valine. Expression of these genes under different growth conditions was analysed. We also studied the expression of transcription factors that

regulate these genes, and the interaction of these factors with the regulatory region of the permease genes. We found that in the absence of amino acids only Gap1 is expressed although the negative factor Dal80 is acting on its promoter. After addition of leucine, the transcription factors Dal81 and Stp1 bind to AGP1 promoter, Stp1 binds to BAP2 promoter, and consequently expression of AGP1 and BAP2 rises. In the presence of a rich nitrogen source and leucine, Bap3 seems to be the only permease capable of incorporating amino acids into yeast cells. The expression of these permeases is regulated so that an amino acid is incorporated only by one or another permease, depending on the growth conditions.

ST-P14
RSPO3 IS A GLYCOPROTEIN SECRETED BY MAMMARY TUMOR CELLS THAT INDUCES WNT-PATHWAY ACTIVATION AND EMT

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R-spondins (RSPOs) is a family of secreted proteins that have been implicated in significant processes like embryonic development, tissue differentiation and human diseases. They have been also postulated as potent stem cell growth factors. In addition, we and other groups have determined that RSPOs are frequently mutated in Mouse Mammary Tumor Virus (MMTV)-induced tumors, particularly RSPO3. Our results show that mouse and human tumorigenic and non-tumorigenic mammary cells express Rspo3 mRNA, but the levels are higher in basal-like cells. They secrete the protein, which associates with the extracellular matrix and/or the cell membrane, since their conditioned medium can be enriched by treatment with soluble heparin. Rspo3 treatment of non-tumorigenic mammary epithelial cells induces changes associated to epithelial-mesenchymal transition (EMT). In addition, down-regulation of its expression in cultured basal mammary cancer cells causes inhibition of migration, vimentin expression and repression of the canonical WNT signalling pathway. When transplanted into mice, these RSPO3-KO cells resulted less tumorigenic than control cells. Our results indicate that RSPO3 is a potent oncogene, which promotes cancer development through WNT-pathway activation in mammary epithelial cells.

SP-P15
MECHANISM OF SIGNAL DETECTION AND SIGNAL TRANSDUCTION IN THE THERMOSENSOR DESK

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The Des circuit in *B. subtilis* is designed to adjust the composition of the membrane lipids in response to the growth temperature, regulating the expression of an acyl-lipid desaturase. This circuit is controlled by the DesKR two-component system. DesK is a transmembrane protein with five segments (TMS) which has kinase and phosphatase activities at 25 or 37° respectively. What biophysical feature gives DesK the ability to act as a sensor? Recently we designed a chimerical protein having a single TMS, which works like full length DesK, and was called minimal sensor. This discovery allowed us to study a complex phenomenon using a simple model. We found a serine zipper motif at the C-terminus of the TMS, located at one face of the helix. The zipper tends to form inter-helical hydrogen bonds at low temperatures, when the membrane is thicker and dehydrated. To test our model of activation, which implies bringing two TM helices closer to stabilize a dimer interface, mediated by the serine zipper, we replaced zipper serines by residues with strong or negligible capacity to form hydrogen bond, using the method of Quick change. Variants were cloned into the expression plasmid pHPKS, which were used to transform *Bacillus subtilis* and then measure β-galactosidase activity at 25ºC or 37°C. We found that residues with hydrogen bond forming capacity stabilize the active DesK conformation.

ST-P16
INSULIN RECEPTOR: PRESENCE AND ACTIVATION IN PHOTORECEPTOR NUCLEI FROM BOVINE RETINA

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We have recently reported a novel light-dependent activation of diacylglycerol kinase (DAGK) and protein kinase C at the nuclear level of photoreceptor cells. We have also observed that ex-vivo light exposure of bovine retinas

modulates insulin-related signaling pathways, such as PI3K-Akt and MAPK pathways, in photoreceptor nuclei. The aim of the present study was to analyze the presence of insulin receptor (IR) in photoreceptor nuclei and the possible DAGK modulation by insulin. A nuclear fraction enriched in small nucleus from photoreceptor cells (PNF) was obtained for the study of IR presence and phosphorylation state as well as local insulin action in lipid kinases in PNF. Immunofluorescence and western blot studies revealed the presence of IR in PNF and a light-dependent increase of nuclear IR. Polyphosphoinositides and phosphatidic acid (PA) formation were found to be increased when isolated nuclei were exposed to 0.8 µM insulin plus 0.2 mM vanadate in nuclei from retinas exposed to light with respect to the darkness condition. Our results i) demonstrate the presence and the functional state of IR in PNF from bovine retinas, ii) suggest a light-mediated localization of IR in PNF and iii) reveal local insulin effects on nuclear lipid enzymes involved in the regulation of lipid second messengers, such as DAG and PA.

ST-P17
EFFECTS OF PHOSPHATIDYLCHOLINE-BILE SALT NANOPARTICLES OVER MCF-7 BREAST CANCER CELLS

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Phosphatidylcholine-bile salt mixed micelles are nanoparticulated systems extensively studied and proposed for the delivery of therapeutics. A recent trend in nanotechnology has been to investigate the interactions of nanoparticles with biological systems, since their effects might depend on their properties (i.e., chemical composition). The aim of the present work was to analyze the effects over cell viability and signaling mediators, of soybean phosphatidylcholine (SPC) and sodium cholate (SC) mixed micelles prepared at different SPC:SC molar ratios (1:1, 1:2, 1:4, 1:8) over the MCF-7 cell line. Cell viability was assessed by the MTT cell viability assay and Erk1/2 and Akt activation was determined by western blotting. Results showed that mixed micelles containing the highest proportion of SC are citotoxic after 24 hs incubation. Increased phosphorylation of Erk1/2 and Akt after 24hs incubation correlated with the cytotoxic effects. To ascertain if the described effects accounted on SC, cell viability and activation of Erk1/2 and Akt was assessed after incubation with different SC concentrations. In accordance with results found for the MM containing high SC proportion, SC at high concentration was cytotoxic and induced activation of Erk1/2 and Akt. Sustained activation of the kinases might be involved in the cytotoxic effect of high SC concentrations over breast cancer cells.

ST-P18
INVOLVEMENT OF ERK1/2 PATHWAY IN THE MIGRATION OF COLON CANCER CELLS INDUCED BY PTHRP

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Parathyroid Hormone-related Peptide (PTHrP) is implicated in several human cancers such as colon carcinoma. The pathogenesis of this disease involves several processes, including enhanced cell survival, proliferation, migration and angiogenesis. Migration of cancer cells is the crucial step in the complex process of metastasis. Recently we obtained evidence that in Caco-2 cells, a cell line from human colorectal adenocarcinoma, exogenous PTHrP increases the number of live cells and cell cycle progression via Erk1/2, p38 MAPK and PI3-kinase. The aim of our study was to investigate the role of PTHrP on migration and angiogenesis of these tumor cells. Wound healing results revealed that cell migration increased after PTHrP treatment for 24 h. Furthermore, western blot showed that the hormone (1 h) induced threonine/serine phosphorylation of p90 ribosomal S6 kinase (RSK), an enzyme recently involved in cell motility. In addition, inhibition of Erk1/2 reversed the phosphorylation of RSK and cell migration induced by PTHrP. Finally, using real-time PCR, we observed that the expression of the pro-angiogenic VEGF increased by PTHrP treatment (20 h). Taken together, our results indicate that PTHrP induces the phosphorylation of RSK and cell migration via the Erk1/2 pathway and suggest a potential role of the hormone on tumor angiogenesis

ST-P19
VITAMIN D AGONISTS AND PROTEASOME INHIBITION UPREGULATE BIM PROTEIN IN ENDOTHELIAL CELLS

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The pro-apoptotic Bim (Bcl-2 interacting mediator of cell death) is a key regulator of tissue homeostasis. We have previously shown that vitamin D agonists, 1α,25(OH)2-Vitamin D3 [1α,25(OH)2D3] and its less calcemic analog TX 527 inhibit the proliferation of endothelial cells (SVEC) and transformed by the viral G protein-coupled receptor associated to Kaposi sarcoma (vGPCR) inducing Bim expression and apoptosis. Bim expression can be controlled by transcriptional and post-transcriptional mechanisms. We further explored the mechanism of regulation of Bim and the role of vitamin D receptor (VDR) in its expression. We found that increasing Bim by 1α,25(OH)2D3 or TX 527 was suppressed when VDR expression was blocked by stable transfection of shRNA against VDR. Bortezomib, a proteasome inhibitor used to inhibit NFkB pathway highly activated in vGPCR cells, similarly to vitamin D agonists, also increased Bim protein levels in a dose-dependent manner (0.25-1 nM). This was accompanied by a reduction in the phosphorylation state of Akt and ERK1/2. Moreover, FOXO3a phosphorylation was also decreased in vGPCR cells. Since Bim increase can be regulated by Akt/FOXO3a pathway and by reduction of its degradation by inhibition of ERK, these results suggest that vitamin D agonists-dependent Bim increment might be regulated by the inhibition of ERK and Akt in SVEC and vGPCRs cells.

ST-P20
FETAL BOVINE SERUM UP-REGULATES MKP-3 MESSENGER LEVELS THROUGH AN ERK-DEPENDENT MECHANISM

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MDA-MB-231 is a human line of breast cancer cells carrying mutations leading to persistent ERK activation. MAPK phosphatases (MKPs) like MKP-1, -2 and -3 are an enzyme family specifically involved in MAPK inactivation. This work analyzed the expression of these MKPs in this cell line and studied the effect of fetal bovine serum (FBS) 10%, a mitogenic stimulus. mRNA levels were analyzed by RT-PCR in cells starved for 24 h (C) or FBS-stimulated for 1h (MKP-1) or 2h (MKP-2 and MKP-3) after starvation (E). Two isoforms of MKP-2 and MKP-3 mRNA (S and L) were detected in both conditions, L being the most abundant, which is a novel finding for MKP-3 in this cell type. Results demonstrate that starvation does not abrogate the basal expression of any of these MKPs, in contrast with results described in other cell types, while FBS stimulation increases mRNA levels of MKP-3 only. This result agrees with the fact that MKP-3 is specific to ERK1/2 and induced only by mitogenic stimuli. Preincubation with PD98059 (PD), a compound that prevents ERK activation, abrogates the effect of FBS on mRNA levels (E=1,43±0,20; E+PD=0,52±0,20, $p<0,05$). Western blot analyses show high MKP-3 protein levels in cells stimulated for 2h (C=0,30±0,05; E=1,22±0,25, $p<0,05$) but not for 3h. To conclude, FBS stimulation tightly regulates MKP-3 in MDA-MB-231 cells so as to counteract persistent ERK activation.

ST-P21
Q-RICH REGION OF TPK2 CATALYTIC SUBUNIT OF PKA AFFECTS STRESS RESPONSE

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It has been previously described that the presence of glutamine-and/or asparragine rich (Q/N-rich) domains in P-bodies (PBs) components promotes their efficient accumulation. Catalytic subunit of cAMP dependent Protein Kinase, TpK2, has a Q-rich region in its N-terminus region that is not present in any of the other isoforms, Tpk1 or Tpk3. We analyze whether the Q-rich region had any effect on the Tpk2 aggregation on PBs in response to stress. *In vitro* kinase assay of affinity purified samples from a strain expressing Tpk2Bcy1-TAP or *tpk*[2] *Q*ΔBcy1-TAP shows that deletion of Q-rich region does not affect kinase activity or its interaction with the regulatory subunit Bcy1. However, *tpk*[2] *Q*Δ-GFP mutant aggregation to cytoplasmic foci is reduced under glucose starvation, severe heat stress

and stationary phase. Stress granules and PBs aggregation are affected during stationary phase in strains expressing *tpk*^{2} *Q*Δ mutant, whereas only PBs granules formation is reduced during heat-shock or glucose starvation. Since the presence of mRNA foci increases cell survival, we tested cell survival of wild type and *tpk*^{2} *Q*Δ strains under heat stress. We found that *tpk*^{2} *Q*Δ mutant increases cell survival. Taken together these data suggest that Tpk2 Q-rich domain might be involved in a mechanism of assembly of PBs and SGs in a stress type-dependent manner.

ST-P22
PKA AFFECTS PROTEIN ABUNDANCE OF RPG1 AND EIF4G1 TRANSLATION FACTORS IN *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae* protein translation is decreased during stationary phase, being initiation the most regulated step of this process. Previously, we described that PKA has a role in the translation inhibition that occurs during entry into stationary phase. Here we show that in exponentially growing cells, the limiting translation factors eIF4G1 and Rpg1 are highly expressed. In contrast, their protein abundance is severely reduced during stationary phase. We analyse the role of PKA activity on eIF4G1 and Rpg1 protein abundance. In strains with reduced PKA activity, eIF4G1 and Rpg1 are not expressed neither in exponential nor in stationary phase cells. However, high PKA activity promotes high expression of eIF4G1 and Rpg1 proteins even during stationary phase of growth. We also determine that the half-life of these factors is lengthened in a strain harbouring high PKA activity versus exponentially growing wild-type cells. Moreover, eIF4G1 and Rpg1 mRNA levels were constant in both exponential and stationary phase of growth in cells with high or reduced PKA activity. Protein abundance of eIF4G1 and Rpg1 in strains with impaired proteasome activity showed that the degradation of these factors during stationary phase is proteasome-mediated. Taken together, we demonstrate that PKA increases the eIF4G1 and Rpg1 proteins abundance through a post-transcriptional mechanism.

ST-P23
EXPRESSION REGULATION OF YEAST PKA CATALYTIC SUBUNIT, TPK1, BY UORF AND NCRNA

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Upstream open reading frames (uORFs) are regulatory elements located in 5' untranslated regions, which can repress the translation of downstream coding sequences. In S. cerevisiae, the catalytic subunit of protein kinase A (PKA) is encoded by three genes TPK1, TPK2 and TPK3. In TPK1 an uORF was described in the 5' UTR (five codons length). In order to study the role of TPK1 uORF, the full-length 5´ sequence was cloned into a β-galactosidase (β-Gal) reporter (WT-UTR) and a version with the uORF start codon mutated (mut-UTR) was constructed. β-Gal activity and mRNA levels were measured. The uORF regulates negatively TPK1 translation and stabilized the mRNA. Under heat shock stress, both β-Gal activity and Tpk1 mRNA level were upregulated for WT-UTR and β-Gal activity but not mRNA level was upregulated for mut-UTR. Therefore the translational efficiency resulted higher for the mut-UTR construct. In other hand, a ncRNA antisense (AS) was also identified overlapping 600pb on CDS 3' end. The AS level was measured in log and stationary growth phases and under heat shock stress. The AS level showed an inverse correlation with TPK1 mRNA, during the stress conditions, indicating a possible role in repression of TPK1 expression. Thus, uORF and antisense ncRNA could contribute to regulate the expression of PKA Tpk1 subunit at transcriptional and post-transcriptional levels respectively

ST-P24
DIFFERENTIAL REGULATION OF YEAST PKA SUBUNIT PROMOTERS IN FERMENTATIVE AND OXIDATIVE GROWTH

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Glucose is the preferred energy source of Saccharomyces cerevisiae. Three signaling pathways are required to correctly respond to glucose availability: the Rgt2/Snf3, the Snf1/Mig1 and the cAMP/PKA pathways. When glucose is limited, Snf1 kinase induces genes involved in the metabolism of alternative carbon sources. In S.cerevisiae three

genes encode the PKA catalytic subunit, TPK1, TPK2, TPK3; while one gene, BCY1, encodes the regulatory subunit. Employing reporter gene methodology we studied the activity regulation of PKA subunits promoters in fermentative (glucose) or non fermentative (glycerol) growth. We found that the activity of all promoters is stronger in glycerol than in glucose medium. In glucose, TPK1 and TPK2 promoters are inhibited by Mig1. TPK2 promoter was inhibited by Mig1 and also Mig2 transcription factors. The four promoter activities are regulated by Snf1 kinase; and the Snf1 downstream effectors Cat8 and Sip4 regulate TPK2, TPK3 and BCY1 promoter activities. In cells grown in glycerol, the four promoters are upregulated by Snf1/Cat8. TPK1 promoter activity is downregulated by Mig1, and BCY1 by Mig2 and Mig3; while TPK2 and TPK3 promoters are upregulated by Mig1, Mig2 and Mig3. Results of ChIP assays show the presence of Cat8 in the TPK1 promoter transcriptionally active. These results were confirmed by mRNAs quantification by qRT-PCR

ST-P25
CELL WALL INTEGRITY AND TRANSCRIPTIONAL REGULATION OF TPK1 SUBUNIT OF PKA FROM *Saccharomyces cerevisiae*

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A signal transduction cascade, the CWI (cell wall integrity) pathway of *Saccharomyces cerevisiae* contains cell wall stress sensors that lead to the activation of a mitogen-activated protein kinase (MAPK) cascade. Signaling through the MAPK cascade leads to expression of transcription factors that facilitate biosynthesis of cell wall components and actin organization. To manage the diverse stress conditions, the CWI pathway cross talks with other pathways or proteins, such as the protein kinase A pathway. PKA is composed of catalytic and regulatory subunits. The catalytic subunits are encoded by TPK1, TPK2 and TPK3 genes. We analyzed the regulation of TPK1 expression during heat stress by the CWI pathway. Wsc1-3 functions as the cell stress sensors to activate the CWI pathway in response to heat shock and activate the MAPK cascade. Using β-galactosidase reporter and quantification of mRNA by qRT-PCR we demonstrate that the three isoforms of the Wsc receptor are not redundant for heat-shock response. Wsc1 upregulates and Wsc3 downregulates TPK1 expression. Further, using different deletion strains, the involvement of MAPK kinase pathway in TPK1 expression regulation was demonstrated, although the results also show that other parallel pathway should be also involved in this regulation. The results indicate that exists a cross talk among MAPK and PKA pathway during heat shock.

ST-P26
TCVPS15 AS A POSITIVE REGULATOR OF AUTOPHAGY IN *Trypanosoma cruzi*

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Autophagy is a tightly controlled degradation process of all eukaryotes. In yeast, the phosphatidylinositol 3-kinase (Vps34) and its membrane targeting unit, the protein kinase Vps15 have been shown to be important regulators of autophagy. However, their role in this process remains elusive in *Trypanosoma cruzi*. Previously, we have characterized the first class III PI3K in *Trypanosoma cruzi*, named TcVps34, which has a role in vital processes such as osmoregulation, acidification and endocytosis. Afterward, we biochemically characterized the regulatory kinase TcVps15 in this parasite. In the present work, we aimed to assess the function of TcVps15 in autophagy. Immunofluorescence studies demonstrated that under starvation conditions TcVps15 colocalize with Atg8, an autophagosome marker in *T. cruzi*. Moreover, assays using acridine orange showed a higher level of acidification in TcVps15 transgenic parasites under nutritional stress. To further study the structure of TcVps15, we generated a mutation in its catalytic domain. Biochemical assays using the recombinant protein showed a six-fold increase in the Km value. We are currently assessing the effects of this mutation in transgenic parasites through immunofluorescence and starvation assays. Taken together, these results provide the first evidence for a function of TcVps15 as a positive regulator of autophagy in *Trypanosoma cruzi*.

ST-P27
ROLE OF RAB3IL (A RAB3 GEF) IN HUMAN SPERM ACROSOMAL EXOCYTOSIS

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Acrosome reaction (AR) is a type of regulated exocytosis that leads to the release of the acrosome granule content. Rab3 and Rab27 constitute the two main Rabs implicated in regulated exocytosis. They localize to vesicles and secretory granules and control their recruitment, tethering, and/or docking to the plasma membrane. We have shown that, like Rab3A, Rab27 is required for exocytosis in human sperm. Both Rabs exchange GDP for GTP in response to exocytosis initiators. We were able to show that Rab3A–GTP and Rab27–GTP localize to the acrosomal region of the sperm head and active Rab27 increases the exchange of GDP for GTP on Rab3A. The molecular mechanism for this activation appears to be through a Rab–GEF cascade. A novel protein designated GRAB (guanine nucleotide exchange factor for Rab3A, also called Rab3IL) displays GEF activity for Rab3A. In this work, we present direct evidence about the localization of Rab3IL in the acrosomal region of human sperm. We also report that Rab3IL participates in an early stage during membrane fusion. And that an anti–Rab3IL antibody impairs exchange of GDP for GTP on Rab3A but not on Rab27. Finally, we demonstrate that Rab3IL interacts with Rab27–GTP bound form. Together, these results confirm the hypothesis suggested about a possible Rab–GEF cascade in human sperm and postulate Rab3IL as the possible GEF.

ST-P28
14-3-3-PROHIBITIN INTERACTIONS. IN VIVO AND IN SILICO STUDIES

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The 14-3-3 protein family is a phosphoproteome master regulator. It binds more than 2000 cellular proteins, recognizing two structural features in its client proteins: a serine or threonine phospho residue and a region of about 30 consecutive aa rich in residues that promote intrinsic structural disorder. Although 14-3-3 is described as a ubiquitous protein family, most of its complexes found in the literature include either cytosolic or nuclear proteins. Here, we show the interaction of 14-3-3 with Prohibitin 1 and 2 (PHB1, 2), a mitochondrial and fully structured protein, therefore an atypical 14-3-3 partner. Both, 14-3-3s and PHBs, are hubs for many signaling pathways triggered by growth factors, the immune response, and steroid hormones regulating metabolism, mitochondrial biogenesis, cell migration, division, and survival. We used in vivo Föster Resonance Energy Transfer (FRET) of genetically encoded fluorescent CFP-14-3-3 beta and PHBs-YFP to demonstrate the interaction between these two proteins and to show the subcellular localization of the complex. Transfected HeLa cells showed positive FRET signal specifically localized in the mitochondria, and no signal was observed when a nuclear 14-3-3 (CFP-14-3-3-NLS) was used as control. A possible structure of the complex is proposed by in silico molecular docking using the structures of both proteins from the PDB (1LU7 and 1A40).

ST-P29
14-3-3 PROTEIN FAMILY LEVELS VARIATIONS DURING DIFFERENTIATION OF 3T3-L1 PREADIPOCYTES

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The 14-3-3 protein family comprises phospho-Serine/Threonine-binding proteins consisting of seven mammalian paralogs. This protein family regulates several cellular processes including cellular differentiation. In adipogenesis, 14-3-3 regulates the subcellular localization of TAZ, a co-repressor of PPAR gamma dependent gene transcription. We studied the mRNA and protein levels of the 14-3-3 family before and after differentiation of NIH 3T3-L1 preadipocytes by using qPCR and Western Blot (WB) with specific primers and antibodies for each 14-3-3 member respectively. The results revealed, after seven days of differentiation, a decrease in mRNA levels of 14-3-3 beta, epsilon and zeta and an increment of mRNA levels of 14-3-3 gamma, eta and tau, compared to undifferentiated cells. Protein levels of each paralog were either conserved or decreased after one week differentiation. A particularly pronounced decrease was observed in eta isoform protein. Therefore, an apparent discrepancy was observed between mRNA and protein levels of 14-3-3 eta, showing a specific degradation in response to differentiation signals in 3T3-L1 cells. Our findings indicate that the differential expression and/or postranslational regulation of 14-3-3 family

members levels during 3T3-L1 adipogenesis may be a mechanism to indirectly modulate differentiation to the adipose linage.

ST-P30
ESTROGEN AND TESTOSTERONE PROTECT AGAINST APOPTOSIS VIA p53, p66SHC, PKCδ AND JNK IN C2C12 CELLS

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In aged skeletal muscle, a prominent apoptosis associated to a deficit of sex hormones is observed, contributing to the pathogenesis of sarcopenia. We have previously demonstrated that 17β-estradiol (E2) and testosterone (T) inhibit H_2O_2-induced apoptosis in C2C12 muscle cells. This work further characterizes the molecular mechanisms involved in the antiapoptotic action of both steroids. We observed that H_2O_2activates p53 in a time-dependent fashion; and this activation is reduced by T treatment. P53 induces apoptosis via p66Shc activation, an adaptor protein that amplifies the generation of mitochondrial H_2O_2. We found that both T and E2 reduce the H_2O_2-induced p66Shc mRNA level. Additionally, the steroids inhibit PKCδ and JNK activation, resulting in the inhibition of the phosphorylation and translocation to mitochondria of p66Shc, events associated to oxidative stress. Tetramethylrhodamine methyl ester (TMRM) staining showed that E2 protects the mitochondrial membrane potential in line with the inhibition of p66Shc translocation to mitochondria. Finally, we demonstrated by qRT-PCR that both steroids diminish the H_2O_2-induced mRNA levels of the apoptotic proteins PERP and Puma and increase those of the antiapoptotic protein Bcl-2. These data provide insights into the molecular basis of sarcopenia associated to sex hormones deficit states.

ST-P31
SCREENING FOR *AKT1* (E17K) MUTATION IN BREAST CARCINOMAS AND CONTROL SAMPLES

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AKT (also known as protein kinase B) is a subfamily of serine/threonine protein kinases and is a major downstream target of growth factor receptors that signal through phosphatidylinositol 3- kinase. Remarkable increase in the Akt kinase activity has been found in approximately 30% to 40% of breast cancer specimens. Recently, a somatic mutation in the PH domain of AKT1 was identified in a subset of human carcinomas, including breast cancer. This mutation results in the substitution of glutamic acid at codon 17 of *AKT1* with lysine (E17K) and alters the lipid-binding specificity of AKT, leading to pathological membrane association and constitutive signaling. The aim of this work was to screen *AKT1* (E17K) mutation in 29 breast carcinomas and 52 control samples from Posadas, Misiones. Blood samples were collected and DNA was extracted by *Salting out* method. DNA extractions were amplified by polymerase chain reaction (PCR), using the primers previously described in the literature. We performed mutation analysis by direct sequencing of PCR products. The sequencing analysis for exon 3 of the human *AKT1* gene in all breast carcinomas and control samples revealed the absence of the point mutation G>A at nucleotide 49 (E17K). Although we did not find the mutation, this could be due to the size limitations of our study.

ST-P32
β4 INTEGRIN MUTATION R1281W IN A WOMEN POPULATION WITH BREAST CANCER FROM MISIONES

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Integrins are heterodimers consisting of an alpha (α) and a beta subunit (β), which are involved in cell essential tasks such as: attachment, migration, proliferation and cell growth. The β4 subunit is encoded by *ITGB4* gene and beside α6 subunit form the α6β4 integrin, which is part of the anchoring joints or hemidesmosomes (HD) and seems to be involved in malignancy of breast cancer. A pathogenic mutation at nucleotide position 3841 of *ITGB4* gene result in an amino acid change at position 1281 of the β4 subunit, R1281W (rs121912467). This residue would be located at a critical point of integrin and its presence would affect the structure of the HD, decreasing cell adhesion, which is a requirement for cancer development. The aim of this work was to determine the presence of R1281W mutation in *ITGB4* gene in 64 women with breast cancer and 30 controls from Posadas, Misiones. Blood samples were collected and DNA was extracted by *Salting out* method. R1281W mutation was analyzed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). We found that all individuals from both cases and controls groups were homozygous for wild type genotype CC (Trp), we did not find any mutant allele carrier homozygous

(TT) or heterozygous (CT) at the *ITGB4* codon 1281 mutation. Although we did not find the mutation, this could be due to the size limitations of our study.

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Gallo G CB-P18
Galván AE EN-P01
Gambarte J CB-P14
Gándara L CB-P63
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Garavaglia B, MI-P26/PL-P64
Garavaglia BS MI-P25
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Garcia Fabiani MB CB-P32
García IA CB-S01/CB-C02
Garcia L PL-C08/PL-P20
García S BT-P07
García Solá M ST-C06
García Véscovi E MI-C05/MI-C06/
CB-P17/MI-P08/
MI-P07/MI-P49
García-Labari I BT-P04
García-Ramírez E PL-P18
Garda H CB-P31
Gardiol D BT-P08/CB-P25/
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Gargantini PR BT-C07
Garrido MN MI-P11
Garro A BT-P03
Garry R BT-P18
Gastaldi V PL-P22

Gaveglio VL NS-P04
Genero M PL-P44
Gennis R EN-P01
Genova ML SB-P01
Genta SB CB-P54/CB-P56
Gentili C ST-P18
Genti-Raimondi S CB-P27/CB-P42/CB-P43
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Ghio S MI-P29/MI-P62
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Giacomelli JI BT-C04
Gil GA CB-C11
Gimenez A MI-P01
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Gimenez-Mart E PL-P36
Giojalas LC CB-P21/CB-P22/CB-P23
Giono LE CB-P33/CB-P35
Giordano WF MI-P11/MI-P23
Girardini J LI-C01/BT-P04
Girardini JE CB-P30
Giri A BT-P08
Giri GF MI-C06
Gismondi M PL-P19/PL-P44
Giusto NM NS-P04/ST-P16
Glass F PL-S02/PL-P41
Glikin GC CB-P59/CB-P60
Glikmann G BT-P15/BT-P16
Goddio MV ST-P14
Godoy Herz MA PL-P28
Gojanovich AD ST-P29
Goldbaum FA SB-C03/SB-P02
Goldraij A PL-P01
Goldy C PL-P59
Gómez Acuña LI CB-P36
Gomez DE BT-P02
Gomez-Casati DF EN-P05/PL-P02/PL-C05/PL-P36/PL-P37/PL-P51
González A CB-P06
González Bardeci N SB-P05
Gonzalez Baro MR CB-P32
Gonzalez DH PL-C08/PL-P20/PL-P21/PL-P22/PL-P23
González L ST-P17
Gonzalez MC CB-P31/ST-P31
Gonzalez ME PL-P53
Gonzalez Montoro A CB-C01
González Pardo V ST-P19
González RM PL-P27
González Wusener AE CB-P06
Gonzalez, SN MI-P79
González-Calvar S ST-P20
Gorostizaga A ST-P20
Gottig N CB-C05/MI-P25/MI-P26/MI-P56/PL-P60/PL-P64
Graham H BT-P22/CB-P17/CB-P42/MI-C11/MI-P34/MI-P42/MI-P43/PL-P64
Gramajo HC BT-C06/BT-P09/MI-P77
Gramajo HG MI-P41
Grande A ST-C03
Grasa M CB-P31
GrassoD MI-P29
Grecco HE ST-S02
Griet M MI-P10
Grillo-Puertas M MI-P20
Groppa M PL-P33
Grosso RA CB-C04
Grotewold E PL-P03
Guaimas, F MI-P03
Guaytima EV CB-C15
Guerreño M CB-P61
Guerrero Molina MF PL-P35
Guerrero SA EN-P03/EN-P04
Guevara MG BT-P10/ MI-P78/PL-P08
Guido ME CB-P24/NS-P02
Guidobaldi HA CB-P21/ CB-P22
Guidolin, LS MI-P01
Gutierrez MG CB-S02

H

Hael Conrad V PL-P58
Hajirezaei MR PL-P45
Hartman MD EN-P11
Hatfull G MI-C02/MI-C12
Heckel SB CB-P04
Hedin N EN-P05
Heinz R PL-P56
Helary C BT-P06
Heras H S B-P03
Heredia V BT-P03
Hermoso JA SB-C02
Hernadez A PL-P55
Hernández E BT-P12
Hernández MA LI-C07
Herrera Aguilar N CB-P18
Hoffschir F CB-C16
Honoré SM CB-P55/CB-P56
Hopiavuori B L-03
Hozbor FA LI-P07
Huang Y CB-S03
Humbert MV MI-P21
Hurbain I CB-S04
Iannone MF PL-P33

I

Ibarbalz, FM MI-C04
Igal RA LI-S02
Iglesias AA EN-P03/EN-P04/

EN-P05/EN-P07/
EN-P08/EN-P09/
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PL-P09
Iglesias M BT-P07
Ilina N EN-P12
Ilincheta de Boschero MG ST-P16
Inda ME ST-C04/SP-P15
Ingallinella, AM MI-P56
Insani M MI-P29
Irala D NS-C03/NS-P03
Irazoqui A P ST-P05
Issoglio FM EN-P14
Ituarte S SB-P03
Iusem ND PL-P27

J

Jaldín-Fincati JR CB-P11
Jancic C CB-C03
Jawerbaum A LI-S03
Jeitany M CB-C16
Joshi S CB-S03
Junier MP CB-C16

K

Katz MJ CB-P63
Keime C L-06
Kevorkian ML MI-P80
Killian A ST-C04
Klinke S SB-C03/SB-P02
Koltan M BT-P11/BT-P19
Kordon E ST-P14
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CB-P33/CB-P34/
CB-P36/PL-P28
Krapf D ST-C01/ST-P03
Krapp AR PL-P34/PL-P38
Kun A CB-P20

L

La Colla A ST-P30
La Spina FA ST-C01
Lacreu ML CB-P44
Lacunza E CB-P32
Laczeski ME MI-P13
Lafon-Hughes L CB-P20
Lagares (h) A MI-P53
Lami MJ BT-P20/BT-P21
Lanfredi-Rangel A CB-C05
Langer, T MI-P05
Lanzarotti E MI-C02
Lara MV PL-P19/PL-P43/PL-P44/PL-P59
Lara J MI-P43
Lario LD PL-P31
Lasaga M MI-P58/NS-P01
Latini C MI-C12
Lavado L PL-P55
Lavatelli A CB-P38
Layana C CB-P37
Leaden L PL-C05
Lechner L PL-P16
Ledda A CB-P31
Ledda MF NS-C03/NS-P03
Lee KZ L-06
Lenardis A PL-P62
Lenaz G SB-P01
León P PL-P29
Leonardi PI LI-C06
Leonhard V BT-P03
Lepek, VC MI-P24/MI-P70
Leporace Guimil J CB-P41
Lestradet M L-06/MI-C05/MI-P07
Lia VV PL-P56
Lia V MI-P62
Liegeois S L-06
Limansky A MI-P68/MI-P72
Limmer S L-06
Lis A PL-C04
Lis AT MI-P39
Lisa AT SB-C04/MI-P11/MI-P38
Liu Y LI-S01
Llarrull LI MI-P63
Llorens MC ST-P06
Lo Cicero A CB-S04
Lodeyro AL PL-C09
Lofeudo JM CB-P32
Lopez FE MI-P46
López López LI PL-P08
López P CB-P01/NS-P01
López, MC MI-P48
López-Rivero A EN-P06
Lorca, GL MI-C10
Lorenzi CB-P17
Lorenzini Campos MN ST-P32
Lorenzo R NS-P05
Lozano M BT-P16
Lozano ME BT-P18
Lucero LE PL-P22/PL-P23
Lujan HD BT-C07
Luna Pizarro P MI-P31
Luque AG MI-P60
Luquet C CB-P61

M

Mac Cormack W BT-P12
Machinandiarena, F MI-P27
MacIntosh G PL-P01
Magni C MI-C10/MI-P06/
MI-P30/ MI-P57/
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Maiale SJ PL-P38
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Mailliet P	CB-C16
Maloberti PM	CB-C12
Maloberti P	CB-P01/NS-P01/ST-P02
Malone LM	MI-P28
Malvicini R	LI-P06
Mammi P	ST-S01
Manacorda CA	PL-P54
Manarin R	CB-P13/CB-P50
Mancini MP	CB-P53
Mans R	PL-C04
Mansilla MC	MI-S01/CB-P38/MI-P45
Mansilla N	PL-C08
Mansilla, MC	MI-P40
Maranzana E	CB-P03/CB-P39/ SB-P01
Marasco LE	CB-P36
Marchesini I	MI-P03
Marchesini MI	MI-P01
Marchetti MF	PL-P41
Marchiaro P	MI-P72
Marfil CF	PL-P26
Margara, LM	MI-C09
Margarit E	CB-C09
Marina M	PL-P63
Mariscotti JF	MI-P49
Marquez MG	CB-C15
Márquez VE	EN-P03
Marti DA	PL-C07
Martí M	SB-P06
Martí, M1	MI-C12
Martin L	LI-C06
Martín M	SB-P06/PL-P51
Martín MJ	ST-P18
Martin MV	PL-S03/PL-P16
Martin N	MI-S01
Martin V	PL-C10
Martina, MA	MI-P36
Martinez HE	CB-C02
Martínez Álvarez L	BT-P12
Martinez H	CB-S01
Martínez MJ	CB-P29
Martinez Noel G	PL-P16
Martínez Zamora G	PL-P07
Martinez Zamora MG	PL-P35
Martinez, MG	MI-P15
Martino G	MI-P64/MI-P67
Martino RA	BT-C07
Martino, G	MI-P06
Marziali F	CB-P25
Marziali FE	CB-P26
Maselli GA	SB-P04
Masias, E	MI-P75
Masuelli RW	PL-P26
Mateos MV	MI-P10/ST-P16
Matic I	L-09
Matteo, M	MI-C12
Mattera VS	CB-P18
Maugeri DA	MI-P79
Maurino VG	PL-P42
Mayol G	CB-P57
Mayorga L	CB-C06/CB-P40
Mayta ML	PL-C09
Mebert AM	BT-P06
Mediavilla MG	MI-P08
Medina MV	ST-C06
Medina R	MI-P31
Melino G	CB-C08
Melli LJ	BT-C01
Méndez AA	PL-P18/PL-P47
Méndez C	ST-P20
Menendez-Bravo S	BT-C06/BT-P22/ MI-P77
Menzella HG	BT-C02/BT-C03/ BT-P13/BT-P14
Mercante, V	MI-P24
Méresse S	CB-P17
Merli, ML	MI-P50
Merwaiss, F	MI-P17
Mesri E	ST-C06
Michaut M	CB-P40
Michavila G	BT-P19/BT-P20/ BT-P21
Michels PAM	TS-S04
Milanesi L	ST-P30
Militello RD	CB-C7
Miller M	LI-S01
Minahk CJ	EN-P01/LI-P01/ MI-P09/MI-P75
Miyazaki SS	MI-P33
Mobashery S	SB-C02
Mogk A	PL-C03
Molina MC	BT-P17
Molino V	CB-P21
Mondino S	MI-P43
Monerris GJ	CB-P48
Monesterolo NE	CB-P48/CB-P49
Montanaro MA	CB-P32
Montaner A	LI-C01
Monti L	PL-P43/PL-P44
Monti MR	MI-C09/EN-P02
Moor F	CB-C04
Mora MC	BT-C04
Mora-García S.	SB-P04
Morán-Barrio J	MI-P68/MI-P72
Morbidoni HR	MI-P71/MI-P76
Mordoh J	CB-P44
Morel Gómez ED	LI-P06
Moreno A	CB-P21/CB-P22
Moreno J.E	PL-P46
Moreno S.	SB-P05
Moreno-Piovano G.S	PL-P46
Morera LP	NS-P02
Morgan PE	CB-P41
Mori Sequeiros García M	ST-P20
Morriss S	PL-P01
Morrone Seijo S	MI-P03

Mortera P MI-P30
Mosser G BT-P06
Motta, MC MI-C03
Mouguelar VS CB-C10
Moussatova A ST-C04
Mozzi F MI-P65
Muchut RJ EN-P09
Müller GL PL-P10
Muñoz FF PL-P08
Muñoz M ST-P02
Muñoz MJ CB-P33
Murua, Y MI-P62

N

Nahirñak V PL-P56
Najle SR BT-P17
Natalini PM ST-P16
Natalucci Cl PL-P11
Neuman I ST-P02
Nicola JP ST-C05
Niemirowicz, GT MI-C01
Nieto Moreno N CB-P33/CB-P35
Nievas FL MI-P23
Nigra AD CB-P48/CB-P49
Nizovoy P BT-P12
Novosak MG MI-P13
Nudler S ST-P20

O

Ocampos MN PL-P20
Oddi SL EN-P03
Oitavén PA PL-P06
Olivera NL BT-P07
Olsen KW EN-P10
Orellana ML BT-P15
Orlando U CB-C12/CB-P01
Orsi R CB-P18
Ortiz Flores R CB-P09
Osella AV PL-P17
Osorio J MI-P06
Ostrowski M CB-C03
Otegui MB PL-C07
Otero L SB-C02
Otero LH SB-C03/SB-P02
Ottado J MI-P25/MI-P26/MI-P56/PL-P60/PL-P64

P

Pacini V MI-P56
Pagani MA PL-C05/PL-P36
Paganini, J MI-P77
Pagano E EN-P12
Pagano EA PL-P12/PL-P13/PL-P61
Pagano M CB-P47
Pagnussat G PL-C10
Pagnussat GC PL-S03/PL-P39/PL-P40/PL-P41/PL-P42
Pagnussat PL-P14
Pagura, L MI-P50
Palatnik JF PL-C02
Palavecino MD ST-P12/ST-P13
Panzetta, ME MI-P12/MI-P36
Panzetta-Dutari G CB-P27/CB-P42/CB-P43
Paoletti L CB-C19/CB-P30
Paratcha G NS-C03/NS-P03
Parcerisa IL PL-P05
Paredes E CB-P61
Pariani S PL-P02
Parisi, MG MI-P78
Parma M MI-P29
Parodi AJ CB-P18
Parsza CN CB-P28
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Pasteran F MI-P72
Pautasso C ST-P24/ST-P25
Paz C ST-P20
Paz EC MI-P35
Pecci A ST-P07
Pedraza AB BT-P10
Pedro MP CB-P16
Peirú S BT-C02/BT-C03/BT-P13/BT-P14
Pelletán LE LI-P03
Pelliza L SB-P02
Pellon Maison M CB-P32
Peluffo L PL-P56
Pena LB PL-P18/PL-P47
Peñalva DA LI-P07
Pepe A BT-P10
Peralta DA PL-P37
Peralta DR BT-P19/MI-P35
Perato M PL-P35
Perato SM PL-P58
Perdomo V CB-P13
Peressutti Bacci N MI-P76
Pereyra Gerber P CB-C03
Pérez MM CB-P45
Pérez NG CB-P41
Pérez SE ST-P17
Perez, DR MI-P14
Pérez V1 MI-C04
Peris D MI-P63
Perotti VE PL-P37
Pescaretti, MM MI-P09
Pesce A BT-P02
Pescio LG CB-C15
Pescio LG LI-C05
Peton A PL-P13
Petrillo E PL-P28
Pezza RJ EN-P02
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Piattoni C EN-P09/PL-P09
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Pieckenstain FL PL-P38/PL-P53/PL-P63
Pierella Karlusich JJ PL-P45
Pietrasanta L CB-C03
Piñas GE MI-P74
Pineda JR CB-C16
Pissinis AM MI-P39
Piuri M MI-C02/MI-C12/MI-P28/SB-P06
Podestá EJ CB-C12/CB-P01/ST-P02
Podestá FE EN-P09
Poestch A MI-P81
Poggi S MI-C12
Pomares MF BT-P11/BT-P19/MI-P35
Pontel, LB MI-C07
Popovich C LI-C06
Porcel de Peralta M CB-C10
Porrini L MI-P45
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Posadas, DM MI-P04/MI-P05/MI-P54
Pozzi B ST-S01
Pregi N CB-C18
Presello DA PL-P57
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Pronsato L ST-P30
Prospitti A PL-P11
Prucca CG CB-C13/NS-C01
Pryciak PM ST-P10
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Pucci O CB-C11
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Q

Quaglino A CB-P34
Quesada-Allué LA CB-P45
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R

Rabinovich GA L-08
Rabossi A CB-P45
Racca AC CB-P42/CB-P43
Radicella JP CB-C18
Raimunda D MI-P51
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Rapisarda VA MI-P20
Raposo G CB-S04
Ravasi P BT-C02
Ravasi P BT-C03
Raya R MI-P31/MI-P32/MI-P44/MI-P65
Raya RR MI-P66
Ré M PL-P04
Reca S ST-P24
Reinheimer R PL-P49
Reinoso EB MI-P58
Renner ML LI-C02
Repetto M ST-P10
Requena Serra FJ PL-P35
Reyna L CB-P27/CB-P43
Rial DV BT-P05
Ribone P PL-C01
Ridano M CB-P27
Ridano ME CB-P42/CB-P43
Righini Aramburu S PL-C06
Rintoul MR MI-P20
Risso G ST-S01
Ritacco V MI-P73
Ritagliati C MI-C03/CB-P50
Rius S PL-P30/PL-P48
Rivarola, M MI-P62
Rivas M BT-C01
Rivelli JF CB-P49
Rivera Pomar R CB-P37
Rizzi YS PL-P32
Rocha, GF MI-P78
Rochin L CB-S04
Rodriguez EJ MI-P34
Rodríguez Berdini L NS-C02
Rodriguez JM MI-P31
Rodriguez MV PL-P48
Rodriguez AV MI-P10
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Rodríguez-Concepción M PL-P29
Rodriguez-Walker M CB-P15
Rojas BE PL-P09
Rojas HJ PL-P01
Rojas-Altuve A SB-C02
Roldan, JS MI-P15/MI-P16
Rondón L MI-C12
Rópolo AS CB-C05/CB-P57
Rosano GL PL-C03/PL-P05
Rosati R MI-P60
Rossi F PL-P38
Rossi FA CB-P46/CB-P47
Rossi FR PL-P63
Rossi M CB-C08/CB-P46/CB-P47
Rossi MA EN-P06
Rossi S SB-P05/ST-P23/ST-P24/ST-P25
Rotblat B CB-C08
Roulet J BT-P09
Ruberto L BT-P12
Rubinstein M L-10/NS-P05
Ruggieri GM PL-P10
Ruiz Oa PL-P52
Ruiz-Ranwes, V MI-P05/MI-P04
Rupil LL BT-C07

Russo SJ CB-P51
Russo de Boland A ST-P18/ST-P19
Russo DM MI-P54
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S

Saavedra L MI-P32/MI-P65
Saavedra ML MI-P66
Sabatini DD L-01
Sabatini M BT-C06/BT-P22
Sabljic I PL-P61
Sadañoski MA EN-P13
Saigo M PL-C06/PL-P15
Saka, HA MI-P12
Salassa BN CB-C04
Salerno GL PL-P16
Salva S MI-P10
Salvador GA CB-P58/LI-C06/MI-P10
Salvatierra Fréchou DM, CB-P62
Salzman V MI-P42
Sampieri L CB-C02/CB-S01
Sánchez DG MI-P38
Sánchez MC CB-P11
Sánchez SS CB-P54/CB-P55/CB-P56
Sánchez, CM MI-P24
Sanguinetti G MI-P56
Sansberro Pa PL-P52
Santacreu BJ LI-C04
Santander F MI-P38
Santander VS CB-P48/CB-P49
Santecchia, I MI-P57
Santillan Zabala V ST-P07
Sapochnik D ST-C06
Saragusti AC CB-P10/CB-P12
Sardoy P EN-P12
Sarti, GC MI-P33
Sartorio M MI-P69
Sasiain, MC MI-P73
Sasoni N EN-P04/EN-P11
Saura A BT-C07
Scampoli NL MI-P47
Scandiani MM MI-P60
Scarpeci TE PL-P24
Schlesinger M MI-P80
Schmitz J PL-P42
Schoijet AC ST-P26
Schurig-Briccio L EN-P01
Schwab Y L-06
Schwarzbaum PJ, CB-P62
Scodelaro Bilbao PG LI-C06
SegatoriVI BT-P02
Sequeiros C BT-P07
Serra EC MI-C03/CB-P13/CB-P50
Serradell MC BT-C07
Serrano MA CB-P55
Sesma FJM MI-P66
Sesma F MI-P32
Sigaut L CB-C03
Silva Belmares SY PL-P08
Silva I MI-P58
Silvestre DC CB-C16/NS-C01
Silveyra MX CB-P29
Sisti, F MI-P18/MI-P22
Smal C SB-P02
Smania, AM MI-P19/MI-P55
Smehilová M PL-P47
Soares B MI-P58
Sola C MI-P61
Solamen L EN-P10
Solano AR CB-C12
Solari CA ST-P21/ST-P22
Soldano A SB-C01
Soncini FC BT-C05/MI-C07/MI-P21/MI-P47/MI-P48
Sonnewald S PL-P45
Soria G Bocco JL ST-C05
Soria M MI-P73
Sosa MA CB-P51/CB-P52
Sosa GM BT-P04
Sossi ML PL-P04
Soto D PL-C10/PL-P42
Souza M MI-P58
Spagnoletti F PL-P55
Spampinato CP MI-P60/PL-P31
Spanevello RA MI-C06
Spera JM MI-C08/MI-P02
Sperandio V MI-S02
Srebrow A ST-S01/CB-P34
Stella CA CB-P53
Stella EJ BT-P08
Sterin Speziale NB CB-C15/LI-C04/LI-C05/LI-P05
Sternlieb T ST-P26
Stival C ST-C01
Stockwell B PL-C10
Stringa P CB-P32
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Suares A ST-P19
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Suárez NE MI-P32/MI-P66
Suárez, AG MI-C06
Suhaiman L LI-P03
Svetaz LA PL-P59
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T

Tabares LC MI-P37
Takenaka M PL-S02
Talia P MI-P62
Tavernelli LE CB-P13
Tellado M CB-P59
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Tempesti T NS-P02
Temprana CF BT-P15/BT-P16
Terán L MI-P44/MI-P65
Teran V MI-P31
Thomas AH LI-P04
Tieleman P ST-C04
Tobares RA MI-P19
Tocci J ST-P14
Todaro LB B-C14
Tofolón E SB-P05
Toledo J CB-P31
Tomas Grau RH PL-P35
Tomassetti M CB-P17/PL-P64
Tomes CN ST-P27
Tonda A CB-C11
Torcat M PL-P62
Torres Nágera MA MI-P78/PL-P08
Torres, MA MI-P09
Torrez Lamberti MF MI-P46
Touz MC CB-C05/CB-P57
Travaini ML BT-P04
Triassi A PL-P10
Trifone CA BT-P16
Tronconi M PL-C04
Trucco LD ST-C05
Tsai, YT MI-P42
Turjanski A MI-C02

U

Uberti Manassero NG PL-P23/PL-P49
Uchiya PA PL-P53
Udovin LD LI-P05
Ugalde JE BT-C01/MI-C08/MI-P02
Uhart M ST-P28/ST-P29
Uranga RM CB-P58
Urdániz E MI-C12/SB-P06
Urrutia M CB-P44
Urtreger AJ B-C14
Russo DM MI-P04
Uttaro AD BT-P17

V

Valdez DJ NS-P02
Valdez Taubas J CB-C01/CB-P02
Valdivia RH MI-P12
Valentini G PL-P43/PL-P59
Valguarnera E MI-C08
Valle E PL-P04
Valle EM PL-P17/PL-P24/PL-P50
Valverde C MI-P52/MI-P53
Van Maris A PL-C04
van Niel G CB-S04
Van Nieuwenhove C MI-P31
Vaquer C LI-P03
Vargas-Roig LM CB-P51
Vasconsuelo A ST-P30
Vasen G ST-P09
Vazquez D SP-P15
Vázquez S BT-P12
Vázquez-Ramos JM PL-P18
Vazquez-Rovere C PL-P56
Vegetti A PL-P49
Velazquez FN CB-C13/NS-C01
Ventura AC ST-P11
Venturino A CB-P61
Verara-Jaque A SB-C04
Verbitskiy D PL-S02
Vergara AN CB-C04
Vergara, MI MI-P13
Verra DM NS-P02
Verstraeten SV CB-P62
Verstuyf M ST-P19
Viale A MI-P72
Viale AM MI-P68
Viarengo, G MI-C06
Vignoni M LI-P04
Vilcaes AA CB-P15
Vilcaes AA CB-P16
Vilchez Larrea SC CB-P20/MI-P80
Villalba, LL MI-P59
Villaverde MS CB-P59/CB-P60
Villegas LB EN-P13
Villena J MI-P10
Vinacour ME PL-P18
Vincent PA BT-P11/BT-P19/BT-P20/BT-P21/MI-P35
Vinderola G MI-P31
Viola IL PL-P21
Visconti PE ST-C01
Voelker DR LI-S04
Vogelstein B LI-S01
Von Bilderling C CB-C03

W

Wappner P CB-P63
Weber K LI-P06
Wehrendt DP CB-P07

Welchen E PL-C08
Whiteheart SW CB-S03
Winters M ST-P10
Wolosiuk R PL-P12

Y

Yamba WM L-06
Yandar Barahona NY MI-P14/MI-P74
Yanovsky MJ ST-S03

Z

Zabaleta E PL-C10
Zabaleta EJ PL-S03/PL-P39/
PL-P40/PL-P41/PL-P42
Zalguizuri A MI-P24/MI-P70
Zamboni D MI-S04
Zamponi N CB-C05
Zanor MI PL-P17/PL-P24/PL-P50
Zapata PD PL-C07/EN-P13/
MI-P59/ST-P31/
ST-P32/MI-P13
Zavala J EN-P12
Zavala JA PL-P61
Zehrmann A PL-S02
Zelaya H MI-P10
Zenoff AM BT-P20
Zhang J CB-S03
Zlocowski N CB-P57
Zorreguieta A MI-P04/MI-P05/MI-P54
Zuljan F MI-P30/MI-P67
Zupan AL ST-P12
Zurbriggen M PL-P45

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Arrange sections of the MANUSCRIPT FILE in the following order:

- Title page
- Abstract (with no sections nor references, and not exceeding 300 words)
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Begin each component on a separate page. Number all pages (starting with the title page), tables and figures in Arabic numerals. Do not number lines.

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Nicholas FISHER, John MILLER, Anthony BAKER.

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5. Title of the corresponding author (e.g., Professor, Dr., Mr., Mrs., Ms.), full name and email address. The title is only intended for correspondence and it will not be used in the printed article.

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The text should be written in clear, concise English, and it should be easily understandable to a broad readership. Sentences should not begin with abbreviations or numerals.

Main headings should be Introduction, Material and Methods, Results (or Systematic Description), Discussion and Acknowledgements (if necessary) and they should be centered and in capital letters, while just one level of subsidiary headings should be on the left, in italics). Do not number headings. Combining Results and Discussion is not allowed.

Accepted abbreviations for statistical parameters are: *P*, *n*, SD, SEM, df, ns, ANOVA, *t*.

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Physical units, as well as their symbols and abbreviations, should be those of the Système Internationale d'Unités (International System of Units, SI).

Adequate statistical treatment of the data is essential.

Linnean scientific names should be in italics, while higher than generic taxa should not.

The generic name of drugs, as well as all other common names, should be written in low case.

Gene designations should be in low case and in italics, while protein

designations should be in regular capital letters.

References:

References in the text should take one of the following forms:

Fisher and Miller (2007) or (Fisher and Miller, 2007). If there are more than two authors: Fisher et al. (2008) or (Fisher et al. 2008).

The following sample phrases may help:

"Contrariwise to the report of Fisher and Miller (2007) some others have shown (Baker et al., 2008)… "

"Contrariwise to an earlier report (Fisher and Miller, 2007) Baker et al. (2008) have shown …"

The list of references must be presented exactly as follows (an Output Style for use with EndNote can be downloaded from our webpage or may be requested from the Editorial Office). References should be arranged alphabetically. Full journal titles should be typed in italics and cited in full with just the volume number in bold. Repeat authors' names and journal titles in multiple entries.

The first and last page of each article should be included. Abstracts of presentations in scientific meetings may be cited, but only if published in a journal, and with the indication "abstr." after the volume and page numbers.

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Fisher N, Miller J (2007). An ectaquasperm-like parasperm in an internally fertilizing gastropod. *Science* **256**: 1566-1570.

Fisher N, Miller J, Baker A (2003). Connexins in paraspermatogenesis in the Annelida. *Proceedings of the National Academy of Sciences (USA)* **256**: 1566-1570.

Fisher N (2007). *Paraspermatogenesis.* University of Cuyo Press, Mendoza.

Fisher N (2008). Connexins in paraspermatogenesis. In: *Perspectives in Invertebrate Reproduction* (J Miller, A Baker, eds.), p. 67-86. University of Cuyo Press, Mendoza.

Fisher N, Miller J, Baker A (2003). Connexins in paraspermatogenesis in the Annelida. *Biocell* **25**: A156, abstr.

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They should be self explanatory without reference to the text. Statistical evaluations should indicate the test used. Micrographs magnification should be indicated by scale bars on them, and the size of the scale bar should be indicated in the legend, not on the micrograph. When the same magnification is used for several panels of the same figure, a single scale bar should be used.

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