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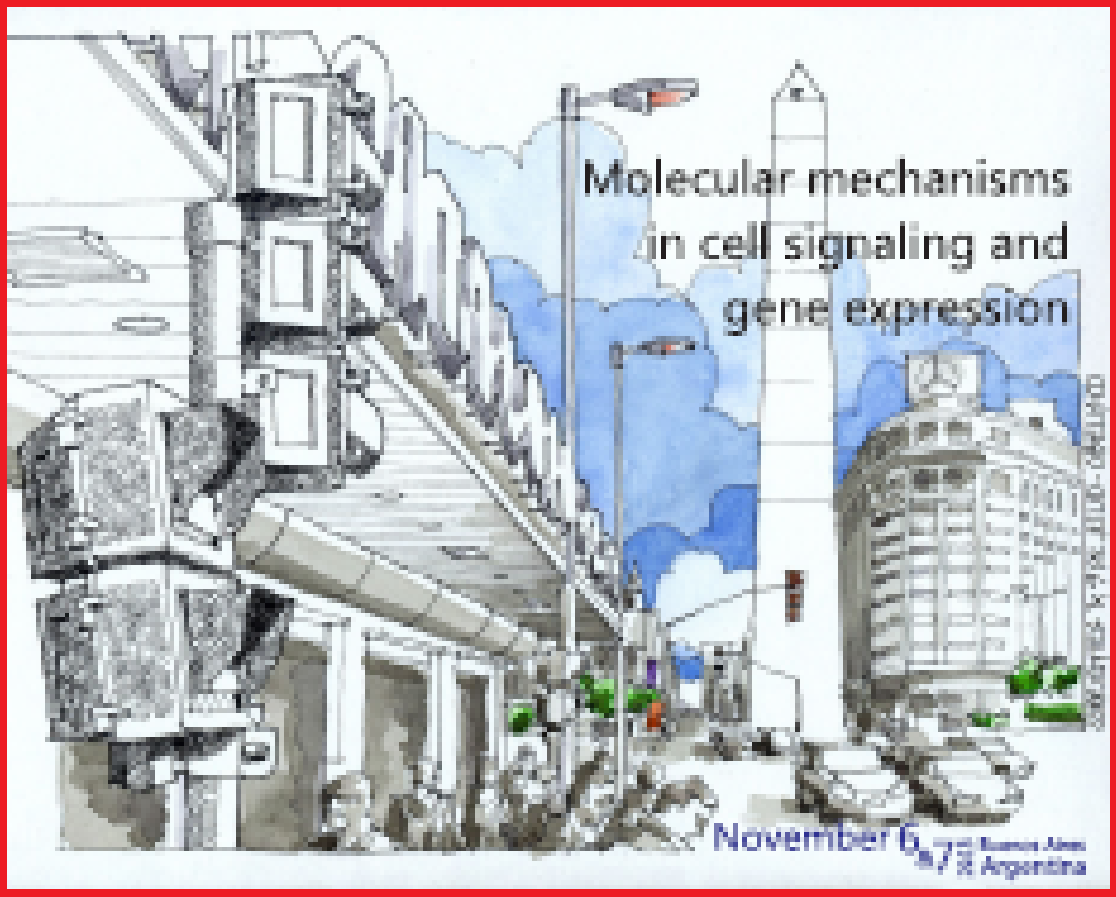
Argentine Society for Biochemistry and Molecular Biology Research

Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular



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- SAIB -

«Molecular mechanisms in cell signaling and gene expression»

Argentine Society for Biochemistry and Molecular Biology

Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

November 6 - 7, 2013
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THE BIRTH OF SAIB: a perspective from one of its founders

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 acopañados 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 Sahli 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 este 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 Brenner

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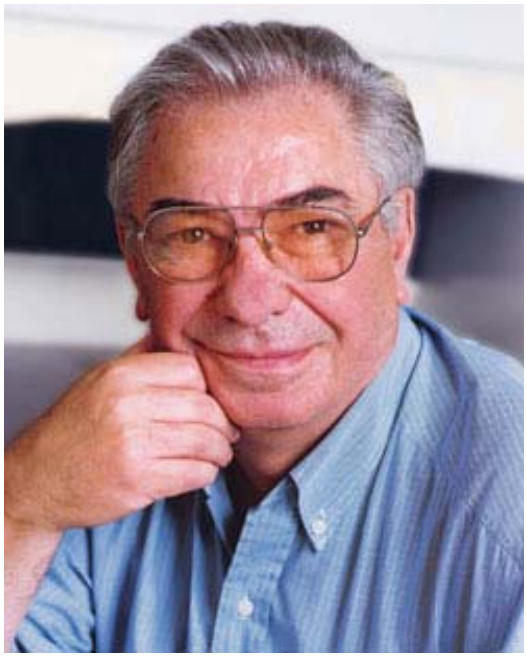
1970-1970	RODOLFO BRENNER
1969-1969	RANWEL CAPUTTO
1965-1968	LUIS F. LELOIR

IN MEMORIAM OF HÉCTOR TORRES



Héctor Norberto Torres, professor emeritus at the University of Buenos Aires and founding director of Argentina's Institute for Molecular Biology and Genetic Engineering, died on April 2, 2011 of a sudden heart attack. He was 75.

Torres, or Doc, as his students and close colleagues called him, had a distinguished career as one of Argentina's leading biological chemists that started with his joining Nobel laureate Luis F. Leloir's research group at the Institute of Biochemical Research, Fundación Campomar, in 1959, immediately after finishing medical school. There, Torres studied the mechanisms that regulate glycogen biosynthesis and earned a doctorate degree from the University of Buenos Aires in 1966.

Working mostly with graduate students and his lifelong collaborator and spouse, Mirtha Flawiá, in the early 1970s Torres discovered that the adenylyl cyclase-cAMP signaling system, which had recently been shown to mediate actions of peptide hormones and biogenic amines in vertebrates, also existed in the primitive fungus Neuropora crassa and proved that cAMP is a developmental cue in this organism.

After 1983, Torres focused on the molecular nature and roles of signaling pathways in the development of trypanosomes, specifically *Trypanosoma cruzi*, a protist that is one of the most primitive eukaryotes and is the etiologic agent of Chagas disease. Torres characterized enzymes controlling glycogen metabolism through phosphorylation/dephosphorylation mechanisms involving cyclic nucleotide phosphodiesterases, cyclic AMP and Ca/diacylglycerol stimulated protein kinase, adenylyl cyclase, nitric oxide synthase, G proteins and energy transducing systems – all possible targets of intervention to attack this parasite.

Among Torres' later contributions are the findings that the signal by which an intermediary nonpathogenic form of *T. cruzi* progresses to the pathogenic form is the second messenger cAMP, generated in response to a peptide generated from globin in the hindgut of the transmitting insect; the discovery of a nitric oxide synthase in *T. cruzi* and the assignment of a role for nitric oxide in regulating the parasite's motility; the discovery of phosphoarginine in *T. cruzi*; and the finding that the biosynthetic enzyme arginine kinase is evolutionarily related to arthropod arginine kinase, suggesting horizontal gene transfer.

More recently, Torres' group cloned and characterized a *T. cruzi* SR-like protein and proved that it is the functional orthologue of a classic mammalian mRNA splicing factor. This proved that *T. cruzi* has the same machinery for splicing RNA as higher eukaryotes.

Torres remained scientifically active until the end. Death found him working on mechanisms that control osmoregulation in *T. cruzi* epimastigotes, on the regulation of poly (ADP-ribose) metabolism as it affects the DNA damage-response and cell death pathways, and on manipulating *T. cruzi*'s redox equilibrium to affect detoxification and pro-drug transformation, thus connecting basic research results to a possible solution for a major public health problem in his country.

Torres was a stalwart of Argentine science. Except for a short time in the U.S. as a Guggenheim Fellow, he worked exclusively in his home country, where he assumed multiple leadership roles. Torres served on the executive councils of both the University of Buenos Aires and the Argentine National Research Council. In 1983, under the auspices of Leloir's Institute and the Argentine Research Council, he founded South America's first Institute of Molecular Biology and Genetic Engineering. As INGEBI's director, Torres fostered the development of young graduate students into mature scientists in an atmosphere of political freedom.

After 1985, in a newly democratized nation after twenty years of political oppression, Torres became dean of the University of Buenos Aires School of Sciences (1988 – 1990). In his role as dean, he is credited with having greatly diminished the wounds caused by the 1966 military intervention in academic affairs that all but destroyed that school's scientific standing and prevented academic freedom under its roof.

Torres and his collaborators at INGEBI successfully mentored more than 138 graduate students and organized numerous advanced graduate courses with faculty members drawn from around the world, including the U.S. and Europe. INGEBI, which Torres directed until 2009, is home to 35 independent investigators, including Pew Latin American Fellows and Howard Hughes Medical Institute investigators, attesting to the high scientific standard Torres was able to attain for the research institute.

Deservedly, Héctor Torres was well recognized by his peers and won honors and awards. He was a member of the Argentine National Academies of Science (1998) and Medicine (2005) and corresponding member of the Brazilian (1999) and Chilean (2002) Academies of Science. Among his awards were the Premio Odol in Biology (1969), the Konex Platinum Award in Genetics (1993), the Luis F. Leoir Award in Chemistry (1996), the Bunge y Born Award in Molecular Biology (2000) and the J.J. Kyle Award of the Argentine Chemical Society (2005).

On a personal note, between 1964 and 1967, I was the Doc's first graduate student. I'll always remember when I first met him in his laboratory at the Fundación Campomar as a prospective graduate student with a "research plan" in hand, he said "Here, we do not speculate, we pipet," thus seeding my research career as an experimentalist. I am forever thankful to him for infusing in me enthusiasm for researching the wonders that make us living beings.

Lutz Birnbaumer

IN MEMORIAM OF RANWEL CAPUTTO

JOURNAL OF NEUROSCIENCE RESEARCH
Volume 12, Issue 2-3, 1984, Pages: iii–iv, L. F. Leloir

«Scientists cover a wide spectrum of personalities. At one end are those who are spectacularly clever, know all the answers, but rarely discover new facts. At the other are those who are quiet, profound, and have the ability and the luck of making discoveries. Undoubtedly, Ranwel Caputto is near the latter end of the spectrum. Without haste and without getting nervous, he has had many successes in his career. His style in research is not the systematic type, nor does he bother too much about details. I have the recollection of a talk with him many years ago in connection with cancer research. He was saying that he was tempted to get into the field, so I asked him how he would tackle such a difficult problem. He said; «Well I think I would start playing about with cancer cells, and then something might come out of it.» He never did any work on cancer, but he did play around with mammary glands, vitamin E, nervous tissue, gangliosides, and several other materials. In each of those fields, he contributed with signal advances.

Dr. Caputto was punctually born on the 1st of January, 1914, in Buenos Aires, Argentina, and he studied medicine at the University of Cordoba, where he was graduated in 1940. In Cordoba he started research with Dr. Alberto Marsal on the alkaline phosphatase of the mammary gland. In 1945, he was awarded a British Council Fellowship and he went to the biochemical laboratory of Cambridge University. There he worked with Malcolm Dixon and was successful in crystallizing triosephosphate dehydrogenase of muscle. Crystallizing an enzyme in those times was a rather important feat. On his return to Argentina, he became a member of a group formed by C. Cardini, L. Leloir, A. Paladini, and R. Trucco which succeeded in isolating glucose 1-6 diphosphate, uridine diphosphate glucose, and other sugar nucleotides. Dr. Caputto's contribution was invaluable, since he carried out some of the crucial experiments and produced some of the best ideas.

In 1953, he left for U.S.A. and worked first at the University of Ohio and then at that of Oklahoma. Those were productive years, during which he was interested in vitamin E and gastric juice proteins. Back in Cordoba in 1963, he initiated a strong research group in the university, a group that became one of the best in Latin America and the nucleus of the faculty of Cordoba University.

The main field of research of that group has been that of brain gangliosides, their mechanism of biosynthesis, their properties, their role in membrane structure, and their changes under stimulation of nerve cells. Besides that, the addition of amino acids to tubulin was studied and yielded novel results. In all these fields, the contributions of the Cordoba group have been very important.

For those of us who have had the privilege of working with Dr. Caputto, it is a pleasure to wish him the best on the occasion of his seventieth birthday.»

L.F. Leloir

PROGRAM

"Molecular mechanisms in cell signaling and gene expression"

Argentine Society for Biochemistry and Molecular Biology scientific meeting

"SAIB 2013"

Buenos Aires, Argentina, November 6th, 2013

9:00-10:00 REGISTRATION

10:00-11:00 **OPENING LECTURE**

Luis Serrano
EMBL/CRG Systems Biology Research Unit, Center for Genomic Regulation, Pompeu Fabra University. Barcelona, Spain
"Could we fully understand in a quantitative and predictive manner a living system?"
Chairman: Luis Mayorga

11:00-11:30 **Coffee break**

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

María Fernanda Ceriani
Fundación Instituto Leloir, IIB-BA-CONICET. Buenos Aires, Argentina
"Developmental switch in signaling pathways involved in the circadian clock"
Chairman: Mario Guido

12:30-15:00 **POSTER SESSION AND LUNCH**

Structural Biology (SB P01/02)
Enzymology (EN P01/03)
Neuroscience (NS P01/02)
Plants Bioch. & Mol. Biol. (PL P01/15)
Lipids (LI P01/16)
Signal Transduction (ST P01/23)

15:00-17:00 **SYMPOSIUM**

UNMASKING ROLES FOR KNOWN PLAYERS IN CELL SIGNALING

Chairpersons: Claudia Tomes / Gabriela Salvador

Omar Coso
IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales, UBA. Buenos Aires, Argentina
"Signaling pathways regulate gene expression acting at multiple levels"

Holger Rehmann
Department of Molecular Cancer Research, University Medical Center Utrecht, The Netherlands
"Structural basis for the selective activation of Epac by cAMP-analogues"

Alex Toker
Harvard Medical School, Boston, USA
"Specificity and diversity in the PI 3-K and Akt signaling pathway in cancer"

Daniel Raben
The Johns Hopkins University School of Medicine, Baltimore, USA
"Regulation and roles of diacylglycerol kinase-theta"

17:00-17:30 **Coffee break**

17:30-18:30 **DEDICATION TO RANWEL CAPUTTO**

"Ranwel Caputto: a tribute on the 50th anniversary of the Department of Biological Chemistry of Córdoba"
Chairman: Hugo Maccioni, Córdoba, Argentina

18.30 SAIB GENERAL ASSEMBLY

Buenos Aires, Argentina, November 7th, 2013

10:00-11:00 **LECTURE**

Daniel Klionsky
Life Sciences Institute, University of Michigan, USA
"The regulation of macroautophagy"
Chairman: Juan José Cazzulo

11:00-11:30 **Coffee break**

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

Tamotsu Yoshimori
Graduate School of medicine/frontier biosciences, Osaka University, Osaka, Japan
"Mammalian autophagy: its machinery, membrane dynamics, and role in diseases"
Chairperson: María Isabel Colombo

12:30-15:00 **POSTER SESSION AND LUNCH**

Cell Biology (CB P01/45)
Biotechnology (BT P01/09)
Microbiology (MI P01/10)

15:00-17:00 **SYMPOSIUM**

NEW ADVANCES IN THE CONTROL OF GENE EXPRESSION
Chairpersons: Hugo Luján / Norberto Iusem

Bettina Malnic
Department of Biochemistry, University of Sao Paulo, Brazil
"Epigenetic regulation of odorant receptor gene expression"

Rubén Agrelo
Institut Pasteur, Montevideo. Uruguay.
"Cancer epigenetic contexts"

Raquel Chan
IAL-CONICET, Universidad Nacional del Litoral. Santa Fe, Argentina.
"Secrets of the plant family of transcription factors homeodomain-leucine zipper I"

Javier Palatnik
IBR-CONICET, Universidad Nacional de Rosario, Argentina.
"Control of leaf size and shape by microRNAs in plants"

17:00-17:30 **Coffee break**

17:30-18:30 **BIOCHEMICAL JOURNAL LECTURE**

Guy Salvesen
Sanford-Burnham Medical Research Institute, La Jolla, California, USA
"Caspases, paracaspase, and the space between death and survival"
Chairman: Marcelo Kazanietz

19:30 **CLOSING CEREMONY – AWARDS**

Chairpersons: Alberto Kornblihtt / Luis Mayorga / Carlos Andreo

Buenos Aires, Argentina, November 8th, 2013

Free day

Puerto Varas, Chile, November 9th to14th, 2013

PanAmerican Society of Biochemistry and Molecular Biology "PABMB" meeting
http://www.pabmbcongress2013.cl/index.html

L01.

COULD WE FULLY UNDERSTAND IN A QUANTITATIVE AND PREDICTIVE MANNER A LIVING SYSTEM?

Serrano L.
EMBL/CRG Systems Biology Research Unit, Center for Genomic Regulation, Pompeu Fabra University. Barcelona, Spain.

L02.

DEVELOPMENTAL SWITCH IN SIGNALING PATHWAYS INVOLVED IN THE CIRCADIAN CLOCK.

Ceriani MF.
Laboratorio de Genética del Comportamiento. Fundación Instituto Leloir. IIB-BA/ CONICET.

Living organisms use biological clocks to maintain their internal tempo and anticipate daily environmental changes. In *Drosophila*, circadian regulation of rest-activity cycles is controlled by 150 neurons; among them, the sLNvs expressing the PIGMENT DISPERSING FACTOR (PDF) neuropeptide define key circadian properties in the absence of external cues. However, it remains unclear how different clusters communicate to result in a coherent output. Through a forward genetic screen we identified a new acute regulator of circadian period. Manipulation of gene expression levels showed that retrograde BMP signaling is necessary for normal circadian behavior. Importantly, pathway activation is crucial for period determination in an adult-specific fashion through downregulation of *Clk* transcription, providing evidence for a novel function of this pathway in the adult brain. We also showed that proper sLNv circuitry requires PDF signaling during early development, yet, in its absence axonal defects become apparent only after metamorphosis, suggesting a delayed retrograde signaling pathway turned early on. We have uncovered a novel mechanism that provides an early "tagging" of synaptic targets that will guide axonal re-extension later in development. Thus, throughout development, the PDF and BMP signaling pathways act in concert to define essential properties of circadian pacemakers.

L03.

RANWEL CAPUTTO: A TRIBUTE ON THE 50TH ANNIVERSARY OF THE DEPARTMENT OF BIOLOGICAL CHEMISTRY OF CÓRDOBA

Maccioni HJF.
CIQUIBIC (UNC-CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba.

The origins of the Department of Biological Chemistry of what is today the School of Chemical Sciences of The National University of Córdoba are closely linked to the character of its founder, Dr. Ranwel Caputto. In this occasion I will first describe his essential role in the creation of the Department and briefly recapitulate the academic evolution of the Department up to date. Additionally, the impact of Dr. Caputto activities on the development of Chemical Sciences in Córdoba will be highlighted.

L04.

THE REGULATION OF MACROAUTOPHAGY

Klionsky DJ.
Life Sciences Institute, University of Michigan, Ann Arbor, MI, U.S.A., 48109-2216.

Over thirty genes have been identified in yeast that are specific to macroautophagy, and homologs have been identified for many of these genes in higher eukaryotes. Although some of the detailed mechanism of macroautophagy has been elucidated, many questions concerning the molecular basis of the pathway remain unanswered. For example, how is the initial sequestering compartment, the phagophore, nucleated? What is the origin of the membrane used for expansion of the phagophore to form the autophagosome? What are the roles of the various autophagy-related proteins in the process of autophagosome biogenesis?
We have been analyzing the regulation of macroautophagy in *Saccharomyces cerevisiae*. One of the central autophagy-related (Atg) proteins is Atg8, a ubiquitin-like protein that is conjugated to phosphatidylethanolamine. The amount of Atg8 determines the size of autophagosomes; however, the factors controlling the expression of *ATG8* have not been determined. Recently, we have been analyzing the regulation of *ATG8* transcription. Because of the conserved nature of this process the insight we gain from understanding regulation in the genetically tractable yeast system will provide additional direction for analyses in higher eukaryotes.

L05.

MAMMALIAN AUTOPHAGY: ITS MACHINERY, MEMBRANE DYNAMICS, AND ROLE IN DISEASES

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Autophagy is an evolutionarily conserved membrane trafficking from the cytoplasm to lysosomes. In autophagy, the unique double membrane-bound autophagosomes transiently emerge in the cytoplasm, sequester portion of the cytosol and organelles, and eventually fuse with lysosomes to degrade the contents. In addition to the basic role in nutrient supply under starvation conditions, the process unexpectedly functions in development, longevity, immunity, and suppression of various diseases including tumorigenesis, neurodegeneration, infectious diseases, inflammatory diseases, and lifestyle related diseases.

My group has been working on unraveling the molecular machinery and roles of mammalian autophagy. LC3, a first protein we identified, has been mostly used golden marker in autophagy studies. This single paper (EMBO J, 2000) has been cited in over 2,000 papers. Recently, we have provided new insights into biogenesis of autophagosome, which have been topic of longstanding debate. We showed that autophagosome forms at the ER-mitochondria contact site (Nat Cell Biol 2009; Nature 2013). Furthermore, we found that autophagy eliminates invading pathogenic bacteria (Science 2004). We have unraveled how autophagy recognizes bacteria (J Cell Biol in press, etc.). We also found a new role of autophagy in selective elimination of damaged lysosomes to defense against diseases including nephropathy (EMBO J 2013).

L06.

CASPASES, PARACASPASE, AND THE SPACE BETWEEN DEATH AND SURVIVAL

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Apoptosis is said to be dependent on caspases. But it has become pretty clear now that supposedly pro-apoptotic caspases can, paradoxically, also trigger non-apoptotic events. Moreover, the universe of caspase homologs includes proteases with distant relationship to caspases, which program entirely different cell signaling pathways. This talk will focus on the biochemical mechanisms that allow caspase-8 and its distant homolog (paracaspase) to program apoptotic cell death *versus* an opposing pathway to cell survival and clonal expansion.

S01.

SIGNALING PATHWAYS REGULATE GENE EXPRESSION ACTING AT MULTIPLE LEVELS

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Living cells react to extracellular signals eliciting a variety of responses that include gene expression regulation. Phosphorylation of transcription factors by kinase cascades that trigger promoter activity and increase mRNA levels is a phenomena well characterized. However, current knowledge of the research field has extended the repertoire of characters involved in gene expression processes to include molecules involved in processing of the RNA transcripts and stability of mature mRNAs. Our observations show that triggered by the same stimuli, early activated MAPK cascades act upon transcription factors to turn on promoter activity of early responsive genes while late activated MAPK cascades regulate mRNA binding proteins controlling the decay of mature mRNAs. Not only the quantity but also the quality of mRNAs expressed is regulated by extracellular stimuli. In addition to alternative splicing, alternative polyadenylation is similarly regulated by the cell environment.Current status of our research is pointing that gene expression regulation is not restricted to promoter activity but also that signaling cascades, mRNA binding proteins and miRNAs participate in the regulation of RNA processing and stability in a coordinated fashion. This is a well-extended phenomenon that regulates the expression of a variety of genes being *Itgb1* and *c-Fos* our main targets of interest.

S02.

STRUCTURAL BASIS FOR THE SELECTIVE ACTIVATION OF Epac BY cAMP-ANALOGUES

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The second messenger cyclic adenosinemonophosphate (cAMP) acts in higher eukaryotes by the activation of protein kinase A (PKA), cyclic nucleotide regulated ion channels and the Guanine Nucleotide Exchange Factors (GEFs) Epac1 and Epac2. Epac1 and Epac2 causes signalling by the small G-protein Rap by catalysing the exchange of GDP bound Rap for GTP. Epac1 is involved in the control of cell-cell and cell-matrix adhesion whereas Epac2 enhances glucose induces secretion of insulin from the pancreas. Epac exist in an auto-inhibited conformation in which the access of Rap to the catalytic side is blocked by cyclic nucleotide binding (CNB) domains. Binding of cAMP induces conformational changes, which liberates the catalytic site. Certain cAMP analogues, which were designed in a semi-rational approach, activate Epac with higher potency than cAMP itself and allow selective activation of Epac by discriminating against other cAMP receptors. The molecular basis for the potent and selective activation of Epac was explained by the determination of the crystal structure of the auto-inhibited Epac2 as well as of Epac2 in complex with the substrate Rap and several cAMP analogues. The obtained model of Epac activation was confirmed by a mutagenesis study and the biophysical characterisation of the regulation process in an in vitro assay with purified proteins.

S03.

SPECIFICITY AND DIVERSITY IN THE PI 3-K AND Akt SIGNALING PATHWAY IN CANCER

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Akt/PKB (protein kinase B) is a proto-oncogene that plays a critical role in cell survival, proliferation and metabolism. There are three mammalian Akt isoforms: Akt1, Akt2 and Akt3. They share a high degree of amino acid similarity and are activated by similar mechanisms. The current paradigm is that all three Akt isoforms promote cancer cell survival and growth. However studies from several laboratories have recently demonstrated that Akt isoforms share non-overlapping functions in breast cancer progression, whereby Akt1 inhibits breast cancer cell invasive migration. Conversely, Akt2 has been shown to promote breast cancer cell migration *in vitro* and *in vivo*. We are conducting studies to explore the mechanistic basis for this selectivity. Using a combination of shRNA and phospho-proteomic approaches we have identified several novel substrates of Akt1 and Akt2 that modulate cell motility in an isoform-specific manner. In addition, we are exploring mechanism by which PI 3-K signaling is mediated in an Akt-independent manner, and have shown that the serum and glucocorticoid-inducible kinase 3 (SGK3) isoform is a PIK3CA effector that promotes invasive migration. The mechanism of activation of SGK3 by PIK3CA will be presented. Finally, studies focusing on phenotypes associated with breast cancer progression mediated by somatic mutations in Akt1 E17K, as well as a newly identified Akt3 translocation event will be presented.
In summary we have identified novel substrates of Akt isoforms in cancer cells and tissues and are evaluating their contribution to progression in variety of human solid tumors, as well as exploring novel mechanisms of Akt-dependent and -independent signaling in cancer.

S04.

REGULATION AND ROLES OF DIACYLGLYCEROL KINASE-THETA

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Diacylglycerol kinases are important mediators of lipid signaling cascades. Our studies have uncovered new insights into the regulation and physiological roles of one DGK isoform designated DGK-θ. Using purified DGK-θwe discovered that this isoform is under dual regulation by polybasic factors and specific acidic phospholipids. Interestingly the stimulation of DGK-θ by these phospholipids is dependent on the presence of a positively charged protein or peptide. Polybasic cofactors lower the K_M for diacylglycerol at the membrane surface ($K_{M(surf)}$), andwork synergistically with acidic phospholipids to increase activity 10 to 30 fold, suggesting that the enzyme is auto-inhibited. Further, our studies suggest that a triad of enzyme, acidic lipid and basic protein are necessary for interfacial activity. In terms of function, our studies illuminate an important role for this enzyme in regulating synaptic vesicle recycling in the central nervous system. These studies have uncovered potentially important role for the enzyme, its substrate (diacylglycerol) and product (phosphatidic acid), in modulating the recycling of synaptic vesicles during prolonged stimulation. These and other new insights into the regulation and roles of DGK-θ will be presented.

S05.
EPIGENETIC REGULATION OF ODORANT RECEPTOR GENE EXPRESSION
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Each olfactory sensory neuron transcribes one single odorant receptor gene allele from a large family of odorant receptor genes. The mechanisms involved in this monogenic and monoallelic expression remain unclear. Different studies have indicated that regulation of gene expression may be affected by gene positioning in the cell nucleus. Here we analyzed the nuclear organization of olfactory sensory neurons and asked whether the position of the two alleles of a given odorant receptor gene within the nucleus correlates with their activity. We performed 3D DNA-FISH experiments to determine the position of odorant receptor gene alleles in relation to different nuclear compartments. Our results show that olfactory neurons have a characteristic nuclear organization which differs from other cell types. Odorant receptor genes that are located on different chromosomes are frequently associated to a large common constitutive heterochromatin block which is located in a central region of the nucleus. In contrast, the OMP gene alleles, which are biallelically expressed in all mature olfactory neurons, are not associated to this heterochromatic block. Moreover, we found that in a given nucleus, the two homologous OR gene alleles are segregated to separate compartments. Our results indicate that nuclear architecture must play an important role in OR gene expression.

S06.
CANCER EPIGENETIC CONTEXTS
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In mammals, silencing of one of the two X chromosomes is necessary to achieve dosage compensation. The non coding RNA *Xist* triggers X inactivation. Gene silencing by *Xist* is only possible in certain developmental contexts that exist in cells of the early embryo and specific hematopoietic progenitors [Agrelo and Wutz, EMBO Mol Med. 2010; 2(1): 6-15]. A potential reason for restricting the activity of this silencing pathway might be to safeguard against illegitimate changes of cell identity which could lead to malignant transformation. Critical silencing factors may only be present in these contexts giving an explanation of why *Xist* is not operative outside these contexts. It has been demonstrated that *Xist* is functional in lymphoma tumor cells, where SATB1 was identified as a silencing factor for *Xist* mediated chromosome silencing [Agrelo *et al.*, Dev Cell. 2009; 16(4): 507-16]. Importantly new results suggest that loss of p16 is a prerequisite for SATB1 to exert its function. [Agrelo *et al*., Oncogene, 2013; doi: 10.1038/onc.2013.158] Analyzing *Xist and SATB1* function in different tumor models could have potential implication for diagnosis and therapy.
Acknowledgments: *Prof Anton Wutz, Institute of Molecular Health Sciences, ETH, Zürich, SWITZERLAND. Transgenic and Experimental Animal Unit. Institut Pasteur de Montevideo, Montevideo, Uruguay.*

S07.
SECRETS OF THE PLANT FAMILY OF TRANSCRIPTION FACTORS HOMEODOMAIN-LEUCINE ZIPPER I
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HD-Zip proteins are transcription factors unique to plants, exhibiting a homeodomain (HD) associated to a Leucine zipper (Zip) motif. These proteins were associated to plant responses to environmental conditions. All the studied members of the subfamily bind the same pseudopalindromic sequence CAAT(A/T)ATTG and exhibit similar expression patterns. However, when they were constitutively expressed in transgenic plants, different phenotypes were generated. A deep sequence analysis of HD-Zip I subfamily enabled the identification of conserved motifs in the C terminal region of most of the 178 proteins studied. These conserved motifs seem to be responsible for the different functions of these TFs. In order to test this hypothesis, molecular and functional studies were carried out in our lab.
Among the 17 Arabidopsis thaliana HD-Zip Imembers, some are apparently duplicated. *Compositae* species exhibit divergent proteins not present in model plants. Functional characterization of four Arabidopsis HD-Zip I (AtHB7, AtHB12, AtHB1 and AtHB13) and two of sunflower (HaHB4 and HaHB11) confirmed the involvement of these TFs in developmental events in response to abiotic stresses. A few could be biotechnological tools as transgenes conferring stress tolerance and improved yield to improve crops. Molecular basis of similarities and differences as well as open questions will be discussed.

S08.
CONTROL OF LEAF SIZE AND SHAPE BY MICRORNAS IN PLANTS
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Plants and other multicellular organisms need precise spatio-temporal control of gene expression, and this regulatory capacity depends, in part, on small RNAs. MicroRNAs are one class of small RNAs that recognize complementary sites in target mRNAs and guide them to cleavage or translational arrest. In plants, microRNAs usually regulate a few target genes and most of the ancient miRNAs control transcription factors involved in development and hormone signaling.
We have been studying two microRNAs that regulate cell proliferation and differentiation in plants, miR319 and miR396 that regulate transcription factors of the TCP and GRF class, respectively. While miR319 avoids cell differentiation and stimulates cell proliferation, miR396 performs an opposite function by repressing cell division. MiR396 levels steadily increase during the organ development and antagonize the pattern of expression of its targets the GRF transcription factors. MiR396 accumulates preferentially in the distal part of young leaves, restricting the expression of GRF2 to the proximal part of the organ, which in turn coincides with activity of cell proliferation. The balance between miR396 and the GRFs is a key element determining the number of cells in leaves. In turn, the miR319-regulated TCP4 induce miR396, so that the two regulatory networks of miR319 and miR396 are interconnected. The functions of miR319 and miR396 go, however, beyond cell division and control several aspects of the plant development including the morphogenesis of the plant.

CB-P01.

NATURAL ANTISENSE TRANSCRIPTS (NATS): FUNCTIONAL ROLE ON ACYL-COA SYNTHETASE 4 (ACSL4) EXPRESSION

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The acyl-CoA synthetase 4 (Acsl4), an enzyme involved in arachidonic acid metabolism, is crucial in steroidogenesis and in pathologic processes such as tumorigenesis Acsl4 is rapidly induced in response to hormones and has a short half-life; however the mechanisms regulating its expression are not fully described. NATs are endogenous RNAs complementary to mRNA, which act on transcription, maturation, transport, stability or translation of its counterparts. We have identified an Acsl4 NAT which is a long non-coding RNA complementary to an Acsl4 mRNA alternative splicing variant. The aim of this study was to evaluate the physiological role of Acsl4 NAT in the mechanism of Acsl4 expression and action. Hormonal regulation of this NAT was assessed. Sequence-specific RT-PCR was performed on MA-10 Leydig cells treated with gonadotropin. Hormone and cAMP treatments increase its expression in a time-dependent manner, reaching a maximum level after 5 h of stimulation. Acsl4 NAT overexpression impacts differentially on Acsl4 transcripts levels, increasing mRNA var 2 expression, protein synthesis and steroidogenesis. Acsl4 NAT knockdown via asymmetric shRNA showed opposite effects. Acsl4 NAT is one of the few described NATs that are hormonally regulated. Therefore, Acsl4 NAT is a new player in the regulation of Acsl4 mRNA expression and activity, thus influencing steroidogenesis regulation.

CB-P02.

A MEMBER OF FK506 BINDING PROTEINS FAMILY PARTICIPATES IN THE REGULATION OF THE AUTOPHAGIC PATHWAY

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Autophagy is a process in which a double membrane vesicle sequesters cytoplasmic material destined to degradation. Initially, autophagosome formation requires membrane remodeling to generate a vesicle which subsequently matures by fusion with other compartments. FKBP38 is one of the members of the FK506 binding proteins which play a role in some critical cellular functions such as protein trafficking and folding. FKBP38 forms a complex with Bcl2 and regulates the localization of this anti-apoptotic protein, which also has a critical role in autophagy by interacting with Beclin 1. We have demonstrated that overexpression of FKBP38 stimulates the pathway whereas knock down of the protein leads to inhibition of autophagy. The participation of FKBP38 seems to be at an early step of the autophagic pathway because the protein colocalizes with Beclin 1 and Atg14, members of the PI3K complex, but not with LC3, a marker of later events in autophagosome formation. Surprisingly, starvation increased the colocalization between FKBP38 and markers of the endoplasmic reticulum and mitochondria, organelles that are the places where PI3K is recruited to initiate the membrane deformation.
Taken together, our data indicate that FKBP38 regulates the autophagic pathway modulating early stages of autophagosome formation.

CB-P03.

VALIDATION OF PREDICTED CNBP TARGET GENES BY *IN VITRO* AND *IN VIVO* APPROACHES

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Cellular Nucleic acid Binding Proteins (CNBP) is a highly conserved single-stranded nucleic acid binding chaperone. Although CNBP is essential for craniofacial development, its molecular targets are unknown. Monohybrid assays identified mouse and zebrafish genomic sequences as putative CNBP targets. Bioinformatic analyses revealed a G-enriched 14-nucleotide consensus binding site (NGGGGG(A/T)GGGGGGN) and predicted a reduced list of CNBP putative targets based on gene ontology terms and synexpression with CNBP. Here, we validated CNBP regulatory function on some putative target genes by studying their expression changes in CNBP knocked-down zebrafish embryos treated with specific *morpholino*. Among the tested genes, three of them (*tbx2b*, *wnt5b* and *smarca5*) were confirmed as CNBP targets *in vitro* and *in vivo*. Indeed, electrophoretic mobility shift assays and ChIP showed that CNBP specifically binds to the consensus and consensus-like sequences present in the promoters of identified genes and, RT-qPCR and whole-mount *in situ* hybridizations on knocked-down zebrafish embryos revealed changes in transcript abundance of the three candidate genes. In conclusion, we report the identification of three novel CNBP target genes functionally related in a regulatory network relevant for vertebrate embryonic development.

CB-P04.

CNBP: A NEW MEMBER OF THE *WNT* ANCIENT PATHWAY?

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CNBP is a nucleic acid chaperone highly conserved among vertebratesrequired for proper craniofacial development. TheCNBP molecular targets identity is still limited. By using the MEME/MAST software, we searched for genes from human, mouse, chicken, amphibian and fish containing the recently determined DNA-binding site consensus in their promoters. Bioinformatic analyses retrieved 16 putative targets conserved among the five species, mainly comprisingtranscription factors, membrane receptors and signaling proteins.Gene ontology analysis retrieved terms related to development and general metabolism.Noteworthy, threeof the identified genes (*ptk7*, *tcf7l2*, *cdk14*) are involved in the canonical *Wnt* pathway. CNBP binding to the promoters of these genes was validated by ChIPonDNA samples of 24-hpf zebrafish embryos. The role of CNBP on the expression of the target genes was assessed by knocking-down or overexpressing CNBP in 24-hpf zebrafish embryos. Data from RT-qPCR and whole mount in situ hybridization showed that CNBP enhances the expression of all the tested genes. Arecent report shows that CNBP negatively controls the expression of *wnt5b*, a member of the non-canonical Ca^{2+}-*Wnt* pathway. Collectively, our data suggest a role of CNBP in balancing the canonical and non-canonical*Wnt*pathway during embryonic development.

CB-P05.

NANOSCALE ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN CELL-MATRIX ADHESIONS: REGULATORY IMPLICATIONS

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Our previous work suggested that the ER-bound tyrosine phosphatase PTP1B has the potential to modulate the phosphorylation state of multiple components of cell-matrix adhesions. Here we used Bimolecular Fluorescence Complementation (BiFC) for direct visualization of PTP1B-substrate complexes in cells. This approach is based on complementation and restoration of fluorescence when two non fluorescent fragments of the yellow fluorescent protein are a few nanometers apart. These fragments were fused to PTP1B and candidate substrates resident of adhesions. Identification of PTP1B substrates was facilitated by using the substrate trapping mutant PTP1B (D181A). We visualized BiFC between PTP1BD181A and α-actinin, paxillin and FAK at peripheral adhesions. BiFC was undetectable in cells pre-incubated with pervanadate, and in cells expressing wild type PTP1B. PTP1BD181A-paxillin and PTP1BD181A-α-actinin BiFC complexes overlapped with the adhesion marker vinculin, but they segregate spatially to the distal and proximal poles, respectively. Elimination of the SH3-ligand motif on PTP1BD181A did not abolished BiFC. We are currently mapping the residues targeted by PTP1B on the substrates. Our results suggest that PTP1B could regulate adhesion-cytoskeleton coupling through dephosphorylation of α-actinin, and signaling through dephosphorylation of FAK and paxillin.
Supported by ANPCyT.

CB-P06.

CHOLINE KINASE ALPHA EXPRESSION DURING RA-INDUCED NEURONAL DIFFERENTIATION

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The morphological hallmark of neuronal differentiation is neurite sprouting and elongation. This process increases the demand of membrane components, and phosphatidylcholine (PtdCho) is the major component of mammalian membranes. PtdCho biosynthesis increases during neuronal differentiation due to the induction in the transcription of two genes encoding key enzymes in the CDP-choline pathway for PtdCho biosynthesis; the Chka gene for choline kinase (CK) alpha isoform and the Pcyt1a gene for the CTP:phosphocholine cytidylyltransferase (CCT) alpha isoform. We report that the stimulation of CKa expression during RA (Retinoic Acid) induced differentiation depends on a promoter region that contains two boxes, which are highly conserved in the Chka gene of different species. The Box1 includes two CCAAAT/ Enhancer-binding Protein-betha (C/EBPb) sites and the Box2 is a inverted repeat sequence GGGGCCTTGGCCCC. We demonstrate that during neuronal differentiation of Neuro-2a cells, RA induces Chka expression by a mechanism that involves both identified boxes: RA promotes ERK1/2 activation, which induces C/EBPb expression. Elevated levels of C/EBPb bind to the Chka proximal promoter (Box1) inducing CKa expression. However, the Box2 is essential for reaching the high levels of expression induced by RA.

CB-P07.

PHOSPHATIDYLCHOLINE: ROLE DURING EMBRYONIC NEURAL STEM CELLS DIFFERENTIATION

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Stem cells represent a unique cell type characterized by the ability to form, maintain and regenerate tissues they reside in. At later developmental stages, multipotent stem cells can be obtained from different embryonic tissues, such as neural stem cells (NSCs) isolated from the developing central nervous system. The identification of the mechanisms that promote neuronal differentiation of NSCs is vital for future stem cell repair strategies. We hypothesize that the differentiation process in embryonic NSCs could be modulated by lipidic metabolites acting as external or internal signals.
To test this hypothesis, we established the conditions for culture embryonic NSCs from C57/BL mouse. The rate of differentiation was evaluated by inmunostaining coupled to fluorescence microscopy. After phosphatidylcholine (PtdCho) treatment for 7 days we observed a significant increase in the number of cells that express β-III tubulin suggesting that PtdCho promotes neuronal differentiation. In order to characterize the molecular mechanisms, we analyzed the involvement of the ERK pathway on PtdCho-induced neuronal differentiation. We observed that the effect is abolished by the inhibitor UO126. Taken together these results suggest that PtdCho or any derivative metabolite could promote neuronal differentiation from NSCs by a mechanism that involves the ERK pathway.

CB-P08.

PHOSPHATIDYLCHOLINE TURN OVER INDUCES NEURONAL DIFFERENTIATION IN NEURO-2A CELLS

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Biosynthesis of Phosphatidylcholine (PtdCho), the most abundant phospholipid in membranes of eukaryotic cells, is induced in different processes that require membrane biosynthesis like cell cycle progression or neuronal differentiation.
During Retinoic acid (RA)-induced differentiation of neuroblastoma cells PtdCho is not only important as structural membrane building blocks, but they are also involved in the signalling pathways driving neuronal differentiation.
The addition of PtdCho liposomes to the growing media promotes neuronal differentiation in the absence of any neurotrophic factor (RA). We found that PtdCho-induced differentiation requires the activation of ERK pathway. Supplementation of medium with intermediates of PtdCho synthesis (Choline, P-Choline and CDP-Choline) and PC derivative metabolites (lysophosphatidylcholine (LPtdCho) and phosphatidic acid), except lysophosphatidic acid and arachidonic acid induce neuritogenesis. The treatment with different phospholipase inhibitors demonstrated that neuronal differentiation requires citoplasmatic phospholipase A2 (cPLA2) activity, suggesting that LPtdCho could be the signal that triggers this process. The other metabolites would induce PtdCho biosynthesis and produce LPtdCho through cPLA2 activity.

CB-P09.

CORTICOSTERONE HALTS DIFFERENTIATION OF A NEURONAL CELL LINE VIA ACETYLCHOLINE RECEPTOR MODULATION

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In previous work we determined that prenatal stress affects the expression of α7 nicotinic acetylcholine receptor (α7-AChR) in the frontal cortex of adult rat offspring. The aim of the present study was to investigate whether corticosterone (CORT) affects the biology of AChRs. A neuronal cell line (CNh) derived from cerebral cortex and exhibiting a cholinergic phenotype was treated with CORT (1μM). Whole-cell voltage-clamp recordings showed functional AChR expression at the plasma membrane. Upon treatment with CORT, CNh cells expressed lower levels of α7-AChR (~60% of that in control cells). As a control, α4-AChR was found not to be affected. Morphometric analyses showed that CORT delayed the acquisition of the mature CNh cell phenotype. After 48 h in differentiation medium, cell cycle analysis using flow cytometry showed that control CNh cells were arrested in the G0/G1 phase (~65-70%), whereas cells grown in the presence of CORT remained undifferentiated (G0/G1<60%). Nicotine treatment affected the differentiation of CNh cells and exerted a synergistic effect together with CORT (G0/G1~40%, G2/M~55-60%). Transfection of CNh cells with GFP-tagged α7-AChR abolished the alterations in the cell cycle in CORT-treated cells. Thus, α7-AChR could act as a modulator of the differentiation of CNh cells and CORT, through this receptor, could impair the acquisition of a mature phenotype.

CB-P10.

MOLECULAR MOTORS AND THEIR ADAPTORS GOVERN STRESS GRANULE DYNAMICS AND COMPOSITION

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Stress granules (SGs) are discrete cytoplasmic ribonucleoparticles (RNPs) that store silenced mRNA and assemble transiently in response to cellular stress. SGs are highly dynamic, with mRNA and protein components shuttling in and out by mechanisms that involve mainly microtubule-based transport. Studies from our lab have shown that the molecular motors cytoplasmic dynein and kinesin-1 mediate SGs assembly and disassembly, respectively. However, the selective transport of target mRNA molecules and their associated proteins (RBPs) remains elusive. We speculated that molecular motor's adaptor proteins are responsible for such selection. Using Drosophila S2 cells together with RNAi technology and high-resolution confocal microscopy we found that Egalitarian (egl), but not Lisencephaly-1 (Lis1), two dynein adaptor proteins, are required for assembly of SGs. Conversely, Barentsz (Btz) and zipcode-binding protein (ZBP), adaptors of kinesin-1 are necessary for the timely dissolution of SGs. In addition, we observed that Fragile-X mental retardation protein (dFMRP) and Staufen-1, essential components of SGs are delivered by different adaptor proteins. Our data suggest that cargo-specific recruitment of adaptor proteins during SG formation is a key mechanism to maintain specificity in translation regulation during cellular stress.

CB-P11.

TYPICAL 2-CYS PEROXIREDOXINS REGULATE STRESS GRANULES FORMATION

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Stress granules (SGs) are cytoplasmic accretions that form transiently in all cell types undergoing acute stress. SGs contain mRNAs trapped in abortive translation initiation complexes and RNA-binding proteins involved in reprogramming mRNA translation and decay, and are linked to pathogenic protein aggregates (Thomas *et al*., Cell Sig 2011). SG assembly and disassembly depends on: I) destabilization of polysomes (Thomas *et al*., MBoC 2005, JCS 2009). II) Retrograde transport by dynein and bicaudal (Loschi *et al*., JCS 2009, III)Aggregation through specific proteins, IV) Disolution and dispersion mediated by stress-induced chaperones and kinesin (Loschi *et al*., JCS 2009; Thomas *et al*., CS 2011).

In a high-throughput RNAi-screen in Drosophila S2R+ cells we identified 32 positive regulators and 15 inhibitors of SG formation (Perez-Pepe *et al*, PLoS ONE 2012). In addition, we investigated the role of the typical 2-Cys Peroxiredoxins (2C-Prx), which are peroxidases with stress-regulated chaperone and kinase activities. In addition, 2C-Prxs bind RNA and associate to ribosomes under normal conditions. We find that 2C-Prxs facilitate SG formation upon oxidative stress induction. Both reduced and overoxidized Prxs are excluded from SGs. Collectively, these results suggest that Prx peroxidase or chaperone activities are involved in SG regulation.

CB-P12.

c-FOS-ACTIVATED SYNTHESIS OF NUCLEAR PTDINS(4,5)P_2 PROMOTES GLOBAL TRANSCRIPTIONAL CHANGES

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In addition to the canonical activity of c-Fos as a transcription factor, we previously showed that cytoplasmic c-Fos activates phospholipid synthesis by an AP-1independent mechanism. For this, c-Fos associates with particular enzymes of the pathway of synthesis of lipids at the endoplasmic reticulum. Since lipid synthesis has been shown to occur in the nucleus, and different phospholipids have been assigned transcription regulatory functions, herein we examine if c-Fos acts as a regulator of phospholipid synthesis also in the nucleus. We also examine if c-Fos is able to modulate transcription through its phospholipid synthesis activator capacity.

In vitro and *in culture* studies showed nuclear-localized c-Fos associated with and activating Phosphatidyl Inositol-4-phosphate 5 Kinase, but not Type III β Phosphatidyl Inositol 4 Kinase, thus promoting Phosphatidyl Inositol-4,5-bisphosphate [PtdIns(4,5)P_2] formation. c-Fos promoted increased PtdIns(4,5)P_2 formation promotes AP-1-independent transcriptional changes.

The regulatory transcriptional functions of c-Fos can now be extended to its capacity to activate phospholipid synthesis. We propose c-Fos as a key regulator of nuclear polyphosphoinositides synthesis in response to growth signals and hypothesize that both c-Fos-AP-1 independent and c-Fos-AP-1 dependent mechanisms will work coordinately when a cell re-enters the cell cycle.

CB-P13.

INSIGHTS INTO RIBOFLAVIN METABOLISM OF *Trypanosoma cruzi, Trypanosoma brucei* AND *Leishmania mexicana*

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Riboflavin is an essential vitamin. Plants, fungi and prokaryotes synthesize it *de novo* while metazoans obtain it from their diet through specific transporters. In this work we study riboflavin metabolism in *Trypanosoma cruzi, T. brucei* and *Leishmania mexicana*. Bioinformatics analysis of *T. cruzi*'s genome suggested it was auxotrophic for riboflavin and we found a putative transporter for this vitamin, similar to that of *Saccharomyces cerevisiae*. The same sequence was found in *Trypanosoma brucei* and *Leishmania mexicana*.
Proliferation of *T. cruzi, T. brucei* and *L. mexicana* was tested under different concentrations of riboflavin and analogs. The growth rate of the three parasites was reduced in sub-optimal concentrations of riboflavin, as in presence of analogs, in a dose dependent manner. ^{3}H-riboflavin transport assays of *T. cruzi* epimastigotes showed that the parasites incorporate riboflavin (initial velocity: 0.7 fmol/min/10^{-7} cells at 2.5 μM riboflavin).
Our results suggest that riboflavin is essential for the proliferation of trypanosomatids. The putative transporter identified in the genome of these parasites is substantially different to those found in mammals. This makes it a potential target for inhibition or transport of toxic analogs, in the development of new trypanocidal molecules.

CB-P14.

INTERPLAY BETWEEN RAB7 AND RAB24 IN THE AUTOPHAGIC PATHWAY

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Autophagy is characterized by the formation of a double membrane structure called autophagosome. Autophagosomes have been reported to fuse with lysosomes. Thus, autophagosome maturation is a multi-step process, which includes several fusion events with vesicles originating from the endo/lysosomal pathway. It was demonstrated, by using a dominant negative mutant of Rab7, that this protein is necessary at the amphisome/lysosome fusion event. Rab24 is an atypical member of the Rab GTPase family whose distribution in interphase cells has been characterized. Rab24 was implicated in the autophagic pathway since upon autophagy induction this protein changes its distribution colocalizing with the autophagosomal markers LC3. It was also reported that Rab24 partially colocalize with Rab7, which connect late endocytic structures and autophagic vacuoles with lysosomes. In this study, we have analyzed the distribution of Rab7 and Rab24 in cells co-expressing both proteins either wt or mutants. Interestingly, in cells cotransfected with RFP-Rab24wt and GFP-Rab7T22N (a dominant negative mutant), we observed a marked change in Rab24 intracellular distribution, loosing its colocalization with LC3. On the other hand, the reverse cotransfection also altered the Rab7 distribution. Our results suggest that both small GTPases share interacting proteins and are likely involved in a common pathway.

CB-P15.

***Chlamydia trachomatis* RECRUITS AND ACTIVATES PKCä AND PKCε AS SURVIVAL STRATEGY**

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Chlamydia trachomatis is an obligate intracellular bacterium that replicates in a vacuole called inclusion and causes genital and ocular infections in humans. We have shown that *C. trachomatis* activates the Akt/AS160 pathway to ensure the arrival of Rab11- and Rab14-vesicles full of sphingolipids to the inclusion. Depending on cell type, PKCε activates or inactivates Akt, either by direct phosphorylation or by the action of a phosphatase. In addition, *C. trachomatis* recruits PKCδ as a protective mechanism against apoptosis. We postulate that *C. trachomatis* recruits both PKCδ and PKCε not only as a strategy to inhibit apoptosis, but also to ensure the arrival of Rab11- and Rab14-vesicles full of lipids. We transfected HeLa cells with different PKC isoforms (PKCβII, PKCα, PKCδ and PKCε and PKCζ), and we observed that only PKCδ and PKCε are recruited to the chlamydial inclusion. Infected cells were treated with several kinase inhibitors, like Rottlerin (PKCδ), Gö 6983 (PKCα) and H89 (PKA), and only Rottlerin completely inhibits the bacterial development. Furthermore, treatment with Rottlerin or Calphostin (a general inhibitor of PKC) modifies the intracellular distribution of Rab14 and prevents its recruitment to the inclusion membrane. These data suggest that *C. trachomatis* could selectively activate both PKCδ and PKCε to ensure the arrival of nutrients and to guaranty its survival.

CB-P16.

TRIPLE COLOCALIZATION STATISTICAL SIGNIFICANCE PLUGIN (TCSS)

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The colocalization of molecules using fluorescence images is one of the most common tools to determine if certain molecules are in positions where they can interact with each other. There are several plugins that use diverse math tools in order to calculate double colocalization, and many of these applications can determine its statistical significance comparing the result of double colocalization with random generated images. Despite the amount of available plugins, only a few of them calculate triple colocalization (data that cannot be deduced from the results of the double colocalizations) and if they do, they fail in the determination of the statistical significance committing type 1 error since results are evaluated as significant when they are not. TCSS is a plugin for ImageJ that calculate double and triple colocalization for Pearson, Manders and intersection coefficients and the statistical significance for Manders and intersection coefficients. In addition, it uses a selection channel algorithm for the correct generation of random images and another algorithm of reduction of the mask in order to avoid committing type 1 error.

CB-P17.

***Infectious Bursal Disease Virus* INTERNALIZATION INVOLVES A MACROPINOCYTIC PATHWAY**

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The *Infectious Bursal Disease Virus* (IBDV) causes an immunosuppressive disease in birds, causing economic losses in the poultry area worldwide. We studied the viral entry pathway and found that internalization occurred in a clathrin-, caveolin-, cholesterol- and dynamin independent pathway, so we aimed our study on the macropinocytic pathway.

We examined the effect of actin polymerization inhibitors in IBDV infection employing Western blot and Confocal Microscopy techniques. Subsequently, we assessed the effect of a Na^+/H^+ exchanger selective inhibitor involved in the formation of macropinosomes. Furthermore, ultrastructural analysis was performed to analyse viral internalization kinetics and the effect of actin polymerization inhibitors on IBDV entry by transmission electron microscopy. We found that the actin cytoskeleton integrity and a functional NHE-1 are required for IBDV infection. Moreover, the ultrastructural analysis revealed cell membrane structures associated with virus entry morphologically compatible with formation of macropinosomes. In addition, when actin polymerization was inhibited, we observed morphological changes that support the notion that the macropinocytosis is used by the virus to be endocytosed by cells.

Together, these data strongly indicate that the IBDV uses the macropinocytic pathway as the main mechanism of cell internalization.

CB-P18.

UDP-GLC ENTERS *S. pombe* ER BY BOTH A KNOWN ANTIPORT AND A NOVEL BUT UNKNOWN MECHANISM

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UDP-Glc glycoprotein glucosyltransferase (UGGT, coded by *gpt1* gene) is a glycoprotein folding status sensor in the endoplasmic reticulum (ER) that tags with a glucose moiety incompletely folded glycoproteins, thus favoring their interaction with calnexin and calreticulin. UGGT substrate, UDP-Glc, is synthesized in the cytosol and the mechanism of its import into the ER is still unknown. From six putative nucleotide sugar transporter (NST) homologues in the genome of *Schizosaccharomyces pombe*, only *hut1*$^+$ and *yea4*$^+$ bear an ER retention signal. We first disrupted both genes in a *Δalg5* background (Protein-linked $Glc_1Man_9GlcNAc_2$ formation in *Δalg5* or *Δalg6* mutants is only due to UGGT activity and indicates entrance of UDP-Glc into the ER). ER vesicles purified from *Δalg5Δhut1* but not *Δalg5Δyea4* mutants showed a 50% decrease in UDP-Glc transport. However, *in vivo* labeling of *Δalg5Δhut1* resulted in $Glc_1Man_9GlcNAc_2$. We now disrupted all genes coding for NST in *S. pombe* genome in an *Δalg5* background. Only *Δalg5Δhut1* mutants showed the same growth lethal phenotype at 37ºC and aberrant morphology at 28ºC as *Δalg6Δgpt1* mutants. These results suggest that even though *hut1*$^+$ appears to be involved in UDP-Glc entrance into the ER, at least another unknown pathway exists in eukaryotic cells.

CB-P19.

HEMIN INDUCES AUTOPHAGY IN LEUKEMIC ERYTHROBLAST CELL LINE

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Autophagy is a normal degradative pathway that involves the sequestration of cytoplasmic components and organelles in a vacuole called autophagosome, which finally fuses with the lysosome to degrade the sequestered material. This process has been associated with several physiologic processes as erythroid maturation. LC3 is a protein present in autophagosomal membrane, therefore is considered a bonafide marker of this structure. Our results indicate that in K562 cells, hemin (erythroid maturation inductor) produced an increased number and enlargement of GFP-LC3 positive vesicles labeled with lysotracker or DQ-BSA compared with others maturation inductors. Moreover, we have demonstrated that hemin induces mitochondria membrane depolarization. We have also shown in erythroblastic leukemic cells co-expressing RFP-LC3 and GFPRab11 (a multivesicular bodies marker) that hemin generates an enlarged vesicles labeled with both markers. Likewise, we have performed a western blot to detect the processing of LC3 protein upon hemin incubation. We have observed in this assay an increased total amount and a higher level of processed LC3. Taken together, our results suggest that hemin could induce mitophagy response in K562 cells, probably allowing an efficient and faster maturation.

CB-P20.

OXALATE MICROCALCIFICATIONS INDUCE BREAST CANCER TUMORS

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Microcalcifications are routinely used to detect breast cancer in its early stages, these early detections gives for a better patient outcome. Mammary microcalcifications are mainly composed of calcium oxalate or calcium phosphate. Oxalate has been considered as an inert end product of the metabolism. However, its accumulation is thought to be toxic to living tissues. Exposure of renal epithelial cells to oxalate leads to diverse events as expression of immediate early genes (IEG), cell growth and up or down-regulation of more than 1000 genes.

Herein, we have obtained good evidence supporting the hypothesis that the exposure of breast epithelial cells to excreted oxalate in a chronic way sets the stage for transformation from normal to fully developed breast tumors. Oxalate induced DNA synthesis and IEG over-expression in MCF7 and MDAMB231 human breast cancer cells in culture. Additionally, in human breast cancer tissues we found a strict correlation between oxalate concentration and over-expression of c-Fos and Fra-1. Surprisingly, female mice that were injected with oxalate periodically during a month generated very aggressive tumors a month later, after the treatment was finished. c-Fos and Fra-1 were also found over-expressed in these tumors. Furthermore, the final oxalate concentrations in the mice tumors were analogous to those found in the human breast tumor tissues.

CB-P21.

CHARACTERIZATION OF MUTANT P53-BASED ONCOGENIC MECHANISM: HOW IS CPSF6 REGULATED?

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P53 point mutants, besides losing the tumor suppressor function of the wt protein, may acquire novel activities that promote the development of aggressive and metastatic tumors. We have previously found that the prolyl-isomerase Pin1 cooperates with mutant p53 to alter gene expression in breast cancer cells. In particular, both proteins induce the expression of 10 genes (Pin1/mutant p53 signature) whose expression is associated with the development of aggressive breast tumors. Among them we found CPSF6, which codes for a 68 kDa protein, belonging to the CFIm processing factor that regulates mRNA polyadenilation. Even if several aspects CFIm function in polyadenilation were described, little is known regarding how CPSF6 may be involved in human cancer. In order to understand the mechanisms underlying the pro-oncogenic role of CPSF6, we studied the consequences of overexpressing zebrafish Cpsf6 (DrCpsf6) during embryogenesis. We cloned the sequence of DrCpsf6 and we confirmed that it localizes in paraspeckles upon ectopic expression in cell lines. Surprisingly, we found that upon microinjection in zebrafish embryos, Cpsf6 expression is readily detectable at early stages but becomes almost completely absent at later stages, suggesting the existence of robust posttranslational regulatory mechanisms. Further studies showed that oncogenic signals do not alter subcellular localization.

CB-P22.

UNDERSTANDING ONCOGENIC SIGNALING: EFFECT OF PIN1 OVEREXPRESSION DURING EMBRYOGENESIS IN ZEBRAFISH

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Prolyl-directed phosphorylation signaling regulates several physiologic processes. Pin1 is a key enzyme in this pathway that modulates the function of protein substrates through conformational changes induced by isomerization of peptide bonds in phosphorylated S-P or T-P motifs. The ability to link protein function with prolyl-directed phosphorylation allows Pin1 to act as a global modulator of biological responses. However, incorrect regulation of Pin1 may promote pathological situations. Previously, we identified Pin1 as a critical link between oncogenic signaling and downstream mechanisms of tumor aggressiveness. However, the actual consequences of Pin1 overexpression in cancer are difficult to asses *in vivo*, due to the variety of substrates and the complex array of phosphorylation signals active in individual cell types. To characterize alterations elicited by Pin1 *in vivo* we are modeling Pin1 overexpression in zebrafish embryogenesis, which provides a unique model to study the effect on signaling pathways. We have previously shown that transient Pin1 overexpression affects forebrain development at early stages. We extended the analysis to later stages were we found that the effect of Pin1 on the telencephalon is maintained but also that structures surrounding the mandible are affected. Our results suggest however, that the observed effects do not involve cartilage formation.

CB-P23.

EFFECT OF GROWTH HORMONE ON ARACHIDONIC ACID METABOLISM AND ITS INVOLVEMENT IN LIVER TUMORIGENESIS

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Transgenic mice overexpressing growth hormone (GH) exhibit high incidence of liver tumors and display upregulation of the signaling pathways Erk1/2 and PI3K/Akt, which mediate cell proliferation and survival. These signaling pathways also participate in the activation and expression of enzymes involved in the metabolism of arachidonic acid, including cyclooxygenases (COX) and phospholipase cPLA2α, which are believed to play a role in hepatic carcinogenesis. The objective of this study was to determine the impact that prolonged exposure to GH exerts on COX1 and cPLA2α in the liver. The expression of these enzymes was assessed in the liver of GH-transgenic mice by immunoblotting and qRT-PCR. Young adult mice, which exhibit preneoplastic pathology, and animals of advanced ages, which develop liver tumors, were used. Normal littermates served as controls. Transgenic mice displayed elevated protein levels of COX1, but its gene expression was not altered. Both the protein and mRNA abundance of cPLA2α were higher in transgenic than in normal mice. The results were similar for young and old mice, and were confirmed in both genders. These experiments suggest that prolonged exposure to GH impairs the expression of key enzymes involved in arachidonic acid metabolism, and could contribute to establish the mechanisms involved in the process of liver carcinogenesis in mice overexpressing GH.

CB-P24.

THE CONTRIBUTION OF RECOMBINATION-DEPENDENT MECHANISMS TO THE REPLICATION OF UV-C DAMAGED DNA

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Exposure of cells to genotoxic stimuli causes replication forks stalling at DNA lesions, which might trigger cell death. To preserve viability, cells activate tolerance mechanisms that prevent replication fork collapse. The best understood tolerance event activated by UV light is Translesion DNA Synthesis, which aids DNA replication by recruiting specialized polymerases like pol η to DNA lesions. Another less characterized tolerance event called Template Switching relies on the homology searching capacity of the recombinogenic protein Rad51. In this context, Rad51 facilitates the utilization of the novel DNA generated on the opposite strand as alternative template. Other recombinogenic independent functions of Rad51 have also been reported. In fact, Rad51 protects replication forks from degradation in a manner that depends on its strand invasion capacity.

Weather any or all of these functions of Rad51 are required after UVC irradiation is unclear. I will present data of the effect of Rad51 depletion on the accumulation of a replication stress marker, 53BP1, and on different DNA replication read-outs after UVC irradiation. I will discuss results in the context of the relative contribution of recombinogenic and non recombinogenic functions of Rad51 to the response of cells to the accumulation of UVC damaged DNA.

CB-P25.

THE CONTRIBUTION OF THE CYCLIN DEPENDENT KINASE INHIBITOR P21 TO THE CHK1 KINASE MEDIATED REGULATION

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The cyclin dependent kinase (CDK) inhibitor p21 was recently characterized by our group as the first negative regulator of the DNA replication auxiliary process, translesion DNA synthesis (TLS). p21 inhibits the recruitment of TLS polymerases to UV damaged DNA and impairs replication fork progression, causing cell death and genomic instability. The degradation of p21 after UV irradiation is therefore critical to modulate the proper onset of TLS after UV irradiation. When searching for potential regulators of p21 degradation after UV irradiation I found that the depletion of the checkpoint effector kinase, Chk1, causes a substantial increase in the levels of p21. Given that Chk1 was suggested as a positive regulator of TLS, it is possible that Chk1 could control TLS, at least in part, though the negative modulation of p21 levels in cells. Experiments designed to analyze the potential link between TLS, Chk1 and p21 will be presented and discussed. Moreover, given the central role of Chk1 in the maintenance of genomic stability we will present evidence exploring the contribution of p21 to this important function of Chk1.

CB-P26.

REGULATION OF ALTERNATIVE SPLICING BY INTRAGENIC ALTERNATIVE PROMOTERS AND ENHANCERS

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In order to determine possible mechanisms for the regulation of alternative splicing by intragenic chromatin structure changes, we have studied the activation of intragenic enhancers and alternative promoters on the splicing patterns of neighboring exons. In particular, we are studying the alternative splicing regulation of the cassette exon 107 (E107) of the human SYNE2 gene that bears a transcription start site (TSS) in the downstream intron. ChIP-seq analysis performed in MCF-7 cells reveals an enrichment of the Argonaute 1 (AGO1) protein at the intronic TSS. Upon knockdown of AGO1, we observed enhanced inclusion of E107 as well as an increase in the expression of the mRNA generated from the intronic TSS. In order to determine if the activation of the alternative promoter leads to changes in the resulting splicing pattern of E107, we analyzed available ChIP-seq data of transcription factors and histone marks and found that the alternative promoter has been reported as a binding site for the estrogen receptor alfa (ERa) in MCF-7 cells. Treatment with 17 beta-estradiol caused increased exclusion of E107 in MCF-7 and T47D cell lines, both derived from human mammary tissue. Using this model we are now studying, by ChIP-qPCR of histone marks and RNA polymerase II, the mechanism by which the activation of the alternative promoter leads to changes in the splicing pattern of E107.

CB-P27.

PI3K SIGNALING PATHWAY IS IMPLICATED IN BRADYKININ-INDUCED MIGRATORY PHENOTYPE OF URETERIC BUD CELLS

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In mammals, nephrogenesis is completed postnatally. We have previously shown that primary cultures of renal papillary ureteric bud (UB) cells of 7 days-old rats display a great capacity to form colonies, and interact with the substratum through focal adhesions (FA) immunostained with vinculin. Bradykinin (BK) treatment induces a migratory phenotype, and in some border cells, BK provokes a redistribution of vinculin from focal adhesion (FA) to the cytosol. In the present work, we tested whether PI3K signaling pathway was implicated in these phenomena. We observed that pre-treatment of cultured UB cells with the PI3K–specific inhibitor Ly294002 avoided BK–induced vinculin redistribution. We examined by immunofluorescence the cellular distribution of PI3K, PTEN, AKT and AKT-P in BK stimulated UB cells. PI3K immunostaining is located in the plasma membrane of border cells lacking FA but containing vinculin in the cytosol, and in the cytosol of cells containing FA. The same pattern of labeling was observed for AKT and AKT-P. Interestingly, cells containing FA do not express PTEN in the plasma membrane. We interpret that vinculin accumulation in the cytosol only occurs in those cells whose function is the one to indicate the direction of the collective migration of the UB cells, and PI3K signaling pathway is implicated in BK-induced migratory phenotype of these UB leader cells.

CB-P28.

ALTERATION OF LIPID RAFTS EVOKES THE FORMATION OF NON RECYCLING -VINCULIN CONTAINING VESICLES

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We reported that bradykinin (BK) induces a recycling of vinculin from focal adhesion (FAs) through the recycling compartment, denoted by the appearance of vinculin/ Rab5/ Rab11/ transferrin receptor positive vesicles in the cytosol. Now, we investigated whether the formation of these vesicles is a BK-specific or a general phenomenon. Since FA complexes are located in specific membrane microdomains (raft), we treated cultured renal papillary collecting duct cells with the lipid membrane-affecting agents cyclodextrin (CD) and neomycin (Neo), which caused dissipation of vinculin-stained FAs due to their deleterious effect on membrane microdomains, where FAs are inserted. After CD and Neo treatment of cultured cells, we observed the presence of vesicle-like structures containing vinculin in the cytosol by immunofluorescence. We treated papillary slices with CD and Neo and isolated vinculin-containing vesicles by an immunomagnetic method. Western blot analysis showed that these vesicles do not contain markers of the recycling endosomal compartment. We conclude that the endocytic recycling pathway of vinculin is not a general phenomenon, and could be a physiological mechanism to reuse the internalized vinculin to reassembly new FA which occur after long time of BK stimulation, but not after CD and Neo treatment.

CB-P29.

KINETICS OF THE ACROSOMAL EXOCYTOSIS IN HUMAN SPERM

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Our laboratory has shown that sperm and neuroendocrinal cells use similar molecular mechanism for secretion. However, we report here that the process is orders of magnitude slower. For these experiments, capacitated human sperm were stimulated with progesterone or with the calcium ionophore A23187 in the presence of a fluorescent lectin. The incorporation of the lectin into the acrosome -indicating fusion pore opening- was assessed in fixed or live cells by fluorescence microscopy or flow cytometry. Despite the slow kinetics of the exocytosis, the cytosolic calcium increased triggered by the stimuli occurred in seconds. To assess if acrosomal swelling, a process required for exocytosis, was responsible for the delay, we measured the kinetics of this process and observed that it required several minutes to be completed. We then tested the kinetics of fusion pore opening in sperm that had completed the swelling. For these experiments intraacrosomal calcium was depleted using the photosensitive calcium chelator NP-EGTA and sperm were stimulated with A23187. Exocytosis was completed when the chelator was photo-inactivated. Under these conditions the pores opened in seconds. These observations indicate that the acrosome reaction requires a preparation time, probably related to acrosomal swelling, whereas the fusion pore opening occurs with a fast kinetics.

CB-P30.

SPERM CAPACITATION IS NECESSARY FOR SNARE ASSEMBLY IN NEUROTOXIN RESISTANT COMPLEXES

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Capacitation is a not well defined process required for the acrosome reaction triggered by physiology stimuli. It can be achieved *in vitro* by incubation under specific conditions. The role of capacitation on the preparation of the membrane fusion mechanism responsible for acrosomal exocytosis is unknown. SNAREs proteins are fundamental proteins for membrane fusion inside cells. We have previous shown that neurotoxin sensitive SNARE proteins are necessary for acrosomal exocytosis. In capacitated resting sperm, SNAREs are assembled in neurotoxin resistant cis-complexes. The aim of this work was to study the dynamics of SNARE before and after sperm capacitation. For this purpose, we developed a membrane permeant neurotoxin by crosslinking a polyarginin peptide to recombinant Tetanus toxin light chain. SNAREs were insensible to the permeant neurotoxin in capacitated sperm. However, the toxin efficiently inhibited exocytosis when SNAREs complexes were disassembled upon stimulation. In contrast, SNAREs were sensible to the neurotoxin in not capacitated sperm indicating that SNAREs were not stabilized in cis complexes. Kinetic studies showed that two hours incubation under capacitating conditions were sufficient for SNAREs assembly in stable complexes. In brief, our results indicate that capacitation is important for the maturation of the fusion machinery responsible for acrosome reaction.

CB-P31.

PHOSPHOLIPID SYNTHESIS ACTIVATION BY c-FOS. IMPLICATIONS IN BRAIN TUMOR CELL PROLIFERATION

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c-Fos belongs to the AP-1 family of transcription factors. Our laboratory determined that in addition to this AP-1 activity, c-Fos associates to the endoplasmic reticulum (ER) and activates the synthesis of phospholipids, the main components of cell membranes. We previously observed that c-Fos is over-expressed in brain tumors in contrast with non-pathological tissues where c-Fos is rarely detected. We established a correlation between the malignancy of brain tumors and the % colocalization of c-Fos with the ER markers. The aim of this study is to design and test putative negative dominant mutants of c-Fos that can block its action as an activator of phospholipid synthesis. The domain NA of c-Fos (aa 1-138) associates to the ER but does not activate phospholipids synthesis, acting as negative dominant mutant. To facilitate the future therapeutic application of mutants, we are seeking for the smallest truncated forms of NA that retain dominant negative capacity. We found that NA deletion mutants diminish tumor cell proliferation whereas FRET-FLIM microscopy shows that the presence of NA reduces c-Fos interaction with PI4KIIα, a key enzyme of phospholipid metabolism. NA domain of c-Fos and its truncated forms show promising features to be used as dominant negative peptides to block the action of c-Fos in malignant brain tissues.

CB-P32.

EXARATE ADULT CHARACTERISTICS AND IMAGO FUNCTIONAL SENESCENCE IN THE MEDFLY *Ceratitis capitata*

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Senescence, behavioral and biochemical parameters were analyzed during the life cycle of the Medfly. Average survival was 36.1±1.2 days, whereas maximum longevity was 81.7±2.1 days. On the basis of the unusual first supine behavior (FSP), at day 10, proposed as a predictor of death, we arbitrarily defined the span of youth stage. This young imago period was defined according to the absence of significant death (since exarate adult emergence until day 20). A reduction in the locomotor activity since FSP, during the transition from early to late young stages, was not accompanied by changes in energetic resources (Glycogen and Lipids). However, there was an increase of peroxidated brain lipids (TBARS), starting early during the youth period, whereas the antioxidant capacity decreased until day 30. Nevertheless TBARS only increased in thoracic muscles at day 30, in correlation with the reduction in locomotor activity and survival. These results agree with the functional senescence index, derived from neutral and polar lipid profiles (Pujol-Lereis *et al.*, 2012). In addition to confirm that functional senescence parameters differ depending on the tissue/organ, our results also support the predominant idea about the role of ROS species in peroxidation. This was made evident by different stress-induced experiments.

CB-P33.

IDENTIFICATION OF SNARES INVOLVED IN THE FORMATION OF THE *Trypanosoma cruzi* PARASITOPHOROUS VACUOLE

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Trypanosoma cruzi is the protozoan parasite that causes human Chagas disease. As an obligate intracellular parasite, *T. cruzi* resides transiently in a parasitophorous vacuole (TcPV). It is well established that TcPV must fusion with lysosomes to establish a productive intracellular infection. SNARE proteins are key molecules of the vesicle fusion machinery. The aim of this study is to identify SNARE proteins involved in the parasite infection process. Our results indicated that Vamp7 (v-SNARE) overexpression increased two fold the infection rate and more than 60% of TcPVs recruited Vamp7 to their limiting vacuole membrane. Silencing this SNARE protein, but not the overexpression of the truncated mutant of Vamp7 (Vamp7 NT), caused a marked decrease in the parasite infection rate. Moreover, we have detected in the vacuole membrane the Vamp7 partners (Vti1b, Snap23 and Stx3). In addition, we determined the participation of the motor protein Kif5, a kinesin implicated in the transport of VAMP7-vesicles to the cell periphery, in the infection process. We observed that cells overexpressing a dominant negative mutant of Kif5 reduced two fold the infection rate. Taken together, these results indicate that Vamp7 plays a major role in TcPV biogenesis, likely by facilitating the interaction with the endolysosomal compartment.

CB-P34.

AUTOPHAGIC MODULATION DURING *IN VIVO Trypanosoma cruzi* INFECTION, A DOUBLE-EDGED SWORD

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Autophagy is a pathway involved in the innate immune response against intracellular pathogens. Beclin-1 (Bcln) is a key protein required for this process. Previously, our group showed that induction of host cell autophagy favors *T. cruzi* infection *in vitro*. To evaluate the *in vivo* effect of autophagy in the infection, we studied mortality, parasitemia and histopathology in autophagy competent $Bcln^{+/+}$ (WT) and deficient $Bcln^{+/-}$ (KO) mice infected with *T. cruzi* Y-GFP strain. Additionally, we studied the effect of two autophagy inhibitors, Chloroquine (CQ) and Difluoromethylornithine (DFMO). Our results showed that the infection can be divided in two stages. During the first stage there is a local infection characterized by higher parasitemia at 5 and 7 days post infection (dpi) in WT animals, similarly to the *in vitro* mechanism. Conversely, the second stage of systemic infection was more aggressive in KO mice, which developed significantly higher parasitemia in subsequent days (10 and 13 dpi). This higher parasitemia was correlated with an earlier death ($p=0.002$) and a larger number of cardiac amastigotes nests. Interestingly, WT mice treated with both DFMO and CQ evolved similarly to the KO mice. Overall, these results demonstrated that, in contrast to the *in vitro* effect in non-phagocytic cells, the autophagic process protects against infection by *T. cruzi* in the *in vivo* model.

CB-P35.

FBXO11-CDT2 INTERACTION CONTROLS THE TIMING OF CELL CYCLE EXIT

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F-box proteins and DCAF proteins are the substrate binding subunits of SCF (Skp1-Cul1-F-box protein) and CRL4 (Cul4-RING protein Ligase)ubiquitin ligase complexes, respectively. Using affinity purification and mass spectrometry, we determined that the F-box protein FBXO11 interacts with CDT2, a DCAF protein that controls cell cycle progression, and recruits CDT2 to the SCFFBXO11complex to promote its proteasomal degradation. In contrast to most SCF substrates, which exhibit phosphodegron-dependent binding to F-box proteins, CDK-mediated phosphorylation of Thr464 present in the CDT2 degron inhibits recognition by FBXO11. Finally, our results show that the functional interaction between FBXO11 and CDT2 is evolutionary conserved from worms to humans and plays an important role in regulating the timing of cell cycle exit.

CB-P36.

MANNOSE-6-PHOSPHATE RECEPTORS IN BULL EPIDIDYMIS: THEIR ROLE IN THE DISTRIBUTION OF LYSOSOMAL ENZYME

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One of the most striking features of the epididymis is the intense endocytic and secretory activity of the epithelium. In bulls, as in other mammals, hydrolytic enzymes might play a role in sperm remodelling in the epididymal fluid. In order to study the mechanism of enzyme secretion in bull epididymis, we have studied the expression and activity of cation-dependent (CD-MPR) and cation independent (CI-MPR) mannose-6-phosphate receptors, which are thought to be involved in proper delivery of lysosomal enzymes to lysosomes. We have found that the CD-MPR was highly expressed in the epididymis of sexually immature bulls (SI), decreasing significantly in adults (SM). Conversely, the expression of CI-MPR was higher in SM than in SI without regional differences being observed. On the other hand, the high activity of lysosomal enzymes in epididymis of SI and its increased tissularretention accounts for the existence of a developed and active lisosomal apparatus. By binding assays we estimated that a number of CD-MPR sites may be inactive in the SI. Moreover, some acid hydrolases found in the epididymal fluid were found to be ligands for MPRs and they were mostly recognized by CI-MPR in the SI, but not in the SM. We concluded that both MPRs could play different roles during sexual maturation of bulls, and that the CD-MPR might be involved in release of enzymes.

CB-P37.

SUMO CONJUGATION TO SPLICEOSOMAL PROTEINS

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Most eukaryotic genes transcribed by RNA polymerase II give rise to precursor messenger RNAs (pre-mRNAs) containing exons and introns. Among several steps of mRNA maturation, splicing is the process by which introns are removed from the pre-mRNA and consecutive exons are joined. This process is carried out by the "spliceosome", a complex of small nuclear ribonucleoprotein particles (snRNPs) and associated factors that assemble on pre-mRNA in a precise and stepwise manner, recognizing sequence-specific splice sites primarily located at the intron- exon boundaries. Proteomic studies have identified the RNA-binding proteins as one of the major groups among small ubiquitin-related modifier (SUMO) conjugation substrates, including splicing-related proteins. In fact, ubiquitylation of snRNP components modulates spliceosome assembly. Furthermore, SUMO conjugation regulates pre-mRNA 3' end processing and ARN editing. However, a possible role for SUMO conjugation in splicing regulation has not yet been explored. We have shown recently that the splicing factor SRSF1 is a regulator of SUMO conjugation, providing a provocative link between the splicing and SUMO machineries.
We propose to analyze the modification of snRNP components by SUMO conjugation and to generate the tools for further studying the role of SUMO conjugation on spliceosome assembly and, consequently, on splicing regulation.

CB-P38.

SMAUG1-MEDIATED REPRESSION IS INDEPENDENT FROM AGGREGATION AND INVOLVES DEADENYLATION

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Local protein synthesis at the synapse is important for synapse remodelling and memory formation. Mammalian Smaug1 (mSmaug1) is an mRNA repressor specifically expressed in mature neurons, where it forms mRNA silencing foci, termed S-foci, involved in synaptic plasticity. The S-foci respond to stimulation by dissolving and releasing transcripts to allow translation, the CamKII alpha mRNA among others (Baez *et al.*, JCB, 2011; Pascual *et al.*, CIB 2012; Baez and Boccaccio, JBC 2005). We previously found that a conserved D domain is required for mSmaug1 aggregation. Here we show that lack of the D domain does not affect repression in an MS2 tethering assay, thus suggesting that aggregation is not necessary for translation silencing and opening the possibility that RNA recognition is facilitated by Smaug aggregation. The molecular mechanism for mSmaug1-mediated repression is unknown and we performed a co-IP screen for several components of the two major deadenylation pathway: the CCR4/NOT/POP and the PAN2/PAN3 complexes. We found that several molecules of these complexes are recruited to the S-foci, suggesting that mSmaug1 mediates deadenylation. We also found that Ago1 is recruited to the S-foci, indicating a role for the miRNA pathway in mSmaug1-mediated repression. We are currently studying the biological relevance of Smaug1 aggregation in Zebrafish models.

CB-P39.

MOLECULAR DETERMINANTS OF SMAUG 1 AGGREGATION

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Mammalian Smaug1 (mSmaug1) is a novel translational repressor specific of the post-synapse of mature neurons. mSmaug1 knockdown provokes the formation of immature synapses and impairs the response to synaptic stimulation (Baez *et al.*, JCB 2011; Pascual *et al.*, CIB 2012). Both Drosophila Smaug and mSmaug1 repress the translation of reporter mRNAs carrying specific motifs termed Smaug-Recognition-Element (SRE) and form mRNA silencing foci termed S-foci (Baez and Boccaccio, JBC 2005). Self-aggregation is a common feature of RNA-binding proteins involved in mRNA-silencing and here we investigated the mSmaug1 domains involved in S-foci formation. We found that mSmaug1 aggregation is independent of RNA binding and requires two conserved regions, a D domain located at the N-terminus and the C-terminal region. Co-tranfection experiments suggest that both regions interact homotypically thus facilitating mSmaug1 oligomerization. Aggregation of the Drosophila molecule requires the conserved D domain and is further facilitated by a QN-rich domain that is absent from the vertebrate molecule, likely due to its inherent toxicity. Co-transfection of dSmaug and mSmaug1 constructs indicates that the homotypic interaction of the D-domain is specie-specific. We are currently studying the relevance of mSmaug1 aggregation to the repressor activity.

CB-P40.

ROLE OF RETINOIC ACID RECEPTORS α AND γ ON CELL GROWTH AND STEM CELL MAINTENANCE IN BREAST CANCER

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Due to their role in the regulation of cell growth and differentiation retinoids (Rd) are being evaluated in clinical trials for cancer prevention and treatment. Rd exert their functions through the binding to the nuclear receptors RAR and RXR. We focus our studies in LM38-LP, a murine mammary tumor cell line composed by luminal (LEP), myoepithelial (MEP) and stem/progenitor cells (MSC) that expresses all functional retinoic acid receptors. Our objective was to evaluate the effect of RARα and RARγ activation on cell growth, alteration of LEP/MEP proportion and their role on MSC growth and maintenance. AM580 (RARα agonist) and AM580+MM (RARγ antagonist) diminished cell proliferation after 144H treatment. Whereas MM, Ro41 (RARα antagonist), BMS (RARγ agonist) and their combinations had no effect on proliferative potential. Through flow cytometry, we could determine that all treatments decreased LEP/MEP ratio (CD24+CD29+/CD24-CD29+). Regarding MSC, through primary mammosphere formation assays, we observed that AM580, MM, AM580+MM and BMS+Ro41 treatments increased mamosphere number. But, while BMS, Ro41 and BMS+Ro41 increased mamosphere size, AM580 induced the opposite effect. In sum, both RARα and γ decrease the proportion of LEP vs. MEP cells but only RARα induces proliferation inhibition. Besides, RARα would participate in MSC survival while RARγ would be involved in MSC growth.

CB-P41.

ROLE OF RABPHILIN3A IN HUMAN SPERM ACROSOMAL EXOCITOSIS

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Mammalian sperm contain a single secretory granule: the acrosome. The acrosome reaction (RA) is a type of regulated exocytosis that releases the acrosomal granule content in the vicinity of the egg during fertilization. Rabphilin is a secretory vesicle protein that interacts with the GTP-bound form of the small GTPases Rab3 and Rab27. Rabphilin consists of an N-terminal Rab binding domain, a linker region of unknown function, and a C-terminal tandem of C2 domains (C2A and C2B) that interact with the SNARE protein SNAP25 and membrane phospholipids. Both Rab3 and Rab27 exhibit crucial roles during the AR but their effectors have not yet been determined. Here, we report that Rabphilin3a is present in human sperm and localizes to the acrosomal region. By means of a functional assay using antibodies in combination with a photosensitive intravesicular calcium chelator, we found that Rabphilin3a participates in an early as well as a late stages during membrane fusion. When introduced into streptolysin O-permeabilized sperm, both wild type and a V61A point mutant (with impaired binding to Rab3) of Rabphilin3a abolished the AR triggered by calcium. We plan to use several other mutants to gather mechanistic information about the specific interactions of Rabphilin during the human sperm AR and demonstrate that Rabphilin3a is a relevant secretory Rabs effector1370.

CB-P42.

TL(I) AND TL(III) ALTER EGF-DEPENDENT EARLY SIGNALS REQUIRED FOR PC12 CELLS CYCLE ENTRY

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We previously demonstrated that Tl alters PC12 cell cycle and cyclins expression. EGF (10 ng/ml) addition to cells failed to prevent Tl-mediated alterations in cell cycle. In the current study, the effects of Tl(I) and Tl(III) (100 ì M) on the early (15-90 min) activation of EGFR/Ras/Raf/MEK/ERK and PI3K/Akt pathways were analyzed in the presence (EGF^+ cells) or absence (EGF^- cells) of added EGF. EGF^+ cells had maximal p-EGRF levels after 15 min of EGF addition. Tl(I) and Tl(III) did not modify p-EGFR kinetics in EGF^- and EGF^+ cells. At this time, pERK-1 was decreased and pERK-2 increased in EGF^+ cells respect to EGF^- cells. Tl increased pERK-1 levels in EGF^+ cells, effect that positively correlated with cyclin D1 levels after 24 h of cells exposure to Tl. Also, Tl decreased pERK-2 levels, which negatively correlated with cyclin D1 levels. In EGF^- cells, pAKT increased progressively and was not affected by Tl. On the contrary, in EGF^+ cells, Tl significantly increased pAKT levels at 15 min of incubation, which positively correlated with cyclin D1 accumulation at 24 h of Tl exposure. Together, experimental evidence suggest that Tl(I) and Tl(III) cause a misbalance in EGF-dependent cell signaling that ultimately leads to the alteration of cell cycle progression in PC12 cells.

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CB-P43.

CHARACTERIZATION OF A NOVEL REGULATOR OF HIF IN *DROSOPHILA*

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Hypoxia-inducible factors (HIFs) are a family of evolutionary conserved alpha-beta heterodimeric transcription factors that elicit the expression of a broad range of genes in response to low oxygen tension. Molecular mechanisms that mediate HIF regulation operate at the level of the alpha subunit, controlling protein stability, subcellular localization, and transcriptional coactivator recruitment. We show here for the first time that the *Drosophila* HIF-alpha homologue, Sima, is regulated at the translational level. Insertion of *sima* 3´UTR in a luciferase reporter gene leads to diminished luciferase activity, but not in reporter mRNA levels, in normoxia. Point mutations in the 3´UTR prevent this translational repression. Moreover, loss of function of a candidate regulator provokes an upregulation of Sima inducible reporters as well as Sima's target genes in cells and flies. Interestingly, these mutant larvae present melanotic masses (MM), a phenotype dependent on Sima and associated to abnormal hematopoiesis. Generation of MM might be related to the upregulation of Sima in the larval hematopoietic organ, the Lymph Gland, where Sima has a fundamental role in inducing differentiation of one type of hemocyte, the Crystal Cell. Further studies will reveal the physiological relevance of this regulation over Sima/HIF-alpha.

CB-P44.

STUDY AND CHARACTERIZATION OF THE AUTOPHAGIC RESPONSE TO HYPOXIA IN *Drosophila melanogaster*

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Autophagy is conserved intracellular degradative/recycling process in which double-membrane vesicles, autophagosomes, sequester organelles or portions of cytosol and fuse with lysosomes for its degradation. Autophagy can be induced in response to stressful conditions. In mammals and worms it has been described that autophagy plays an adaptative role in the response to limiting oxygen levels. *Drosophila melanogaster* can survive in low levels of oxygen for days, making it an ideal organism to study the cellular response to hypoxia. The role of autophagy in hypoxia has not been described in this organism. We wondered weather autophagy could be induced by low oxygen levels in *Drosophila*. We observed the formation of ATG8 positive foci, a hallmark of autophagy, in fat body cells of larvae grown at 4% oxygen for 12 hours. By analyzing different autophagy markers we can assure that these foci represent bonafide degradative autolysosomes. Our results link the induction of autophagy to the response to hypoxia in *D. melanogaster* for the first time. We are further characterizing this response biochemically and genetically. We will address weather autophagy is necessary for the adaptative response to hypoxia and weather the hypoxia pathway is involved in the induction of autophagy under this stressful conditions.

CB-P45.

OXIDOVANADIUM(IV) COMPLEXES WITH FLAVONOIDS: ACTIVATION OF NFKB AND APOPTOSIS IN HT-29 CELL LINE

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Polyphenolic flavonoids present anti-inflammatory and anti-tumor effects. In particular, Chrysin and Silibinin exhibit anti-proliferative and anti-metastatic effects against various malignant cell lines. In this work we report the biological effects of two complexes of vanadyl(IV) with Chrysin and Silibinin on a human colon adenocarcinoma cell line (HT-29 ATCC HTB 38). VOsil caused an inhibitory effect on cell viability in the range of 75-100 μM while VOchrys only provoked this effect at 100 μM ($p<0.01$). Moreover, both complexes increased the level of Reactive Oxigen Species 250% over basal and decreased the GSH/GSSG ratio in the range of 25-100 μM. ($p<0.01$). Besides, VOsil increased the level of early apoptotic cells (annexin V+/ PI -) at 6 and 24 h and activated the caspase -3. Then, this complex sent the cells to apoptosis (evaluated by the Sub G1 peak). On the contrary, VOchrys only caused cell cycle arrest in G2 phase at 24 and 48 h at 100 μM ($p<0.01$). VOsil also caused a diminished activation of NFκB vía (inducing with TNF), while VOchrys did not show any effect. This result is related to the pro-apoptotic effects showed by VOsil.

These results presented herein demonstrated that VOsil has a stronger deleterious action than VOchrys on the HT-29 cells, whereby suggesting that VOsil is the best potentially good candidate for future use in alternative anti-tumor treatments.

SB-P01.

CRYSTAL STRUCTURE OF THE HISTIDINE KINASE DOMAIN FROM LOV-HK FROM THE BACTERIUM BRUCELLA

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The release of bacterial genomic sequences has greatly expanded the number of known two-component system histidine kinases (HK). A particular group, called the HWE family present in α- and γ-proteobacteria was identified bioinformatically. A member of this group, LOV-HK from the bacterium Brucella, has been shown to mediate the light-induced increase of the virulence of this pathogen. As part of our project, we solved the three-dimensional structure of the histidine kinase domain from LOV-HK by X-ray crystallography. This is the first structure of a histidine kinase from the HWE family. Like other HKs, it presents a Dimerization and Histidine phosphotransfer subdomain (DHp) and a Catalytic and ATP-binding subdomain (CA). The structure shows two different dimer conformations. Both dimers differ in the parallel/antiparallel assembly of the monomers, the regions involved in the dimerization interface and the relative orientation between the DHp and CA subdomains. According to the interface area and the nature of their contacts, both dimers might exist in solution. SLS experiments indicate the HK domain is a monomer in solution. These observations suggest that the HK domain alternates between the two dimeric conformations, which dissociate during gel filtration in SLS experiments. The quaternary structures and their biological relevance will be the next focus of our efforts.

SB-P02.

THE LOV DOMAIN FROM BRUCELLA LOV-HISTIDINE KINASE: THE ROLE OF THE FLANKING REGIONS

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Light-oxygen-voltage (LOV) domains are blue-light signaling modules. They bind the cofactor FMN, which confers sensing function. Light modulates the virulence of the bacterium Brucella through a LOV-histidine kinase. The Brucella LOV domain adopts the alpha/beta PAS domain fold, consisting of a beta-sheet and helical connector elements. Our results point to the beta-scaffold as a key element in the light activation. This beta-sheet, on one side, interacts with the FMN molecule and on the other side communicates with N- or C-terminal helical regions flanking the LOV core or directly with effector domains. According to secondary structure predictions, Brucella LOV-HK harbors a C-terminal helix (J-helix) contiguous to the LOV core, which is estimated to be 37 residues long and a 25 residue-long N-terminal helix (N-helix), with no sequence similarity to other known LOV proteins. Solution experiments show that the N-helix is essential for the formation of a stable dimer. To explore the functional importance of the J-helix, we performed NMR studies with a construct consisting of the LOV core and the 37 C-terminal residues. The J-helix is 17 residue-long, flexible and exposed to the solvent. Val135, located at the N-terminus of the J-helix, changes its chemical environment upon illumination suggesting a pivotal role. These results are discussed in the context of the full-length protein.

PL-P01.

CONTRASTING TOBAMOVIRUS CAUSE GLOBAL CHANGES IN ENDOGENOUS sRNAs AT EARLY STAGES OF INFECTION

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Plant viral infections induce profound changes in plant metabolism and gene expression. Small RNAs (sRNAs) (transcripts regulators) may play an important role on those changes. Several classes of sRNAs have been described; two major categories are miRNAs and hc siRNAs.
We sequenced sRNAs population from Arabidopsis plants infected with two contrasting Tobamovirus (ORMV (severe) and TMV-Cg (mild)) using NGS. Seedlings were mechanically inoculated and systemic leaves were sampled at two and four days post inoculation (dpi), without and with viral presence, respectively.
Viral infection reveals a significant impact on several classes of sRNAs. miRNA and siRNAs derived from transposons, promoters and mRNAs were altered, even at 2dpi without virus accumulation at sampled tissue. More interestingly the same sRNAs were also differentially accumulated between viruses.
Several sRNAs target genes were selected based on their differential sRNA counts between treatments and also for their function like RNA regulation, protein degradation and biotic stress. These genes were subjected to expression levels measurement by qPCR. Some of them displayed differences between treatments, even those whose associated siRNAs mapped on their intronics regions. The origin of this sRNA remains unclear, but it seems to have a role on the regulation of gene expression related to virus disease production.

PL-P02.

SIMULTANEOUS EFFECT OF WATER AND SALINITY STRESS ON SOYBEAN PLANTS

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Water stress as well as salinity is the main factor that impairs soybean growth in our country. On one hand water deficiency is the main reason that affects crop growth and on the other hand in some areas salinity is the most important edafic factor that alters soybean growth.
Plants were grown in 1 kg pots containing three parts of Argiudol and two parts of sand. Different watering conditions were employed: concentration 50 and 80% AU field capacity, in the presence of different NaCl ranging from 0 to 4 dS/M.
Physical and chemical properties were measured in these soil samples. Considering the fenologic state R2, plant height showed no difference respect to controls. Plants treated with 80% AU field capacity and 0 dS/M salinity completed their growth cycle, but plant height diminished 35% respect to controls.
Oxidative damage was determined in soybean leaves assessing lipid peroxidation. All treatments carried out in the presence of 4 dS/M yielded up to 6-fold increment of this parameter respect to controls. Catalase as well as superoxide dismutase and guayacol peroxidase activities were also determined. Results here reported indicate that salinity is more deleterious than drought. Plants treated with both stresses did not show any synergic effect, indicating that salinity stress predominates.

PL-P03.

HO-1 GENE EXPRESION IS NOT EPIGENETICALLY RESTRICTED AND INVOLVES STRESS-RELATED TRANSCRIPTION FACTOR

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Hemo oxygenase-1 (HO-1) plays a protective role against oxidative stress in plants. The mechanisms regulating its expression, however, remain unclear. Here we studied the methylation state of a GC rich HO-1 promoter region and the expression of several environmental stress associated-transcription factors (TFs) in soybean plants subjected to ultraviolet-B (UV-B) radiation. Genomic DNA and total RNA were isolated from leaves of plants irradiated with 7.5 and 15 kJ.m-2 UV-B. A 304 bp HO-1 promoter region was amplified by PCR from sodium bisulfite-treated DNA, cloned into pGEM-T plasmid vector and evaluated by DNA sequencing. Bisulfite sequencing analysis showed similar HO-1 promoter methylation levels in control and UV-B-treated plants (C: 3.0±0.6%; 7.5: 3.14±0.5%; 15: 2.9±0.5%). Interestingly, HO-1 promoter was strongly unmethylated in control plants. Quantitative RT-PCR of TFs showed that GmMYB177, GmMYBJ6 and GmWRKY21 but not GmWRK13 were induced by UV-B irradiation, inferring that the former group mediates HO-1 up-regulation. These results indicate that HO-1 gene expression is not epigenetically regulated. Moreover, the low level of HO-1 promoter methylation suggests that this antioxidant enzyme can rapidly respond to environmental stress. Finally, this study has identified some stress-related TFs involved in HO-1 up-regulation under UV-B radiation.

PL-P04.

STRUCTURAL CHARACTERIZATION OF THE THREE ATXYN1 CBMS FROM *Arabidopsis thaliana*

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The *Arabidopsis thaliana* genome codifies for the xylanase (AtXyn1), which is predominantly expressed in vascular bundles, but not in vessel cells. Like many polysaccharide-degrading enzymes, AtXyn1 displays a modular structure in which the catalytic domain (C-terminal) is attached to three in tandem non-catalytic modules. Those modules where classified in the family 22 (previously family 4_9) of carbohydrate binding modules (CBM) (http://www.cazy.org/CBM22.html), according to sequence similarity. In order to determine if those domains were classified correctly, we performed a sequence and structural analysis, leading to an acceptable tridimensional model of AtXyn1 consisting in a β-sandwich (classic β -jelly roll) with a shallow surface groove that forms the ligand-binding site. Those models were conducted employing a "threading" method using the two CBMs form the Clostridium thermocellum (family 22) xylanase (XylY) (UniProt=XYNY_CLOTM) as template (showing a 17% of identity with the module Xyl2 from AtXyn1). Then, we cloned the three CMBs in the pET28 vector and the protein was expressed, purified and the physicochemical and structural parameters in the absence or presence of several ligands were determined. We conclude that the CMBs of AtXyn1 are correctly classified since its folding and affinity to xylane are consistent with the rest of the members of the CAZy family 22.

PL-P05.

SUBCELLULAR LOCALIZATION OF ASPARTIC PEPTIDASES FROM MILK THISTLE BY TRANSIENT EXPRESSION IN NICOTIANA

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Typical plant aspartic peptidases (APs) (E.C.3.4.23) from thistle flowers are expressed as zymogens. APs are not randomly distributed within plants. Moreover, in each part of the cell, proteolytic activity is performed by a separate protease specifically targeted to particular subcellular compartments. This cellular anatomical specificity of plant APs has suggested a huge number and wide diversity of these enzymes. The aim of this work was to establish an eukaryotic expression system for the study of silpepsin 1 and 2, two APs from flowers of *Silybum marianum* (milk thistle) and to analyse its subcellular localization by confocal laser scanning microscopy. Transient expression in Nicotiana benthamiana leaves was accomplished by Agrobacterium- mediated DNA transfer. Both preprosilpepsins were cloned using GATEWAY BP reaction in pDORN222 and, subsequently subcloned in the binary vector pB7FWG2 by LR recombination reaction. The coding sequences were cloned in-frame with fusion region coding for Egfp under the control of the CAMV 35S promoter. Recombinant clones were selected with spectinomycin. Positive clones were confirmed by PCR. Four-week-old plants grown at 25°C in long-day condition were agroinfiltrated. Results obtained would indicate that silpesin 1 and 2 direct GFP extracellularly in accordance with the expected localization in the secretory pathway.

PL-P06.

EXPRESSION OF THE PLANT DNA GLYCOSYLASE-HOMOLOGUE *ACDI* ON DEVELOPMENT AND STRESS

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DNA glycosylases remove modified bases from DNA participating in the Base Excision Repair (BER) pathway. We are studying the still uncharacterized Arabidopsis gene encoding the DNA glycosylase-homologue *ACDI*. We found that, by alternative splicing, *ADCI* may generate two proteins that conserve the glycosylase domain but differ in their nuclear localization motives. Using *ACDI-GFP* transgenic plants we observed one of these proteins located at the nucleus. Studying *ACDI:GUS* plants we monitored the expression of the gene in different plant tissues and developmental stages, as well as under stress conditions. We observed that *ACDI* expression is highly regulated during plant development and is particularly induced on water-devoid tissues. This pattern was consistent with the presence of elements sensitive to dehydration on the *ACDI* promoter region. Interestingly, *ACDI* over-expressing plants are tolerant to salinity and oxidative stress suggesting this glycosylase-homologue modulates plant stress responses. Putative effects of *ACDI* under these conditions will be discussed.

PL-P07.

CLPS: A NOVEL SUBSTRATE SELECTOR OF THE CLP SYSTEM IN *Arabidopsis thaliana*

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Proteolysis is an essential mechanism in protein quality control. In chloroplasts, this function is accomplished by the ClpPR complex, which acts as a protein-degradation machine. The chaperones ClpC1/2 and ClpD select and unfold substrate proteins and translocate them to ClpPR. However, target selection appears to be aided by adaptor proteins that bind to the chaperones. For example, ClpS is proposed to modulate ClpC1/2 substrate selection and affinity, yet its function is unknown. Interestingly, ClpS from *Escherichia coli* is the discriminator of the N-end rule pathway. The rule states that the half-life of a protein is determined by the nature of its amino-terminal residue. *E. coli* ClpS recognizes N-end rule substrates and presents them to the ClpAP protease. Based on these observations from bacteria and the fact that ClpS is present in plants, the existence of the N-end rule pathway in chloroplast is possible. Using recombinant ClpS from *Arabidopsis thaliana*, we found by gel filtration chromatography that it can form homo-oligomers of 2, 3, 4 and 6 subunits. ClpS was found to interact with ClpC2 and ClpD, which is in line with its proposed biological function. Importantly, ClpS was able to bind substrates recognized by *E. coli* ClpS. Our results suggest that ClpS plays a role in substrate selection for the Clp system in plants, maybe following the rules of the N-end pathway.

PL-P08.

FUNCTIONAL CHARACTERIZATION OF THE HD-Zip TRANSCRIPTION FACTOR AtHB13

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HD-Zip proteins are transcription factors unique to plants, exhibiting a homeodomain (HD) associated to a Leucine zipper (Zip) motif. It has been reported that the overexpression of AtHB13, a member of HD-Zip I subfamily, confers tolerance to freezing temperatures, severe drought and salinity stress. However the natural role of this gene is largely unknown.

Aiming to functional characterize this gene, T-DNA insertion mutant plants (*mut13*) were obtained and characterized. These (*mut13*) plants do not present a distinct morphological phenotype during the vegetative phase, but after bolting, longer inflorescence stems, and smaller siliques with a high percentage of unfertilized ovules than WT controls were observed.

On the other hand, the promoter region of this gene was isolated and plants transformed with a construct in which this promoter directs the expression of the *GUS* reporter gene. Histochemical analyses of these plants indicated that the reporter gene is expressed mainly in the base of the inflorescence stem.

Altogether, these observations suggest that *AtHB13* participates in the regulation of the inflorescence stem development, and although no GUS expression was detected in flowers, AtHB13 seems to be essential for ovule fertilization and silique maturation.

PL-P09.

IMPLICATIONS OF SOYBEAN METALLOTHIONEINS IN ABIOTIC STRESS TOLERANCE

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Metallothioneins (MTs) are a ubiquitous family of cysteine rich proteins, greatly responsive to a wide range of stress factors. Their main functions are related to heavy metal detoxification and/or general oxidative stress defence. Animal and fungus MTs are highly characterized; by contrast, data on plant metallothioneins are limited, even though many MT isoforms are encoded in their genomes. Considering metallothioneins potential in abiotic stress tolerance/ defence in plants of agronomic interest, two soybean MT isoforms were selected for further studies: GmMT1 and GmMT3 -most highly expressed isoforms in roots and shoots, respectively. Recombinant soybean MTs, purified from *E. coli* cells cultured in different metal enriched media, are able to bind Cu, Cd and Zn with high stoichiometries. When these MTs are heterologously expressed in MT-null *S. cerevisiae* cells, both proteins restore Cu tolerance and improve resistance to oxidative stress, but GmMT3 is best for coping with Cd stress. Moreover, *A. thaliana* plants expressing soybean MTs under the CaMV35S promoter are able to grow better than wt plants in media with high Cu, Cd, Zn or with hydrogen peroxide. Altogether, our results confirm the promising potential of metallothioneins to palliate different environmental abiotic stresses in plants.

PL-P10.

MORPHOLOGICAL AND BIOCHEMICAL CHANGES UNDER SALINE STRESS IN BARLEY ROOTS (*Hordeum vulgare*)

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Salt stress is one of the abiotic factors that limit normal plants development. Under these conditions the plants undergo morphological and biochemical changes. In response to saline stress the plants accumulate compatible solutes and lower the toxic concentration of ions in the cytoplasm by restriction of Na+ influx or its sequestration into the vacuole as a protective mechanism from the osmotic effect. The aim of this study was to evaluate the effect of Na+ in the morphological structure and microtubule organization in roots of barley (germinated 4 days under salt stress). Root osmotic potential of salt-stressed plants was -1.4 MPa compared to -0.6 MPa in controls and its intracellular Na+ concentration increased 500%. Image obtained by scan electron microscopy showed several damage in superficial tissue. Although this was not an anatomical change, an increase in the cellular volume was observed. Image confocal microscopy showed a differential organization of microtubules while by western blots assay a decrease of 50% in the α-tubulin level was observed. Results indicates that the saline stress evoked dynamic changes in the microtubules organization of barley roots.

PL-P11.

PROTEOMIC ANALYSIS OF PLANTS OVEREXPRESSING *AtERF019* TRANSCRIPTION FACTOR UNDER DROUGHT STRESS

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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) were more tolerant to water stress conditions, showing improved development, a healthier appearance and higher survival rates than wild type plants. Furthermore, ERF019 transgenic plants exhibited a marked delay in senescence. To uncover the molecular mechanisms that could be involved in regulating the drought tolerance in ERF019 plants, we carried out a proteomic analysis using Col-0 and transgenic plants subjected to a controlled water deficit treatment. We found out five proteins that were present only in ERF019-7 control sample (ATPase subunit, Reticulon-like protein, Branched-chain-amino-acid aminotransferase, ABC transporter and zinc finger transcription factor) and one protein unique to ERF019-7 drought sample (ADP-ribose pyrophosphatase). Some of these proteins are known to function against stress, and could be responsible for the stress tolerance found in ERF019 lines. Results suggest that AtERF019 may be responsible for the drought stress tolerance by increasing the expression of stress protective proteins that together produce physiological adaptations that enable the plant to stand water deprivation.

PL-P12.

DEREGULATION OF HYDROGEN PEROXIDE LEVELS BY A CCT DOMAIN-CONTAINING PROTEIN IN ARABIDOPSIS

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CCT domain-containing proteins are found in a wide range of flowering plants but not in mammals or yeast. They 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. Here we report the identification of a gene encoding a CCT domain-containing protein (UKCCT) which is downregulated upon treatment of Arabidopsis seedlings with the herbicide Methyl Viologen in light. In this work we analyzed sub cellular and tissue specific expression using transgenic plants. Arabidopsis plants expressing the UKCCT-GFP fusion protein showed nuclear localization indicating that UKCCT function might be related to regulatory processes. Using promoter-GUS reporter fusions we observed high expression of UKCCT in shoots, especially in cotyledons and leaves but not in stem and roots. Interestingly, Arabidopsis plants over-expressing UKCCT show a deregulation of H_2O_2 levels, chlorotic and hyponastic leaves resembling the phenotype observed in cat_2 mutants. On the other side, *ukcct* mutant lines showed a better photosynthesis performance and higher seed set. Our working hypothesis is that the function of this protein is related to the regulation of hydrogen peroxide levels and to senescence onset.

PL-P13.

DILUCIDATING THE ROLE OF ATUK80 IN *Arabidopsis thaliana*

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The functional characterization of proteins with unknown function is one of the main challenges in modern biology. AtUK80 was identified in an activation tagging experiment performed in order to find novel oxidative stress related genes. Homologues of this gene are present in other members of the plant kingdom and in a photosynthetic member of the protist kingdom. Analysis of silenced and over expressing Arabidopsis lines revealed that this protein might be involved in the regulation of vesicular trafficking in Arabidopsis. Additionally, AtUK80 co-immunoprecipitate with the multifaceted glycolitic enzyme glyceraldhyde 3P dehydrogenase. Taking into account that this enzyme is a key component of H_2O_2 signaling cascades in plants through activation of Phospholipase Dδ we postulate that AtUK80 might be involved in the H_2O_2 signaling pathway by modulating vesicular trafficking events.

PL-P14.

CLONING OF TWO NEW CDPK ISOFORMS FROM *Solanum tuberosum*

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Calcium-dependent protein kinases (CDPKs) comprise a multigene family of calcium sensors that play important roles regulating plant growth and development and plant responses to environmental stresses. To the moment six isoforms have been characterized in potato plants. *In silico* analysis of *Solanum phureja* genome allowed us to identify other 20 candidate genes encoding potential CDPK isoforms that cluster into four subgroups. Expression of 13 of these isoforms was detected in leaves and stolons from *Solanum tuberosum* cv Desirée plants. Using primers directed against their 5´and 3´utr sequences we amplified isoforms StCDPK25 and StCDPK17 belonging to subgroup 1 and 3 respectively. These fragments were cloned and sequenced. Isoform StCDPK25 shares 99% identity with the *S. phureja* coding sequence and presented all the characteristics of a CDPK. Cloning of StCDPK25 coding sequence in pDEST expression vector in order to obtain the recombinant enzyme tagged to 6xHis is currently in progress. Analysis of its sequence indicated that it could be targeted to chloroplasts; the N-terminal sequence will be fused to GFP and transient expression assays will be performed in *Nicotiana benthamiana.* On the contrary, only a 1 kb fragment containing exons 1, 2 and 7 was amplified for StCDPK17 suggesting that alternative splicing has occurred.

This work was financed by CONICET and UBACYT.

PL-P15.

mRNA OF StCDPK1 IS EXPRESSED IN ROOTS AND VASCULAR SYSTEM AND TARGETED BY micro RNAs

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Plant calcium-dependent protein kinases (CDPKs) are calcium sensors that play important roles regulating plant growth and development and responses to biotic and abiotic stresses. These enzymes are subjected to complex regulation at both transcriptional and post-transcriptional levels. StCDPK1 is expressed during stolon to tuber transition in potato plants. *In silico* analysis of StCDPK1 promoter sequence predicted a wide variety of cis-acting regulatory elements among which a number of defense and stress responsive elements are evident. Here, we show the expression profile of StCDPK1 revealed by potato transgenic lines harboring β-glucuronidase (GUS) driven by StCDPK1 promoter. GUS expression was detected by both histochemical staining and fluorometry. Promoter activity was significant in roots, but also in stems, leaf veins and branching points. We have further confirmed high promoter activity in roots in stable transgenic lines harboring green fluorescent protein (GFP) under control of StCDPK1 promoter. In addition, our preliminary *in silico* analysis revealed two miRNAs (miRNA390 and mRNA414a) that could potentially target StCDPK1. RT-PCR assays validated the presence of these miRNAs in whole plant and stolons. Isolation of precursors and cloning of these miRNAs are in progress.

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BT-P01.

BIOACTIVE PEPTIDES DERIVED FROM WHEY PROTEINS WITH ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITY

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Food proteins encrypt bioactive peptides that can be released during gastrointestinal digestion or food processing by enzymatic proteolysis. Flowers of Carduae tribe, family Asteraceae, contain aspartic proteinases. The aims of the present work were to isolate proteolytic enzymes from flowers of *Arctium minus* (Hill) Bernh. (Asteraceae), to obtain protein hydrolyzates from bovine whey with angiotensin-converting enzyme (ACE) inhibitory activity employing the peptidases present in enzyme extracts (EE), and to isolate bioactive peptides responsible for the ACE inhibitory activity. Crude enzyme extracts were prepared by pestle pounding of A. minus flowers at pH 7.0. Pigments and other phenolic compounds were eliminated by size-exclusion chromatography. EE were obtained in the void volume. Whey bovine hydrolyzates were performed with EE and analyzed by SDS-PAGE and MALDI-TOF/MS. Hydrolyzed whey was ultrafiltered, and low molecular fraction (peptide mass = 3000 Da) showed ACE inhibitory activity. Peptides obtained by hydrolysis were analyzed by RP-HPLC (ÄKTA Purifier). From twelve peaks resulting from chromatograms, 22.4% of total ACE inhibitory activity was recovered in the peak with retention time: 27.26 min. Hydrolyzates and isolated peptides with ACE inhibitory activity could be potentially used in food industry for formulation of nutraceutical products.

BT-P02.

EXPRESSION OF A FUSION PROTEIN, Z (JUNIN VIRUS)-N (MEASLES), FOR THE GENERATION OF RECOMBINANT VLPS

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In the last decades, virus like particles (VLPs) have been widely studied for the development of new vaccines, diagnostic kits and nanocarriers. Due to their importance, researchers have focused their study on new proteins, able to induce VLPs formation. It has been previously described that VLPs can be formed when a fusion protein Z (from Junín virus)-GFP is expressed in 293T cells.

This work is mainly focused on the study of Junín virus Z protein as a tool to generate quimeric VLPs carrying heterologous viral antigens. In line with this idea a C-terminal fragment of the measles' nucleoprotein (NCT) was cloned in frame with both Z and GFP. The plasmid containing the fusion of the three ORFs (pZ-NCT-GFP) was transfected into Cos-7 cells. Expression was studied through flow cytometry and fluorescence microscopy. Moreover, Western blot analysis was used to comfirm protein identity. Transfection was optimized, and a maximum of 65% efficiency was obtained. The fusion protein was found in these cell lysates as it was detected by anti-Z and anti-GFP antibodies. Furthermore, Z-NCT-GFP was also detected in a clarified transfected cells' supernatant, after concentration by ultracentrifugation through a sucrose cushion. In conclusion, these results suggest that Z-protein induced VLPs can be used as an antigen carrier system.

BT-P03.

THE SAME SIGNAL PEPTIDE IS NOT ALWAYS EFFICIENT FOR DIFFERENT TYPES OF MAMMALIAN CELL LINES

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Most of the glycoprotein-based biotherapeutics are produced in mammalian cell lines because post-translational modifications should be identical or, at least, similar to those obtained in humans. Although CHO cells are the most prevalent for producing them, human embryonic kidney cells (HEK), and mouse myeloma (NS0) are being used as highproducing cell lines. On the other hand, signal peptides (SPs), capable of efficiently directing protein secretion in mammals, are key elements in recombinant protein production. In order to study the efficacy of three SPs to direct the secretion of a scFv-Fc fusion protein; CHO-K1, HEK293 and NS0 cell lines, producing the quimeric protein, were generated. In this way, the SPs derived from human albumin, human azurocidin and Cricetulus griseus Ig kappa chain V-III region MOPC 63 like were evaluated. The secretion efficiency of the fusion protein was analyzed by indirect specific ELISA from culture supernatant of each cell line. Although the best results were obtained with the human azurocidin derived SP for CHO-K1 and HEK293 cells, the scFv-Fc protein was not detected in the NS0 culture supernatant using the same SP. Nevertheless, the scFv-Fc protein was expressed by NS0 cells using the other two sequences. In conclusion, the human azurocidin signal peptide, being the best option for CHO-K1 and HEK293 cells, could not extrapolated to NS0 cells.

BT-P04.

CHEMICALLY-ASSISTED FRAGMENTATION AS A TOOL FOR IDENTIFYING BOTHROPS ALTERNATUS VENOM COMPONENTS

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Characterization of the peptide/protein content of snake venoms has a number of benefits for basic research, clinical diagnosis, development of new research tools and drugs of potential clinical use, and for antivenom production strategies. Within – and between – species heterogeneity of venoms may also account for differences in the clinical symptoms observed in accidental envenomation. Transcriptomic analyses were carried out in order to explore putative venom components. In this work a proteomic study was made to identify Bothrops alternatus venom components by using SDS-PAGE and chemically-assisted fragmentation (CAF) with 4-sulfophenyl isothiocyanate before MALDI TOF/TOF mass spectrometry and *de novo* sequencing, combined with database searching. CAF improves fragmentation efficiency and simplifies the interpretation of acquired spectra.

Bothrops is a genus of venomous vipers widely distributed in our country and venoms of snakes from different regions were compared. Several proteins already described in venoms from other species of this genus were identified. Moreover, we were able to find individual differences between venoms from different members of the B. alternatus species. Particularly, a 23 kDa protein that was identified as a glycoprotein Ib-binding in some individual venoms is completely absent in others.

BT-P05.

DEVELOPMENT OF NEW HEK-293 CELL LINES FOR THERAPEUTIC PROTEIN PRODUCTION

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Recombinant protein production in animal cells shows numerous advantages, such as folding and post-translational modifications (phosphorylation and glycosylation). However, the main limitation resides in the difficulty to produce them in high levels. To solve this drawback, in the present study, we aimed to generate HEK-293 cell lines over-expressing a gene expression key protein designated here as "P" (for confidential purposes). This protein is involved in different mRNA metabolism steps, such as stability, transport and translation. These new cell lines were tested for recombinant gene expression. Firstly, EGFP (enhanced green fluorescence protein) expression analysis revealed that the HEK-P cell line produced a 2-fold increase in EGFP levels compared with wild type cells. Then, by means of a cell cloning strategy, individual clones from the HEK-P cell line that showed a 4-fold increase in the fluorescence levels were identified. Interestingly, these clones have shown similar behavior when two therapeutic protein productions (rhEPO and rhIFN-á) were evaluated.
Additionally, other parameters were considered, including growth characteristics, morphology, glucose consumption and lactate and ammonium production. Taken together, these results represent a significant advance in the development of optimized cell lines widely used in the pharmaceutical industry.

BT-P06.

STUDY AND CHARACTERIZATION OF VLPS EXPRESSING RABIES STRUCTURAL PROTEINS IN MAMMALIAN CELLS

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The structural proteins of many viruses have the ability to auto assemble in empty structures, known as Virus-Like Particles (VLPs). VLPs have been widely used in vaccine development. The goal of this work is to evaluate the assembly of rabies VLPs expressing the structural proteins of the virus in HEK293 cells. We evaluated the expression of the RV glycoprotein (G) that is the target membrane protein of neutralizing antibodies. We also expressed the RV matrix protein (M) together with G protein to evaluate whether the presence of this protein improves particle budding.
We generated two recombinant HEK293 stable cell lines that express RV proteins (HEK-G and HEK G/M cell lines). The expression of G was confirmed by flow cytometry, western blot and immunofluorescence microscopy. As there is no available antibody to M protein, we used RT-PCR to detect the expression of this protein in the HEK-G/M cell line. Supernatant of the producer cell lines where concentrated and purified by ultracentrifugation. By Dynamic Light Scattering (DLS) we calculate the hydrodynamic diameter of the particles, obtaining average values of 64 nm and 60 nm for VLPs produced by HEK-G and HEK-G/M cell lines, respectively. Particle morphology was analysed by Transmission Electron Microscopy. These results encourage the study of these VLPs as possible biosafe rabies vaccine candidates.

BT-P07.

CHARACTERIZATION OF COMPOUNDS THAT INHIBIT HUMAN IFN-α ACTIVITY USING A NEW REPORTER GENE ASSAY

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Several pathologies have shown an excessive production of endogenous IFN with undesirable symptoms. For that, it is interesting to find molecules which can inhibit their activity. Following this objective, the WISH-Mx2/eGFP human reporter IFN activity cell line was obtained in our lab and employed to analyze 88 synthetic compounds from the chemical library of the Medicinal Chemistry Group. It has the Mx2/eGFP construction, where the sequence of eGFP is under the control of Mx2 promoter, specifically induced by type I human IFNs. Thus, the eGFP expression is directly correlated with the hIFN-I concentration in the sample. Using the reporter cell line, the eGFP expression in the presence and absence of each compound was compared aiming to find molecules that decrease the eGFP production as a consequence of IFNs-I activity inhibition. As result, 10 compounds were identified and characterized by studying their toxicity, effective dose, their effect on antiviral and antiproliferative IFNs-I activity, their action on cell cycle and the analysis of their apoptotic potential. Finally, 5 of them were selected to analyze their combined effect on the IFN biologic activity by employing the reporter gene assay. An interesting cooperative effect to decrease the IFN activity was observed. Besides, this effect was corroborated by the standard methods routinely used to study the IFN´s potency

EN-P01.
STARCH-BINDING DOMAINS CONSERVATION IN STARCH SYNTHASE III ISOFORMS
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Starch-binding domains (SBDs) are key modules present in numerous polysaccharide metabolism enzymes. These modules are essential for starch-binding and catalytic activity of starch synthase III from *A. thaliana*. In Ostreococcus tauri, a unicellular green alga, there are three SSIII isoforms, known as Ostta SSIII-A, SSIII-B and SSIII-C. In silico studies show that these three isoforms contain two, three and no N-terminal SBDs, respectively. In addition, phylogenetic analysis has indicated that OsttaSSIII-A N-terminal fragment is closely related to SSIII N-terminal sequences from other green microalgae. Besides, we observed variable SBDs sizes and numbers in green algae SSIII enzymes. Furthermore, sequence alignment and homology modeling data showed that 3-D structures obtained and the amino acid residues implicated in starch binding are well conserved in OsttaSSIII SBD, except in OsttaSSIII-B D1, which might possibly undergo a deletion in the n N-terminal region. Preliminary results from affinity gel electrophoresis assays suggest that OsttaSSIII SBDs displayed some promiscuity in the binding to different polysaccharide substrates. These results not only discloses significant information concerning evolutionary and structure–function aspects of SBD domains, but are also crucial to better understand the metabolism of starch in algae.

EN-P02.
FERREDOXIN-NADP$^+$ REDUCTASE FROM *Escherichia coli* CONTAINS TIGHTLY BOUND NADP$^+$
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The Ferredoxin-NADP$^+$ reductases (FNRs) are flavoenzymes that catalyze the reversible electron transfer between NADP(H) and ferredoxin or flavodoxin. *Escherichia coli* contains a bacterial-type FNR (*Ec*FPR) involved in different biosynthetic pathways where reactions favor the reduction of ferredoxin or flavodoxin from NADPH in order to supply redox equivalents to diverse metabolisms. The bacterial-type FNRs are less active enzymes than the plastidic-type reductases. The difference found in the catalytic efficiency is influenced by some structural features such as the conformation of the prosthetic group, the mobility and the structure of the C-terminal region, among others. The NADP$^+$ binding mode may also condition the enzyme activity. We determined that purified *Ec*FPR contains one mol of bound NADP$^+$ per mol of enzyme. *Ec*FPR free of NADP$^+$ was obtained and then, the effect of the NADP$^+$ binding on the enzyme activity analyzed. We observed a marked decrease in the K_M value for the NADPH for *Ec*FPR free of NADP$^+$ suggesting an inhibition of the enzyme by product. *Ec*FPR plays a role in NADPH/NADP$^+$ homeostasis. Thus, this phenomenon could be related to a regulation mechanism of physiological relevance. Additionally, we studied the effect of different NADP$^+$ analogues on the *Ec*FPR diaphorase activity. We observed enzyme activation in the presence of these compounds.

EN-P03.
LEPFNR IS THE REDUCTANT FOR HEME DEGRADATION BY *Leptospira interrogans* HEME OXYGENASE
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Like most pathogens, *Leptospira interrogans* requires an iron source for bacterial metabolism and growth during infection. Within mammalian hosts, free iron is negligible because it is mostly bound to high affinity proteins. However, *L. interrogans* has evolved to acquire iron from host hemoglobin. A crucial protein involved in this process is the heme oxygenase (LepHO), which cleavages the porphyrin of heme and release iron, biliverdin and carbon monoxide. Heme breakdown by bacterial heme oxygenases requires molecular oxygen and an electron source derived from NADPH supplied by ferredoxin-NADP$^+$ reductase (FNR) and ferredoxin (Fd). The observed Fd dependence led us to test FNR and Fd as potential reducing partners of LepHO. Thereby, we studied the LepHO cleavage of heme macrocycle by optical absorption spectroscopy. We established that the plastidic-type ferredoxin-NADP$^+$ reductase from *L. interrogans* (LepFNR), acts as the physiological electron donor without the participation of a ferredoxin. We characterized the interaction between LepHO and LepFNR determining the dissociation constant of LepHO-LepFNR complex. We found that in the presence of NADPH, LepFNR and ferrozine, LepHO releases ferrous iron. Our results indicate that LepFNR and LepHO are involved in the heme-iron utilization pathway, through which *L. interrogans* can successfully acquire iron from host for its own survival.

LI-P01.

INHIBITORY EFFECT OF n-3 POLYUNSATURATED FATTY ACIDS ON CANCER CELL GROWTH BY p53 ACTIVATION

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Growing evidence show that n-3 and n-6 polyunsaturated fatty acids (PUFAs) have regulatory effects on carcinogenesis, however the mechanisms involved in this process remain unknown. In this report, we analyzed the *in vitro* and *in vivo* effects of n-3 and n-6 PUFAs on a syngeneic murine mammary adenocarcinoma cell line (LMM3). Tumor cells viability was measured by Resazurin and apoptosis by Hoechst. The PUFAs tumor cell membrane profile was analyzed by gas chromatography and PUFAs derivatives (eicosanoids) by HPLC. Our results showed that alpha linolenic acid (ALA, C 18:3 n-3) induced significantly higher pro-apoptotic effects than linoleic acid (LA, C18: 2, n-6). Moreover, ex-vivo studies showed that tumor cells from mice fed with ALA enriched diet diminished tumorigenesis by decreasing the tumor cell release of 12 (S)-HETE, a pro-carcinogenic eicosanoid and producing higher levels of pro-apoptotic 12(S)-HHT. In addition, the ALA enriched diet resulted on a higher tumor tissue expression of the pro-apoptotic tp53 tumor suppressor factor. These results provide evidence of the biological pro-apoptotic effects of n-3 PUFAs and particularly, the fatty acid ALA, as the regulator of the signaling pathway tp53. This provided a novel mechanism and a potential target on anticancer treatments.

LI-P02.

DOWNREGULATION OF GLI1-DEPENDENT SURVIVAL PATHWAY UNDERLIES ARACHIDONIC ACID ANTI-TUMORAL ACTIVITY

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Numerous studies have demonstrated a role for essential fatty acids (ePUFAs) during tumor development. However, the molecular mechanism underlying this phenomenon remains elusive. Here, we defined a novel molecular mechanism explaining the ePUFA arachidonic acid (AA) anti-tumoral activity. We used *in vivo* and *in vitro* assays to determine the effect of AA in primary tumor volume, lung micro-metastasis and apoptosis (TUNEL and Caspase 3/7 activation) as well as gene expression (qRT-PCR and WB) and transcriptional activity (Luciferase and ChIP assays). We observed a significant reduction in tumor volume and micro-metastasis incidence in AA-injected animals compared to the control group. Moreover, we demonstrated an increased apoptosis level in AA treated group *in vivo* and *in vitro*. Analysis of the mechanism showed that the AA treatment decreases the expression of the anti-apoptotic molecules Bcl-2 and Bfl-1/A1 by down-regulating their promoter activity. Moreover we found that the AA silencing of the oncogenic transcription factor GLI1 is the underling mechanism controlling Bcl-2 and Bfl-1/A1 expression. Finally, we demonstrated that AA-induced apoptosis can be rescued by overexpressing GLI1 in cancer cells. These results define a novel mechanism used by ePUFAs to inhibit tumor growth and suggest the use of AA for the development of new therapeutic approaches.

LI-P03.

MOLECULAR MECHANISM OF THE LIPIN1 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, key metabolic steps are positively affected, including Phosphatidate Phosphohydrolase (PAP1). PAP1 catalyzes phosphatidic acid (PA) conversion to diacylglicerol. The mammalian enzymes responsible for PAP1 activity are the Lipin family, which are emerging as critical regulators of lipid metabolism. Lipins may govern the pathways by which phospholipids are synthesized and control the cellular content of signaling lipids.

Herein, we studied *in vitro* the activity of mammalian Lipin 1 (responsible of most PAP1 activity in mammals) purified to homogeneity. Lipin1 activity was measured with or without recombinant c-Fos in PA/Triton X-100 mixed micelles varying the assay conditions in order to clarify the activation mechanism. The kcat of the enzyme is doubled upon c-Fos addition, while the Km remains unaltered. Co-immunoprecipitation of the purified proteins demonstrated a physical interaction between c-Fos and Lipin 1. Using c-Fos mutants, we established the molecular determinants of the interaction. Results support our hypothesis that c-Fos physically associates with the phospholipid synthesis enzymes that it activates and reinforce the concept of a protein capable of increasing pivotal enzymatic activities *per se*.

LI-P04.

IMPLICATION OF SPHINGOLIPID METABOLISM IN RENAL EPITHELIAL CELL LINE DIFFERENTIATION

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Sphingosine 1-Phophate (S1P) is an important sphingolipid mediator in cell fate, synthetized by Sphingosine Kinase (SK). We studied the involvement of SK activity in the establishment of differentiated phenotype of MDCK cells induced by external hypertonic media. For this end, confluent MDCK cells were subjected to hypertonic medium with the concomitant addition or not (control) of 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. DHS treatment induces β-catenin redistribution from plasma membrane to intracellular localization and actin cytoskeleton reorganization, resulting in disassembling of AJ. 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, Myriocin (Myr), an inhibitor of the novo synthesis, was used. Myr 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-P05.

SPHINGOLIPID METABOLISM IS A SHARP REGULATOR OF A RENAL EPITHELIAL CELL PROLIFERATION

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Sphingosine Kinase (SK) is a key regulator enzyme in lipid metabolism that regulates the balance between sphingosine and sphingosine-1-Phosphate (S1P), being S1P a lipid involved in several cellular processes. We have demonstrated that SK is strongly expressed in renal epithelial cells and is a key regulator in cell survival. In the present work we evaluated the importance of the tight regulation of SK activity in renal epithelial cell cycle. For this purpose, MDCK cells cultured at low density were seeded with 10% BFS for 24 hs, to allow cell cycle progression. Then, cell were treated or not with D,L-threo-dihydrosphingosine (DHS), a selective SK1 inhibitor at different concentrations(1,5 and 1,75μM). SK inhibition induced a decrease in cell number in a concentration dependent manner after 24 hs of incubation (257,200, 129,650 and 85,768 cells, control, 1.5 and 1.75 μM respectively) with no alteration in cell viability. Cells were arrested in G1 phase (57.9%, 72.7% and 76.1% for Control, 1.5 and 1.75 μM, respectively) and decreased of mitotic cell/field (4.5, 1.4 and 1.3 for Control, 1.5 and 1.75 μM, respectively) showing a cell cycle arrest. In summary we proposed that depending on SK rate activity, the enzyme can be, not only a regulator of the cell survival, but also of the cell cycle progression.

LI-P06.

EFFECT OF FATOSTATIN ON HYPEROSMOLARITY-ACTIVATED LIPID METABOLISM IN RENAL EPITHELIAL CELLS

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In the renal cell line MDCK, we demonstrated that the increase in environmental osmolarity up-regulates phospholipid (PL) and triglycerides (TG) metabolism. The aim of the present work was to evaluate whether or not the increase in lipid metabolism requires the activation of the transcription factor sterol response element binding protein (SREBP). With this purpose, MDCK cells were incubated in isosmolar and hyperosmolar medium (containing 125 mM NaCl) alone or in the presence of different concentrations (10-25 μM) of fatostatin (FATO), a SREBP inhibitor. To evaluate lipid synthesis 0.4 μCi [^{14}C(U)]-glycerol was added to the incubation medium 3 h before cell collection. After 24 h of treatment with NaCl, cells were collected and counted and lipid content and biosynthesis were determined. FATO decreased the number of cells. Hyperosmolarity increased total PL and TG content by 60 and 100%, respectively, unexpectedly FATO increased PL and TG accumulation. FATO (10, 15 and 20 μM) decreased [^{14}C]-glycerol incorporation into DAG (by 60, 70 and 80%) but not change on PL and TG synthesis was observed. These results suggest that hyperosmolarity would activate SREBP which in turn would modulate the up-regulation observed in lipid synthesis under hyperosmotic conditions.

LI-P07.

TRIGLYCERIDE SYNTHESIS DYNAMICS UNDER HYPEROSMOLAR STRESS

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Renal medullary cells are immersed in a hyperosmolar environment due to their function in the urine concentrating system. In order to survive in these conditions, cells must develop protective mechanisms. We showed that the renal papilla is the zone with the highest content and synthesis of phospholipids (PL), which helps to preserve the membrane structure and cell viability. Triglycerides (TG) are a possible source of fatty acids for the synthesis of PL. In this work we study the dynamics of TG and PL synthesis in renal epithelial cells subjected to hyperosmolarity. We studied lipid synthesis in renal medullary slices and in MDCK cells submitted to different hyperosmolar media (from 298 to 579 mOsm/kgH2O) by using different precursors [^{14}C(U)]-glycerol, [^{14}C]-arachidonic acid (AA), [^{14}C]-palmitic acid and [^{3}H]-oleic acid. After treatment lipids were extracted, separated by TLC and quantified by liquid scintillation. We observed that high osmolarities increase TG and PL synthesis in all the samples assayed. All precursors were incorporated to both TG and PL molecules except for the AA which was found only in PLs. Cerulenin, a FAS inhibitor used to suppress fatty acid *de novo* synthesis, decreases TG synthesis and content while PL synthesis remains active. These results show that molecular machinery involved in lipid metabolism is differentially regulated by hyperosmolarity.

LI-P08.

HYPERTHERMIA IMPAIRS SERTOLI CELL FUNCTIONS. EFFECTS ON LIPID METABOLISM

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Spermatogenesis is known to be vulnerable to temperature. Previous work from our group showed that *in vivo* testicular hyperthermia selectively damages germ cells at specific developmental stages and rapidly induces a seminiferous epithelium involution. This concurred with a considerable buildup of lipid droplets in Sertoli cells (SC). The aim of this study was to examine the effects of heat exposure on isolated SC in culture. Warming to 43ºC disrupted SC microtubule network and actin microfilaments. This SC cytoskeleton perturbation was accompanied by increased ROS production, mitochondrial depolarization, and accumulation of triacylglicerols (TAG). The heat exposure-dependent TAG accumulation could be explained by an impairment of mitochondrial fatty acid oxidation and a re-direction of fatty acids into TAG synthesis and lipid droplet formation. The impaired mitochondrial physiology also reduced the *de novo* synthesis of cardiolipin, as observed after incubating SC cultures with [^{3}H]arachidonic acid. The hyperthermia-induced cytoskeletal and mitochondrial alterations could in part respond for the lipid metabolic disorder that SC undergo. Since these cells provide support to germ cells, these changes might be one of the underlying causes of the temporal impairment of spermatogenesis observed *in vivo* during testicular hyperthermia.

LI-P09.

MACROPHAGE DIFFERENTIATION INCREASES GLYCEROLIPID SYNTHESIS IN ACCORDANCE WITH GPAT UP-REGULATION

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Type-2 diabetes and obesity are characterized by an excessive accumulation of triacylglycerols (TAG) partially caused by a deregulation of glycerol-3-phosphate acyltransferases (GPATs) that catalyze the first step in *de novo* glycerolipid synthesis, and carnitine palmitoyl transferase 1 (CPT1) that regulates fatty acid oxidization. In order to study the roles of these enzymes in monocyte-macrophage(MP)-foam-cell transition (a model of TAG accumulation during atherogenesis) we tested their expression by qRT-PCR in the murine MP RAW264 and the human THP-1 monocyte cell lines differentiated into foam cells by oxidized LDL (oxLDL) and into MP by PMA, respectively. Mitochondrial GPAT1 and 2 expressions did not change in either model. We then analyzed the ER isoforms GPAT3 and 4. Interestingly, only GPAT3 expression significantly increased in monocyte to MP transition. These results were consistent with GPAT activity assays, since N-ethylmaleimide (NEM) sensitive activity (GPAT2, 3 and 4) but not NEM resistant activity (GPAT1) increased after oxLDL treatment. We could also prove that CPT1a was up-regulated during RAW264 cell differentiation to foam cells while a significant decrease was observed in the human MP derived from THP1 monocytes, suggesting that β-oxidation is not that active in MP, consistently with the anaerobic metabolism hallmark of M1 pro-inflammatory MP.

LI-P10.

SYNAPTIC PKC ISA DOWNSTREAM EFFECTOR OF PLD DURING IRON-INDUCED OXIDATIVE STRESS. EFFECT OF AGING

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We have previously demonstrated that iron-induced oxidative stress activates phospholipase D (PLD) signaling in cerebral cortex synaptic endings (Syn). The purpose of this work was to study the PLD downstream signaling events during iron-induced oxidative stress in Syn obtained from adult (4 months old) and aged (28 months old) rats. Diacylglycerol (DAG) production was increased in Syn from adult rats exposed to iron. This rise in DAG formation was dependent on PLD1 and PLD2 activities. Western blot assays showed that iron overload activates synaptic PKCα/βII and PKD1 and reduces glutamate uptake, both in adult and aged rats. In adult rats, PLD1 and PLD2 modulated PKCα/βII and PKD1 activation. In contrast, in senile rats, DAG formation catalyzed by PLDs did not participate in PKD1 and PKCα/βII activation, but it was dependent on PKC activities. Moreover, PLD1 and PKC inhibition (with 0.15 μM EVJ and 10 μM BIM, respectively) restored glutamate uptake to control levels only in Syn from aged rats. On the contrary, PLD2 inhibition (with 0.5 μM APV) reduced even more the uptake of glutamate in adult and aged Syn. Our results show a differential regulation of PKCα/βII and PKD1 by PLDs during iron-induced oxidative stress as a consequence of aging.

LI-P11.

DIFFERENT PPARs AGONISTS MODIFY NUCLEAR LIPID METABOLISM IN RAT CEREBELLUM

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Previous research from our lab demonstrated an active phosphatidic acid (PA) metabolism in isolated nuclei from rat cerebellum. We detected and characterized several enzymatic activities related to its metabolism, such as lipid phosphate phosphatase (LPP), diacylglicerol lipase (DAGL), monoacylglycerol lipase (MAGL), phospholipase A (PLA) and lysophosphate phosphatase (LPAPase). Interestingly, we also showed that they are differently regulated by trans-retinoic acid through an unknown non-genomic mechanism. Thus, in this work we study the modulation of these enzymatic activities by different fatty acids (FA) and their derivatives which could interact with nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs). To this end, adult rat cerebellums were homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Using the respective radiolobeled substrates co-incubated with these agonists, we observed that arachidonic acid and docosahexaenoic acid stimulate DAGL activity by 80%. PGE2 also produced a stimulatory effect on this activity but to a major extent (128%) while slightly diminished LPP activity (19%). Taken together, these results demonstrate a lipid metabolism regulated by FA and related molecules in rat cerebellum nuclei that could be involved in gene expression and differentiation, apoptosis or inflammation processes.

LI-P12.

CHANGES IN CERAMIDE METABOLISM CONTRIBUTE TO MDCK CELL DIFFERENTIATION

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Ceramide (Cer) and Glucosylceramide (GlcCer) are involved in many important cellular processes. We have previously demonstrated that GlcCer derived from recycled Cer is essential in MDCK cell differentiation induced by hypertonicity. Cer species are synthesized by six Cer synthases (CerS1-6), each one with different acyl CoA specificity. The aim of this study was to evaluate the expression of CerS1-6, and the different species of Cer and GlcCer during MDCK cell differentiation. Confluent MDCK cells were cultured under isotonicity or subjected to hypertonic media. RT-PCR analyses showed that MDCK cells express CerS2, CerS4 and CerS6 in both experimental conditions. We analyzed the fatty acid composition of Cer and GlcCer species by MALDI-TOF MS. We identified four mayor species of Cer (C16:0, C24:1, C24:0 and C22:0) and six mayor species of GlcCer (C16:0, C24:1, C24:0, C22:0, C20:0 and C18:0). These results are in accordance with the profile of CerS expression detected. Although the composition of sphingolipids was qualitatively the same under both conditions, we found that C16:0 GlcCer have the highest relative intensity in control cells, whereas hypertonicity-cultured cells showed an increase in the relative intensity in C24:0 GlcCer. These changes in Cer metabolism of MDCK cells could provide new clues to understanding the mechanism of renal epithelial cell differentiation.

LI-P13.

DGK-θ AT THE SYNAPSE: A ROLE FOR THE METABOLISM OF DAG IN SYNAPTIC VESICLE RECYCLING?

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Diacylglycerol kinase-θ (DGK-θ) is one of ten mammalian DGK isoforms that regulate the generation and metabolism of two important lipid second messengers: diacylgylcerol (DAG) and phosphatidic acid (PtdOH).Although DGK-θ is primarily expressed in the brain, no physiological role has been identified for this isoform in the central nervous system (CNS). Our data demonstrate this enzyme is involved in regulating glutamate release from cortical neurons. Both shRNA-knockdown of DGK-θ and neurons derived from DGK-θ knock-out mice exhibit a decreased rate of synaptic vesicle (SV) endocytosis compared to control neurons. Importantly, the rate of SV endocytosis is recovered by ectopic expression of DGK-θ in neurons depleted of the endogenous enzyme. In contrast to SV endocytosis, our most recent data show that the rate exocytosis is elevated in DGK-θ knock-out neurons. Our data establish a role for DGK-θ at the presynaptic nerve terminal in the regulation of SV recycling, and suggest that DGK- θsupports synaptic transmission during periods of elevated or sustained neuronal activity.

LI-P14.

PHOSPHOLIPID REMODELING IN DOPAMINERGIC NEURONS: ROLE OF α-SYNUCLEIN VARIANTS AND IRON OVERLOAD

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Increased levels of α-synuclein (α-syn) and iron-overload are pathognomonic signs of dopaminergic neurons in Parkinson's disease (PD) patients. Moreover, iron and fatty acid (FA) availability are predisposing factors for pathological α-syn aggregation. In this work, we characterized the phospholipid remodeling pathways that regulate FA availability in dopaminergic neurons overexpressing α-syn variants (WT and A53T) and exposed to iron-overload. Increased cellular oxidant and lipid peroxidation levels were observed in dopaminergic neurons exposed to iron-overload. The inhibition of calcium-independent phospholipase A^2 (deacylation pathway) provoked an increase in the extent of cellular damage induced by iron-overload. In this connection, phospholipid acylation was differentially affected by iron overload and the presence of α-syn variants. FA incorporation into phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was increased in dopaminergic neurons harboring WT α-syn. This acylation profile was not altered by iron-overload. Neurons expressing A53T α-syn (a variant present in autosomic dominant PD and with high iron affinity) showed a diminished FA esterification in PC and PE. This effect was enhanced in iron overloaded neurons. Our results show that FA availability is differentially regulated by α-syn variants and iron overload in this *in vitro* model of PD.

LI-P15.

CONSEQUENCES OF THALLIUM EXPOSURE ON MDCK CELLS LIPIDS METABOLISM

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Thallium (Tl) is a toxic heavy metal that among other symptoms causes renal damage. In the present study we investigated the effects of Tl(I) and Tl(III) on lipids metabolism in a renal epithelial (MDCK) cell line. Cells were exposed to 10 or 100 μM of Tl(I) or Tl(III) for either 24 or 48 h. The profile of phospholipids (PL), cholesterol (Cho) and triacylglycerides (TG) was evaluated. No changes were observed at 10 μM Tl(I), but increased total PL (24 h: 28%, 48 h: 40%) and Cho (24 h: 30%, 48 h: 52%) contents were found at 100 μM Tl(I). TG content was decreased by 30% and increased by 80% after 24 and 48 h of Tl(I) treatment, respectively. Tl(III) (100 μM) increased both PL and Cho content around 100% after 24 h and 300% after 48 h of incubation. TG content was increased by 60 and 600% after 24 and 48 h, respectively. Such an increase was also evidenced in lipid droplet size. PL profile showed decreased phosphatidylethanolamine and increased phosphatidylcholine contents. After 48 h, both cations decreased the fluidity of the outer monolayer of plasma membrane, increased that of the inner monolayer, and increased the annular fluidity. Tl(III) caused marked alterations in cells ultrastructure. The increase in cell membranes components (PL and Cho) and fatty acid storage (TG) as well as the changes in membrane properties may contribute to renal cell dysfunction in Tl-exposed people.

LI-P16.

HUMAN MILK: EVALUATION OF CREAMATOCRIT METHOD FOR DETERMING CALORIC CONTENT

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Human milk is a fluid capable of providing all the nutrients and defenses necessary for the newborns (NB). The neonatology service of the HIGA San Martin La Plata City has its own Mother's Milk Bank, whose milk is classified according to gestational age in which the birth took place (to find homogeneity with the recipient's mother milk); colostrum (up to 7 days old), transitional milk (7 to 14) and mature milk (over 14). Categorized milk is classified by its caloric content (CC), by the creamatocrit method (CM) which only considers milk lipids (L) whereas proteins (P) and carbohydrates (C) are not quantified (CM:L66.8+290).This allows selecting the milk which best adapts to the NB's needs. The aim of the present study was to determine if CM (at present applied in the Milk Bank) is appropriated to evaluate milk CC. For this purpose milk CC was determined by CM and analytical methods (P:Kjeldakl, L:Gerber, C:Antrona-Sulfuric) and Atwater coefficients. Mature milk had 0.40-0.65 kcal/ml (both methods). However, CM was equivalent to CC only when milk P and C were within normal ranges, if not CC was over/under estimated. In conclusion, we propose milk CC should consider all macrocomponents, in benefit of those neonates undergoing critical stage. IR Spectrometry would be the most convenient method since it is simple, quick, non-destructive and sensitive.

MI-P01.

PROTEOMIC STUDY OF SUMOYLATED PROTEINS IN *Trypanosoma brucei*

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SUMOylation is a regulatory post translational modification involving the covalent attachment of a small ubiquitin-like protein called SUMO to a variety of proteins, participating in diverse cellular processes. The functional consequences of SUMO attachment are based on the alteration of the target interaction surface. SUMO resulted essential for procyclic (PC) and bloodstream (BS) forms of *Trypanosoma brucei.* There is strong evidence supporting the association of SUMO with the expression of the variable surface glycoprotein, responsible for the evasion of the immune response. We intend to accomplish the proteomic identification of SUMO targets in *T. brucei* by performing SUMO chromosomal tagging. This enables SUMO expression at physiological levels avoiding competition with the endogenous form and providing tags for tandem affinity purification of SUMO conjugates. In addition, we have applied an approach to reduce unspecific purification of contaminant proteins by a LysC-based strategy allowing the identification of the modified lysine at the same time.
We succeeded in obtaining PC and BS clones with a double allele knock-in of this SUMO variant. Immunofluorescence and Western blot analysis showed that the tagged SUMO forms have a nuclear localization and display a characteristic SUMOylation pattern. We are now purifying SUMOylated proteins to obtain their proteomic profile.

MI-P02.

EFFECTS OF HDACIS ON *T. cruzi* REPLICATION, DIFFERENTIATION, INFECTIVITY AND GENE EXPRESSION

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Epigenetic events like acetylation and deacetylation of histones affect gene expression in protozoan parasites such as *T. brucei* and *P. falciparum*. Here we show the effect of histone deacetylases inhibitors (HDACis) on *T. cruzi* differentiation, replication, infectivity and gene expression. We found that epimastigotes replication rate is barely affected after treatment with inhibitors Trichostatin A (TSA), Apicidin (Api), Sirtinol (Sir) and Nicotinamide (Nic). Differentiation to metacyclic trypomastioges forms was inhibited with all HDACis tested, but differentiation to amastigote forms was not affected. Also, pre-incubation of cell derived trypomastigote with TSA, Api and Sir produced a 10% increase on *in vitro* infections, but the opposite effect was observed with Nicotinamide. Finally, we tested the effects of Api on trypomastigote gene expression. We found a two fold increase for Bromodomain factor 2 and two *T. cruzi* HDACs transcripts. Genes coding for cell cycle division, cell programmed death proteins, PCNA and a Trypomastigote surface antigen decreased to half comparing to control parasites. These results show that enrichment in acetylated histones derived from HDACis treatment is sufficient to produce changes on gene expression, affecting differentiation and infectivity thus suggesting that this epigenetic event may play a role in controlling transcription rate in *T. cruzi*.

MI-P03.

THE METACASPASES OF *Trypanosoma cruzi*: SCREENING OF INTERACTORS AND SEARCH FOR NATURAL SUBSTRATES

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Apoptosis is a programmed cell death essential for the development and homeostasis in multicellular organisms. In unicellular organisms, on the other hand, its existence and possible role is still controversial. Despite the presence of morphological characteristics compatible with apoptosis, caspase orthologs are absent in the sequenced genomes of these organisms. About a decade ago, sequences with some similarity to those of caspases were identified in the genomes of plants, fungi and protozoans and named metacaspases. This seemed to end the controversy, but doubts arised again when it was shown that these enzymes have biochemical characteristics very different, although they seem to share with caspases some functions, including the regulation of cell cycle and death.
Knowledge of the mechanism of action of metacaspases is still very limited, mostly because the targets of their activity are still unknown. We are searching for natural substrates of *T. cruzi* metacaspases, by two strategies: 1) the screening of a number of interactors (obtained by co-purification from parasite extracts and identified by MS), expecting that some of them will be true substrates, and 2) a detailed study of substrate specificity using a peptide library obtained by a new technique called PICS, which should give us valuable information of the consensus sequence recognized by these proteinases.

MI-P04.

DEVELOPMENT AND OPTIMIZATION OF AN ATTENUATED VACCINE DESIGNED TO CONTROL *Salmonella* INFECTION

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Foodborne diseases (FBD) are highly related to the low income areas and represent one of the main problems worldwide. The most common causative agent of FBD is *Salmonella*. The process of Salmonella infection to non-phagocytic cells (epithelial cells) involves the adhesion, invasion, maturation of *Salmonella*-containing vacuoles and replication steps. The RcsCDB regulatory system is deeply involved in controlling the expression of genes required to establish these stages. In our laboratory, we have obtained the *rcsC11* attenuated mutant, affecting the RcsC sensor, which causes constitutive activation of the system. In this study we evaluated the ability of both the wild-type strain and the *rcsC11* mutant of *S.* Typhimurium to infect epithelial cells (non-phagocytic) and macrophage cells (phagocytes). We determined that the *rcsC11* attenuated mutant was capable of producing a strong immunogenic response. Afterward, the mutant was gradually removed because it was unable to replicate in macrophages, in contrast to other well described *Salmonella* attenuated mutants. Furthermore, we constructed the pMAT13 plasmid vector that was able to replicate in *S.* Typhimurium. The *rcsC11* mutant transformed whit pMAT13 were able to express and secrete heterologous proteins. This finding indicates that the *rcsC11* mutant is a good candidate for the development of new vector vaccines.

MI-P05.

MOLECULAR IDENTIFICATION OF A GLYCOSIDE HYDROLASE-PRODUCING BACTERIUM OF INDUSTRIAL INTEREST

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Cyclodextrin Glucosyltransferase (CGTase), belonging to glycoside hydrolase family 13, catalyzes starch conversion into cyclodextrins and other industrial products. We have isolated a CGTase from a soil bacterium which had been classified as *Bacillus circulans* based on its phenotypic profile. Recently, the use of molecular tools has led to re-classification of microorganisms. The 16S rRNA gene is widely used for phylogenetic studies; however, its use is limited due to intragenomic heterogeneity. In addition, single-copy housekeeping genes are used, although there is no consensus about which of them should be employed. The aim of this work was to identify our CGTase-producing bacterium by molecular phylogenetic analysis of the 16S rRNA gene and the housekeeping genes gyrB, recA and tufA. These genes were amplified by PCR with degenerated primers and sequenced. Multiple sequence alignments including our sequences and curated nucleotide sequences from Bacillus and related genera were obtained with ClustalW software. The substitution model was inferred from each alignment with Modeltest. Phylogenetic trees were reconstructed by Maximum Likelihood and Maximum Parsimony criteria using PAUP. The reliability of each tree topology was performed by bootstrap (1000 pseudoreplicates). We conclude that the bacterium under study must be re-classified within the *Paenibacillus genus*.

MI-P06.

THE *ptsP* GENE CONTROLS THE BACTERIOCIN PRODUCTION AND ROOT COLONIZATION IN *P. fluorescens* SF39a

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Bacteriocins are proteinaceous toxins that can kill closely related species, providing the producer better access to limited resources. Rhizosphere isolate *P. fluorescens* SF39a secretes a bacteriocin active against *P. fluorescens* CTR212. The aim of this work was to identity of genetic determinants involved in the production or regulation of this bacteriocin. Previously, a miniTn5Km1 mutant (*P. fluorescens* 451) affected in bacteriocin production was obtained. In *P. fluorescens* 451, the transposon was inserted into *ptsP* gene which encodes a phosphoenolpyruvate phosphotransferase of the phosphotransferase system PTS^{Ntr}. In this work, heterologous complementation studies were performed. The *ptsP* gene from *P. fluorescens* Pf01 restored the production of bacteriocin, indicating that the observed phenotype in the mutant 451 was caused by inactivation of the *ptsP* gene.

The protease and pyoverdine production, motility, biofilm formation and ability to colonize the rhizosphere of wheat were analyzed in the strain 451. This mutant showed a decrease in biofilm formation and protease production, and an increase in swarming motility and pyoverdine production. In addition, this mutant was less competitive than wild-type strain in the wheat rhizosphere. These results suggest that a mutation in the *ptsP* gene affects a variety of phenotypes that can influence bacterial fitness in the environment.

MI-P07.

PROTEIN-PROTEIN INTERACTIONS BETWEEN TRYPANOSOMATID RNA-RECOGNITION MOTIFS

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Gene expression regulation is mainly exerted at the post-transcriptional level in kinetoplastid parasites. This order comprises human pathogens such as *Trypanosoma cruzi*, the agent causing Chagas Disease. In this picture, RNA-binding proteins play a crucial role regulating mRNA half life and translation efficiency when bound to 3´ untranslated regions in the mRNA. Previously, we have determined that proteins from the TcRBP family containing a single RNA-Recognition Motif (RRM) can shuttle between the nucleus and the cytoplasm. Our latest working model suggests that these proteins bind mRNA targets in the nucleus, and are then exported to the cytoplasm as ribonucleoprotein particles. We have identified the RRM as an unconventional and structural nuclear localization signal allowing active nuclear entry. Now, we have identified TcP22 as a TcUBP1-RRM binding partner by affinity chromatography followed by MS analysis, and confirmed it by *in vivo* immunoprecipitation. *In vitro* experimental work suggests that TcUBP1-RRM uses the β-sheet to interact with TcP22, the same surface that is used to bind RNA. However, TcP22 cannot prevent TcUBP1-RRM binding to RNA. In silico docking analysis is consistent with our experimental work, providing a detailed picture for this unconventional interaction. Ongoing work is focused on the biological role of RRM protein-protein interactions.

MI-P08.

TRANSCRIPCIONAL REGULATION OF AchE, PlcH AND PchP BY ArgR, A REGULATOR OF ARGININE METABOLISM

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The acetylcholinesterase activity (AchE) of *Pseudomonas aeruginosa* is encoded by the PA4921 gene (achE); its expression depends on the use of choline (Cho) as nitrogen (N) source. Hemolytic phospholipase C (PlcH) and phosphorycholine phosphatase (PchP) are also induced by Cho. All have been involved in the pathogenesis of *P. aeruginosa*. Here, we studied the transcriptional regulation of *achE*, *plcH* and *pchP* genes by ArgR, a regulator of arginine (Arg) metabolism. *In silico* analyses of 900bp upstream ATG of *achE* gene revealed two putative binding sites for ArgR (–52/ –96bp). Fragments of different length (LF: 990, MF: 500 and SF: 300bp) of the *achE* putative regulatory region were fused to *lacZ* and integrated into the WT chromosome. In Cho/succinate-grown cells containing LF or MF fragments showed higher level of reporter activity (˜700 MU) than cells with SF. In Arg/succinate-grown cells with LF, MF or SF insertions the activities were similar, suggesting that in the SF fragment is located the ArgR binding site. By the use of a Δ*argR* strain and by EMSA assays we demonstrated that *achE* expression depends on ArgR regulator. Not only the AchE enzymatic activity was increased by the use of Arg as N source but also PlcH, and PchP in a lesser level. Additionally, a consensus sequence resembling the potential binding site for ArgR was found in the regulatory region of the three genes studied.

MI-P09.

ENTEROBACTIN AS AN INTRACELLULAR OXIDATIVE STRESS PROTECTOR ALLOWS *Escherichia coli* COLONY FORMATION

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To acquire iron, *Escherichia coli* synthesize the catechol siderophore enterobactin, along with its specific transport system. Previously, we showed that an *E. coli entE* mutant is unable to develop colonies on agarized M9 medium (M9A). This phenotype was related to an increment in the oxidative stress and was reverted by enterobactin suplementation and by anaerobic culture conditions. In this work, we demonstrate that enterobactin needs to be hydrolyzed, after reaching the cell cytoplasm, in order to allow colony development. We observed that a *fes* mutant, unable to release iron from the enterobactin-iron complex, did not develop colonies in M9A and showed increased ROS levels compared with the wild type strain. The absence of colonies was reverted by addition of the reducing agent ascorbic acid. Meanwhile, enterobactin supplementation did not recover *fes* strain colony formation. On the other hand, we observed that a group of aromatic amino acids when added to the medium, allowed normal colony growth of the *entE* mutant. Furthermore, tryptophan and tyrosine reduced the increment of ROS levels in *entE* cells growing in M9. These results agree with our hypothesis implying enterobactin in the oxidative stress protection and in a critical role on *E. coli* colony development in M9A.

MI-P10.

COMPENSATORY BALANCE BETWEEN EXTRACELLULAR MATRIX COMPONENTS OF BACTERIAL BIOFILMS DETERMINES RESISTANCE TO ENVIRONMENTAL STRESSORS

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Within a biofilm, bacteria are embedded in a complex extracellular matrix that serves at least for two purposes: providing robustness and stability to the biofilm and protecting the cells against a variety of external aggressions. In *Pseudomonas putida*, two large secreted proteins, LapA and LapF, are key elements with distinct roles. LapA is essential for irreversible attachment to biotic and abiotic surfaces, while LapF is involved in cell–cell interactions, microcolony formation and a mature biofilm development. It was suggested that these surface proteins could be interacting with different exopolysaccharides (EPSs). We report the existence of a compensatory effect where *P. putida* reacts to the lack of either LapA or LapF by increasing the expression of the specific EPS encoding genes and the consequent protein synthesis. In spite of this compensatory effect, both mutants showed reduced attachment, microcolony development and biofilm formation. However, *lapA* and *lapF* mutants showed high resistance to a variety of antibiotics, detergents and saline stress, also displaying competitive colonization in the corn rhizosphere. Our data confirm the role of exopolysaccharides in stress protection and indicate the existence of an "allostasis" mechanism by which bacteria would sense and counteract alterations in the composition of the extracellular matrix.

NS-P01.

PREMATURE BIOENERGETIC AND COGNITIVE DEFECTS IN A RAT MODEL OF PRESYMPTOMATIC ALZHEIMER'S DISEASE

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Intraneuronal accumulation of amyloid β (Aβ) has been linked to mild cognitive impairment that precedes Alzheimer's disease (AD) onset. This neuropathological trait was recently mimicked in a novel animal model of AD, the hemizygous (+/-) transgenic (Tg) rats (McGill-R-Thy1-APP). Our aim was to do in Tg(+/-) and controls (WT) a time-course analysis of the bioenergetic profile in cognitive areas of the brain to assess the impact of mitochondria functionality on the cognitive performance. We measured Aβ levels and performed respirometric and ATP synthesis assays with isolated mitochondria from cortex and hippocampus. Cerebral oxidative stress markers were evaluated and behavioral tests performed. Our results showed alterations in respiratory chain functionality and deficient oxidative phosphorylation in 3-month old Tg(+/-) rats as compared to WT. This phenotype was associated to mitochondrial Aβ accumulation, higher levels of anxiety and spatial reference memory impairment. However, episodic-like memory, working memory and spatial learning were preserved. A clear mitochondrial dysfunction was observed in older Tg(+/-) rats (6 month-old) characterized by lower oxygen consumption rate and increments in reactive oxygen species. Conclusions: bioenergetic deficits may be linked to mitochondrial Aâ accumulation and emotional and cognitive alterations at early stage of AD neuropathology

NS-P02.

LOVASTATIN AND NICOTINE MODULATE α7 AND α4â2 ACETYLCHOLINE NICOTINIC RECEPTORS

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Neuronal α7 and α4β2 are the predominant nicotinic acetylcholine receptor (AChR) subtypes found in central synapses and in the hippocampus in particular. The effects of lovastatin (an inhibitor of cholesterol biosynthesis, 10-1000 nM) and nicotine (a general AChR agonist, 1-100 μM) were evaluated on these two AChRs endogenously expressed in rat hippocampal neurons. Chronic (14 days) lovastatin and nicotine treatment augmented cell-surface levels of α7 and α4β2 neuronal AChRs, as measured by fluorescence microscopy and radioactive ligand binding assays. This was accompanied in both cases by an increase in total protein levels as determined by Western blots. α4β2 AChR levels in neurites were more sensitive to nicotine than those in soma, at all concentrations tested. The opposite was the case with α7 AChRs. At low lovastatin concentrations (10-100 nM), the increase in neurite α4β2 AChR was higher than in soma; the opposite occurred at higher (500-1000 nM) lovastatin concentrations. However, neurite α7 AChRs rose more than somatic α7 AChRs at all lovastatin concentrations tested. These results suggest that α7 and α4â2 AChR are regulated in hippocampal neurons by cholesterol levels and agonist exposure. This regulation depends on drug concentration and receptor localization.

ST-P01.

MITOCHONDRIAL FUSION REGULATES GENE TRANSCRIPTION AND ACTIVITY OF A MITOCHONDRIAL PROTEIN StAR

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It is well known that the rate-limiting step in steroidogenesis is the transfer of cholesterol from the outer (OMM) to the inner mitochondrial membrane, mediated by a mitochondrial protein StAR (Steroidogenic acute regulatory). We have shown that StAR's activity depends on ERK phosphorylation and that mitochondrial fusion is essential in steroidogenesis, through the up-regulation of Mitofusine 2 (Mfn2), a central mitochondrial fusion protein. The aim of the present study was to analyze if mitochondrial fusion and StAR phosphorylation are involved in the regulation of StAR levels and localization at the OMM; thus, increasing cholesterol transport. We performed Mfn2 knockdown in MA-10 Leydig cells and observed a significant decrease in mRNA and protein StAR levels by semi-quantitative RT-PCR and immunoblot. This is a reversible process since reestablishment of mitochondrial fusion allows StAR gene expression and mitochondrial StAR localization. MA-10 cells were transiently transfected with StAR S232A, a mutated form of StAR lacking ERK phosphorylation site. Mitochondrial StAR decreased in the presence of StAR S232A. These results demonstrate, for the first time, that mitochondrial fusion regulates the transcription of mitochondrial proteins. In conclusion, StAR retention at the OMM, due to mitochondrial fusion and ERK phosphorylation is a crucial step in determining StAR activity.

ST-P02.

EFFECTS OF TESTOSTERONE ON APOPTOSIS IN C2C12 MURINE SKELETAL MUSCLE 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 demonstrated that testosterone protects against H_2O_2-induced apoptosis in C2C12, murine skeletal muscle cell line, involving the androgen receptor (AR) with classical (nuclear) and non-classical (mitochondria and microsomes) localization. The aim of our research is to deepen the understanding of the antiapoptotic actions of the hormone in muscle. We observed that at short times of exposure to H_2O_2, C2C12 exhibit a defense response showing ERK, Akt and Bad phosphorylation, remaining in these state for 2 hours. Simultaneously, during the 4 hours of H_2O_2 treatment, phosphorylation of proapoptotic protein JNK (1, 2 and 3) was observed. Using Real Time PCR, an increase in mRNA levels of apoptotic proteins was detected while antiapoptotic proteins undergo fluctuating changes during H_2O_2 exposure. Testosterone treatment prior to H_2O_2 maintained Akt phosphorylated throughout the time and reduceed JNK phosphorylation and expression of apoptotic proteins. Moreover, the hormone reduceed the activation of the proapoptotic protein p66Sshc and blocked its mitochondrial localization induced by H_2O_2. The data obtained provide insights into the molecular basis of sex hormone-dependent sarcopenia.

ST-P03.

PHOSPHOLIPASE C ACTIVITY IS REGULATED BY ARF6 DURING ACROSOMAL EXOCYTOSIS

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The acrosome is a large membrane-limited granule that overlies the sperm nucleus. In response to physiological stimuli, sperm undergo exocytosis of this granule. This is a special type of calcium-dependent exocytosis termed the acrosome reaction (AR) which is a prerequisite for fertilization. By using biochemical, exocytosis assays and microscopy techniques we demonstrated that the small GTPase ARF6 is present in, localizes to the acrosomal region of, and is required for calcium and DAG-induced exocytosis in human sperm. Myristoylated and GTPgammaS loaded-ARF6 induces exocytosis in the absence of extracellular calcium and promotes the activation of phosphatidylinositol 4-phosphate 5-kinase leading to phosphatidylinositol 4,5-bisphosphate (PIP2) synthesis. The GTPase-induced AR relies on PLC activity and DAG production. We present direct evidence that active ARF6 increases PLC activity causing PIP2 hydrolysis. Consistent with this result, ARF6 promotes an IP3-dependent intraacrosomal calcium release required for AR. Challenging sperm with calcium or DAG boosts the exchange of GDP for GTP on ARF6, suggesting that both stimuli promote a GEF activity in sperm that activates recombinant ARF6 turning it into an AR inducer. ARF6 has a dual role in human sperm exocytosis inducing both the synthesis and the hydrolysis of PIP2 sitting at a critical point during the exocytotic cascade

ST-P04.

VDR INVOLVEMENT IN 1α,25(OH)2-VITAMIN D3-DEPENDENT CELL CYCLE MODULATION IN SKELETAL MUSCLE CELLS

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We previously reported that 1α,25(OH)2-vitamin D3 [1,25D] promotes muscle cell proliferation and differentiation in murine skeletal muscle cells. We now present data indicating that the vitamin D receptor (VDR) is involved in cell cycle modulation promoting differentiation in C2C12 cell line. When VDR was knocked down by a shRNA against this receptor, the activation of p38 MAPK by 1,25D was abolished. Furthermore, the up-regulation of cyclin D3 and cyclin inhibitors (p21Waf1/Cip1 and p27Kip1) by the hormone also occurred in a VDR dependent manner. 1,25D-induced G0/G1 phase arrest was evidenced by flow cytometry. At the same time, the hormone increased myogenin levels only when VDR was expressed. In C2C12 wild type cells, expression of cyclin D3, p21Waf1/Cip1 and p27Kip1 triggered by 1,25D was not observed in presence of SB203580, a specific inhibitor of p38 MAPK. p38 MAPK was also required for 1,25D-induced CREB transcription factor expression. The results suggest that VDR is involved in p38 MAPK activation by 1,25D, which modulates the cellular cycle in skeletal muscle cells. Further studies are required to clearly understand 1α,25(OH)2-vitamin D3 regulation of myogenesis.

ST-P05.
PHOSPHORYLATION REGULATES THE FUNCTION OF ZEB1 TRANSCRIPTIONAL REPRESSOR
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ZEB1 is a transcription factor important for the epithelial-mesenchymal transition by which many tumors undergo metastasis. ZEB1 is phosphorylated but the role of phosphorylation(P) is unknown.Our studies show that a decrease in P-ZEB1 increases both DNA binding and transcriptional repression of ZEB1 target genes and that its ZD2 domain holds 4 phosphorylation sites (T851,S852,S853,T867). Functional assays with ZD2 mutants show that PMA, or IGF-1 (mediated by ERK) can prevent the transcriptional role of ZD2. GFP-ZD2 clones show that IGF-1 disrupted ZEB1 nuclear localization.Our aim is to expand the significance of P to full length ZEB1 (FLZEB1) and the importance of the T867 site. FLZEB1 mutated at all 4 sites repressed more the activity of gene targets than wtZEB1, which was not obtained by a single mutation T867A suggesting that the 4 sites are needed and a cooperative activation among them.Immunofluorescence of CHO cells transfected with ZEB1-T867A or ZEB1-T851A/S852A/S853A/T867A show the mutants are unresponsive to IGF-1 (they remained nuclear) and that T867 controls cellular location of ZEB1. Transfections assays with ZD2-T867A, ZD2-T867E (P-mimetic) and ZEB1-T867A show that IGF-1 induces its effect through T867 and that ZEB1 has other IGF-1 responsive phosphosites.The results confirmed that ZEB1 biological role is regulated by P-ZEB1 would serve as an integrating factor of external signals.

ST-P06.
CNBP: A NEW MEMBER OF THE *Wnt* ANCIENT PATHWAY?
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CNBP is a nucleic acid chaperone highly conserved among vertebrates required for proper craniofacial development. The CNBP molecular targets identity is still limited. By using the MEME/MAST software, we searched for genes from human, mouse, chicken, amphibian and fish containing the recently determined DNA-binding site consensus in their promoters. Bioinformatic analyses retrieved 16 putative targets conserved among the five species, mainly comprising transcription factors, membrane receptors and signalling proteins. Gene ontology analysis retrieved terms related to development and general metabolism. Among the identified genes, three (*ptk7, tcf7l2, cdk14*) are involved in the canonical *Wnt* pathway and one (*boc*) in the antagonist *Sonic hedgehog* pathway. CNBP binding to the promoters of these genes was validated by ChIP on DNA samples of 24-hpf zebrafish embryos. The role of CNBP on the expression of the target genes was assessed by knocking-down or overexpressing CNBP in 24-hpf zebrafish embryos. Data from RT-qPCR and whole mount *in situ* hybridization showed that CNBP enhances the expression of all the tested genes. A recent report shows that CNBP negatively controls the expression of *wnt5b*, a member of the non-canonical Ca^{2+}-*Wnt* pathway. Collectively, our data suggest a role of CNBP in balancing the canonical and non-canonical *Wnt* pathway during embryonic development.

ST-P07.
Dal81 MEDIATES HIERARCHICAL UTILIZATION OF POOR NITROGEN SOURCES IN *Saccharomyces cerevisiae*
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Nitrogen is an essential nutrient for all life forms. The yeast *Saccharomyces cerevisiae* can use a broad variety of compounds as nitrogen sources. Expression of genes involved in the utilization of poor nitrogen sources is repressed in the presence of rich nitrogen sources. This mechanism has been extensively studied and is known as nitrogen catabolite repression (NCR). Recently, a hierarchical utilization of poor nitrogen sources has been proposed. Dal81/Uga35 is a transcription factor involved in the regulation of genes expressing proteins that participate in the incorporation and metabolism of several poor nitrogen sources such as gamma-aminobutyric acid, urea, allantoin and amino acids. The aim of this work was to determine if there is a hierarchical activation in the use of poor nitrogen sources and to study the role of the transcription factor Dal81 in the establishment of this hierarchy. For this purpose, expression levels of different inducible permeases such as Uga4, Agp1, Bap2, Dal7 and Dur3, and the interaction between Dal81 and the regulatory regions of their genes were measured using RT-qPCR and ChIP assays, respectively. Results clearly show that genes needed for leucine utilization are expressed in higher levels that those for gamma-aminobutyric acid, urea and allantoin and that this hierarchy depends on Dal81.

ST-P08.
CaMKII AND eIF4E PARTICIPATE IN ARRHYTHMIAS GENERATION DURING CARDIAC AGING
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We use *Drosophila melanogaster* for studying cardiac diseases. One transgenic strain containing a fluorescent reporter system that senses intracellular twitch Ca^{2+} transient increases was utilized. We observed changes in the frequency of Ca^{2+} transients with age in semi-intac fly heart preparations and its putative modification by 1) The inhibition of Ca^{2+}-calmodulin-dependent protein kinase (CaMKII), involved in Ca^{2+} cycling and 2) the reduction in eIF4E expression, an eukaryotic translation initiation factor. Results: Flies between 60 and 70 days old showed reduction of heart rate compared with 7 days old flies and widespred distribution of cardiac periods (intervals between transient increases of Ca^{2+}). Arrhythmicity index (standard deviation of the heart period), increased from 0.27 to 0.52 (n=12). CaMKII inhibition reduced dispersion of cardiac periods distribution and arrhythmicity index from 0.27 to 0.19 (n = 21). Lower levels of eIF4E reduced dispersion of cardiac periods distribution and arrhythmicity index from 0.27 to 0.09 in 7 days old (n=18) flies. The results indicate that CaMKII regulates cardiac function in *Drosophila* and is an arrhythmogenic molecule. More important, eIF4E participates in the genesis of arrhythmias independently of its canonical function as translation factor, pointing to this factor as a putative new candidate involved in the pathophysiology of the mammalian heart.

ST-P09.

EFFECT OF LIGHT ON SIGNALING PATHWAYS IN A PHOTORECEPTOR CELLS NUCLEAR FRACTION FROM BOVINE RETINA

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The aim of this work was to evaluate the effect of light exposure on signaling pathways in a fraction enriched in photoreceptor cell nuclei (PNF) from bovine retina. We studied whether the exposure of bovine retina to light causes any changes in the PNF fraction in the activation and localization of MAPKs and proteins involved in two canonical signaling pathways, PIP2-PLC-PKCs and PI3K-Akt. Bovine eyes were adapted to darkness (2 h, 0ºC), and then eye cups were exposed to light (280 cd, 30 min) or to dark. PNF was obtained by ultracentrifugation onto a discontinuous sucrose gradient. Light-induced DNA damage was evaluated by immunofluorescence. PKCα, PKCδ, Akt and ERK1/2 total and activated content was detected by Western Blot. No DNA fragmentation was observed in dark or light condition. Light causes an increase in the content of activated PKCα and PKCδ with respect to the dark condition in the PNF (2.5 fold and 3- fold respectively), with similar levels of total forms. Likewise, an increase in the content of activated Akt (3-fold) without changes in total Akt was seen. A significant increase was also observed in response to light in the content of total and phosphorylated form of ERK1/2 (2-fold) in PNF. Our results demonstrate that, in absence of nuclear damage, ERK and signaling pathways linked to GPCR and RTK were activated in PNF when bovine retina was exposed to light.

ST-P10.

CONCOMITANT MITOGENIC EFFECTS OF GROWTH HORMONE AND EPIDERMAL GROWTH FACTOR OVER MCF-7 CELLS

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Growth hormone (GH) is essential for normal growth and development of the mammary gland. However, high GH levels were associated with pathogenesis and progression of breast cancer. GH modulates epidermal growth factor receptor (EGFR) expression and signaling, broadly involved in cancer pathogenesis, in cell types different from the breast tissue. In this study, we explored the crosstalk between GH and EGF signaling pathways in the epithelial breast cancer cell line MCF-7 to understand how these factors might work together to affect breast cancer behavior. For this purpose, EGFR expression and signalling were assessed in MCF-7 cells after incubation with GH, EGF or both and results were correlated with effects on proliferation. These studies showed that concomitant treatment with GH and EGF during two hours inhibits ligand-induced EGFR downregulation. GH did not synergize with EGF in activating Erk1/2 or Akt; on the contrary, Erk1/2 activation by concomitant treatment with GH and EGF was diminished respect to the addition of their individual effects. Such desensitization was associated with reduced induction of cyclin D1 expression and proliferation of cells treated with GH and EGF. In conclusion, inhibition of ligand-induced EGFR downregulation by GH co-treatment would attenuate Erk1/2 activation, cyclin D1 induction and promotion of cell proliferation of the breast cancer cells.

ST-P11.

EXPRESSION OF PRO AND ANTIAPOPTOTIC PROTEINS REGULATED BY VITAMIN D AGONISTS IN ENDOTHELIAL CELLS

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We have previously shown that $1\alpha,25(OH)_2$-Vitamin D_3 [$1\alpha,25(OH)_2D_3$] 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) through NF-κB inhibition and inducing apoptosis via caspase-3 activation. The intrinsic apoptotic pathway could be activated by mitochondrial disruption changing the balance between pro and antipoptotic proteins. In the present work we study the expression of pro and antiapoptic proteins Bcl-2, Bax and Bim at mRNA and protein levels in SVEC and vGPCR cells stimulated by $1\alpha,25(OH)_2D_3$ and TX 527. Time response qRT-PCR analysis of anti apoptotic protein Bcl-2 demonstrated that mRNA levels decrease at 12 h and then increase at 48 h in $1\alpha,25(OH)_2D_3$ and TX 527-treated SVEC, whereas protein levels remain unchanged through time in both SVEC and vGPCR cells. Anti apoptotic protein Bax remain unchanged whereas Bim mRNA and its protein levels increased in SVEC cells. On the other hand, Bortezomib (0.5 μM), a proteasome inhibitor, similarly to vitamin D agonists, also increases Bim protein levels. Altogether these results suggest that $1\alpha,25(OH)_2D_3$ and TX 527 inhibit the NF-κB pathway and trigger apoptosis inducing and increase in Bim protein that could elicit caspase -3 activation through the intrinsic apoptotic pathway in endothelial cells.

ST-P12.

PHOSPHOLIPASE D PATHWAY MODULATES KEY SIGNALING EVENTS IN ACTIVATED T CELLS

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The T cell receptor (TCR) triggers several intracellular signaling events that are crucial for proper T cell development and function. The aim of the present work was to study the participation of the phospholipase D (PLD) pathway in signaling events elicited by the TCR stimulation in Jurkat T cells, namely: protein kinase D 1 (PKD1), extracellular signal-regulated kinase (ERK1/2) and p21-activated kinase 1 (PAK1). To suppress phosphatidic acid (PA) and diacylglycerol (DAG) generation by the PLD pathway, cells were preincubated for 1 h with 0.4% n-butanol (since in the presence of primary alcohols PLD generates phosphatidylalcohols which cannot be further dephosphorylated to DAG) and the TCR was activated with anti-CD3 antibodies. Western blot assays showed that n-butanol treatment reduced TCR-induced PKD and PAK1 activation while ERK1/2 activation was not affected. Moreover, pull down assays showed that n-butanol also reduced Rac1 activation after 10 min of stimulation with anti-CD3. Previous reports evidenced that in activated T cells PKD phosphorylates histone deacetylase 7 (HDAC7) and induces its nuclear export allowing gene expression. In agreement with the inhibition of PKD, our results showed that n-butanol also restrained the nuclear export of EGFP-HDAC7 in activated Jurkat T cells. Thus, the PLD pathway modulates key signaling events elicited by the TCR engagement.

ST-P13.

DIACYLGLYCEROLS REGULATE DISTRIBUTION OF THE MUSCLE-TYPE NICOTINICACETYLCHOLINE RECEPTOR

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The effects of exogenous and endogenously-generated diacylglycerols (DAG) on the density and distribution of the muscle-type nicotinic acetylcholine receptor (AChR) in CHO-K1/A5 cells and their involvement in possible downstream pathways were evaluated by [125 I]-alpha-bungarotoxin binding, fluorescence microscopy, cell transfection and Western blots.
Treatment with dioctanoylglycerol (DOG) for 4 h augmented cell-surface AChR levels without altering total AChR levels. Co-incubation of DOG with drugs that increase intracellular calcium (A23187 or Thapsigargin) and thus activate classical PKCs, did not produce the same effect. Incubation with Gö 6976, a classical PKC inhibitor, mimicked the DOG effect, increasingAChR levels. Longer exposure to DOG (18 h) led to intracellular accumulation ofAChR without affecting the total amount of AChR. DOG changes on AChR distribution were blocked when cells were co-incubated with Gö 6976 and Rottlerin (inhibitor of PKC delta). Decreasing DAG levels at the Golgi, by silencing Nir2 protein, led to accumulation of AChR intracellularly.
In conclusion, exogenous and endogenous DAG appear to modulate the distribution of muscle-type AChR. Short-term exposure to DOG may lead to classical PKC inhibition, whereas long-term effects of DOG may involve activation of both classical and novel PKC enzymatic mechanisms.

ST-P14.

TRANSCRIPTOMIC LANDSCAPE REGULATED BY ACYL-COA SYNTHETASE4 (ACSL4) EXPRESSION IN BREAST CANCER CELLS

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ACSL4, an enzyme that esterifies arachidonic acid (AA) into arachidonoyl-CoA, is increased in breast, colon and hepatocellular carcinomas. Previously we showed that the transfection of breast cancer MCF-7 cells with ACSL4 cDNA (MCF-7 ACSL4 cells) confers them a highly aggressive phenotype. ACSL4 overexpression increases 5-lipooxygenase (5-LOX) and cyclooxygenase-2 (COX-2) metabolism of AA. Treatment of MDA-MB-231 tumor xenografts with a combination of ACSL4, 5-LOX and COX-2 inhibitors reduced tumor growth in doses that were ineffective when used alone. Thus ACSL4 is a potential therapeutic target for tumor formation. Therefore, the aim of this study was to identify the genetic elements regulated by ACSL4 expression. Applying a massively mRNA sequencing approach to MCF-7 and MCF-7 ACSL4 cells and employing the TopHat and Cufflinks software analysis, we detected 182 differentially expressed loci with $p < 0.05$. Within differentially expressed genes, DNMT1, HDAC, IL20, WNT6, RBL2/p130 and TWIST-1 are genes related to cell aggressiveness regulation and to expression regulation of estrogen receptor and COX-2, targets of ACSL4. The high number of differentially expressed genes demonstrates quite effectively that cell aggressivity regulation by ACSL4 is not through changes in an individual molecule or pathway, but it is the result of global changes in gene expression.

ST-P15.

CHRARACTERIZATION OF PROCESSING AND STRESS GRANULES UNDER DIFFERENTIAL HEAT STRESS STRINGENCY

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We have previously shown that Tpk2 and Tpk3 subunit isoforms of PKA differentially localize to processing bodies (PBs) and stress granules (SGs) evoked by severe and middle heat stress. Here, we investigate in more detail the mechanisms and signaling pathways involved in PBs/SGs granule formation induced by stress severity. Severe heat stress triggers formation of PBs and SGs containing Tpk2 and Tpk3 subunits and leads to the accumulation and aggregation of 48S pre-initiation complex. The aggregation of Tpk does not require sphingolipid signaling. On the contrary, middle heat stress triggers Tpk3 foci formation as well as Tpk3 accumulation on PBs and SGs granules, in a process depending on sphingolipid synthesis. This stress promotes the accumulation and aggregation up to the close loop mRNA complex. To globally characterize the proteins associated to the granules evoked by middle and severe heat stress we identified the protein composition of granular fractions enriched by sedimentation combined with 2D electrophoresis and MALDI MS-MS. We identified proteins exclusively enriched at middle heat stress, proteins exclusively enriched at severe heat stress as well as proteins common to both groups. These results suggest that different degrees of severity of the same stress evoke specific responses on RNP assembly.

ST-P16.

EXPRESSION REGULATION OF PROTEIN KINASE A CATALYTIC SUBUNIT, Tpk1, FROM *Saccharomyces cerevisiae*

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Upstream open reading frames (uORFs) are translational regulatory elements located in 5´ untranslated regions. They can repress the translation of downstream coding sequences (CDS). *S. cerevisiae* protein kinase A (PKA) is composed of a regulatory subunit Bcy1 and three catalytic subunits, Tpk1, Tpk2 and Tpk3, a *TPK1* uORF was described in the 5´UTR (five codons length). We cloned the full-length UTR sequence into a β-galactosidase (β-Gal) reporter. β-Gal activity and mRNA level, quantified by qRT-PCR, were compared between the WT UTR construct and a construct where the uORF start codon was mutated. The results indicated that the uORF regulates negatively *TPK1* translation but does not destabilize the mRNA. The analysis was also made under heat shock stress, as our previous results had shown that Tpk1 expression is upregulated. Both β-Gal activity and Tpk1 mRNA level were upregulated, β-Gal activity increased more than mRNA level. A ncRNA antisense (AS) was also identified overlaping 600 pb on CDS 3´end. The AS level was measured in log and stationary growth phases and order heat shock stress. The AS level went with *TPK1* mRNA levels, indicating a possible role in activation, but not repression of TPK1 expression. Thus, both mechanisms, uORF and antisense ncRNA could be contributing to regulate the expression of PKA Tpk1 subunit at transcriptional and post-transcriptional levels respectively.

ST-P17.
TOR SIGNALING IN *Arabidopsis thaliana*
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Target of rapamycin (TOR) kinase is an evolutionarily conserved master regulator that integrates energy, nutrients, growth factors and external signals to promote survival and growth in eukaryotes. The knowledge of TOR signaling in plants has been hampered because of the embryo lethality of the *Arabidopsis thaliana tor* mutants. The controversial resistance to rapamycin, a TOR inhibitor in other organisms, limits the molecular dissection of the TOR pathway in plants. Thus we analyzed the effect of second generation inhibitors, such as PP242, in *Arabidopsis* plants. Unlike rapamycin, PP242 is a selective inhibitor that targets the ATP domain of TOR. We grew plants in media with different carbon/nitrogen balance and PP242 concentrations. The inhibitor was able to delay germination and affect plant growth. Particularly, root size was severely reduced. Moreover, we show that the synthesis of anthocyanins and the expression of key genes of TOR, carbon and nitrogen signaling were modified by the presence of the inhibitor. Thus, the PP242 inhibitor could be a useful tool to investigate TOR signaling.
This work was supported by CONICET, ANPCyT, UNMdP and FIBA.

ST-P18.
TOR COMPLEX AND *Arabidopsis*-PATHOGEN INTERACTION
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The TOR (Target Of Rapamycin) pathway is a major controller of growth-related processes in eukaryotes. The plant TOR complex comprises the TOR kinase, and the proteins Raptor and LST8. Under favorable environmental conditions, the TOR pathway promotes growth and limits catabolic processes. There must exist a balance between growth and disease resistance, thus TOR signaling should be regulated in a biotic stress situation. We studied plant-pathogen interactions in the model plant *Arabidopsis thaliana*, using mutants in different TOR complex proteins. Plants of 25-d were sprayed with a fungus solution and evaluated for 14-d. We assayed *Fusarium graminearum* and *Botrytis cinerea* strains isolated from Prov Bs As, two important pathogens that cause major agronomic crop losses. We show that the mutant Raptor is tolerant to *Fusarium* but susceptible to *Botrytis*. *Fusarium*-inoculated Raptor plants do not present fungus growth or plant cell death. On the other hand the LST8 mutant resulted susceptible to the infection with both fungi. Infection of LST8 mutant with *Botrytis* was slower than that of wt plants. Gene expression was analyzed in leaves up to 3-d of treatment. Although additional experiments are needed, our results indicate that the TOR signaling pathway could be involved in biotic stress response.
Supported by CONICET, ANPCyT, UNMdP and FIBA.

ST-P19.
GROWTH HORMONE (GH)-SIGNALING THROUGH JAK2/STAT5 PATHWAY IN MUSCLE OF GROWING GH-TRANSGENIC MICE
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Growth hormone (GH) transgenic mice (Tg) present higher GH serum levels than normal siblings (N) since birth but no phenotypic differences are observed until the third week of age when GH-dependent growth begins. JAK2/STAT5 is the main GH signaling pathway related to body growth; muscle and bone are the principal tissues implicated in this process.
In order to asses potential molecular mechanisms involved in the age-dependent growth response to GH, 2 weeks (before GH-dependent growth), 4 weeks (GH-dependent growth) and 9 weeks old (young adult, control) Tg and N mice received an acute stimulus of GH and were sacrificed after 30 minutes. Muscle was extracted and protein activation/content was assayed by immunoblotting. Whereas N mice show high STAT5 phosphorylation levels in response to GH stimulus, with the highest response during the growth period, Tg mice do not respond at any age studied, accompanied by low levels of GHR and high levels of negative modulators. Maximal endogenous STAT5 phosphorylation was found in 2 weeks old mice, with a slight increase in Tg mice at every age studied. Pups also present high levels of negative modulators which could be restraining GH action at this age.
As the activation of JAK2/STAT5 signaling pathway is restricted in Tg mice muscle, other GH-activated pathways would be responsible for the exacerbated growth seen in these mice.

ST-P20.
REGULATION OF AKT ACTIVITY BY SUMO CONJUGATION
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Our laboratory studies the regulation of pre-mRNA alternative splicing by signal transduction pathways. We have reported that Akt activation regulates the activity of two splicing factors of the SR protein family (SRSF1 and SRSF7), altering different steps along mRNA metabolism: alternative splicing and translation. We also revealed Akt as an SR protein kinase capable of phosphorylating these SR proteins. We found that the SR protein SRSF1 enhances SUMO conjugation to several target proteins, including Akt. Here, the further explored this unexpected post-translational modification of Akt. By site directed mutagenesis we mapped SUMO conjugation sites within Akt. We found that Akt SUMOylation is required for its regulatory role on fibronectin and Bcl-x alternative splicing. Consistent with the pro-survival and pro-proliferative role of this kinase, we showed that its SUMOylation favors the production of fibronectin mRNA isoforms characteristic of highly proliferative tissues and tumors, as well as of anti-apoptotic Bcl-x mRNA isoforms. We also found that SUMOylation of Akt controls its function as a regulator of cell cycle progression. These findings reveal a novel level of regulation for Akt function, opening new areas of exploration related to the molecular mechanisms determining that the activation of a single kinase can lead to a vast diversity of cellular responses.

ST-P21.

PKCδ, ERK AND JNK IN 17β-ESTRADIOL ANTIAPOPTOTIC ACTION IN C2C12 CELLS: EFFECTS ON COXIV AND MPTP

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17β-Estradiol (E2) protects several tissues from apoptosis, including skeletal muscle. Aging is characterized by a progressive loss of muscle mass and strength, pathology known as sarcopenia. This is associated to low hormone levels and deregulation of apoptosis. We have shown that E2 prevented apoptosis induced by H_2O_2 in C2C12 skeletal muscle cells, acting on mitochondria. Although the main proteins mediating apoptosis are members of the Bcl-2 family and caspases, signaling molecules such as MAPKs and PKC, have been involved in its regulation. Here we studied further in depth the mechanism of action of E2 protecting C2C12 cells against apoptosis. We showed that E2 induced ERK translocation to mitochondria to regulate mitochondrial permeability transition pore (MPTP) and COXIV activity. We also demonstrated that H_2O_2 stimulated the phosphorylation of PKC δ in tyrosine 311, but pretreatment with E2 abrogated this activation. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays showed that PKC inhibitors prevented apoptosis. Moreover, H_2O_2 phosphorylated JNK in a time-dependent manner but preincubation with E2 suppressed this activation. PKC inhibition blocked JNK phosphorylation, suggesting that PKC δ acts upstream of JNK. Our studies deepen the knowledge of the molecular basis of myophaties linked with deregulation of apoptosis by hormonal deficit states.

ST-P22.

CROSS-TALK BETWEEN ERK AND p38-BASED MAPK PATHWAYS IN THE YEAST *S. cerevisiae*

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Understanding how cells integrate multiple simultaneous stimuli to decide their future behavior is a challenge. We study MAPK cascade pathways in the yeast *S. cerevisiae:* the ERK-based cascaded activated by mating pheromone (PR) and the p38-based one activated by high osmolarity (HOG). Recently, using single cell analysis, we found an unexpected cross-talk between them: in yeast adapted to high osmolarity, PR stimulates HOG (Baltanas *et al.,* 2013). It does so by "creating" an osmotic imbalance functionally similar to an osmolarity shock: PR increases the efflux of glycerol (the osmolyte that yeast use to counteract a high external osmolarity), causing loss of turgor, leading to HOG activation. Now, we isolated a point mutant of the yeast p38 Hog1 that it is only activated by a bona-fide high osmolarity shock and not by pheromone. Our initial characterization suggests that the mutant p38 fails to translocate to the nucleus after pheromone treatment, indicating that the two inputs regulate differently the p38 retention in the cytoplasm, probably by modulating the expression or activity of anchoring proteins. Interestingly, two of the four mammalian p38s isoforms 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.

Keywords: yeast, MAPK cascades, cross-talk.

ST-P23.

TARGETING PI3K/AKT PATHWAY TO OVERCOME ENDOCRINE RESISTANCE IN BREAST CANCER

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The phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR) pathway is commonly deregulated in tumors that do not respond to antiestrogen therapy, being associated to resistance to endocrine and other therapies.

The aim of this study was to target the PI3K/AKT pathway to overcome resistance to endocrine therapy. For this purpose we used the ER-positive IBH-6 human breast cancer cell line. We stably transfected IBH-6 cells to overactivate AKT1 using the myristoylated Akt1 (myrAKT1) construct. While IBH-6 wt cells treated with the antiestrogen ICI182780 (Fulvestrant) decreased cell proliferation, IBH-6-myrAkt1 cells did not. However, the last ones are sensitive to rapamycin.

IBH-6-wt and -myrAkt1 cells were inoculated into NOD/SCID mice and tumors were treated for 15 days with ICI, rapamycin, or the combination of both. IBH-6-wt tumors showed no significant changes in tumor growth curves after rapamycin treatment. However, IBH-6-myrAkt1 tumors treated with rapamycin significantly reduced tumor growth ($p<0.01$), and this inhibition was even greater in combination with ICI. The molecular and cellular mechanisms involved in the response to rapamycin and to other PI3K/mTOR inhibitors are being currently analyzed in this model.

These results support the idea of using PI3K/AKT/mTOR pathway inhibitors to overcome resistance to endocrine therapy in breast tumors with high AKT1 levels.

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