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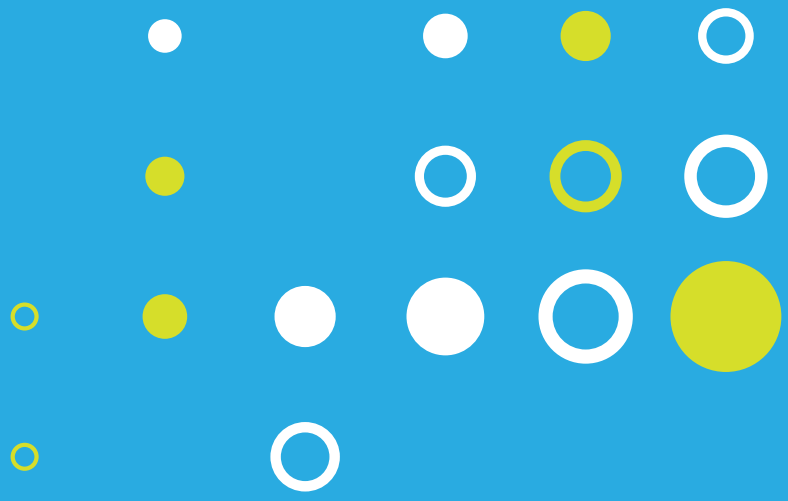

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- SAIB -

48th Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

XLVIII Reunión Anual
Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

October 29 - November 1, 2012

Mendoza
República Argentina

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Universidad Nacional de Rosario

-Plant biochemistry and molecular biology-
Diego Gómez-Casati
CEFOBI-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario

-Signal Transduction-
Claudia Nora Tomes
IHEM-CONICET, Facultad de Ciencias Médicas
Universidad Nacional de Cuyo

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The abstracts were evaluated by the members of the SAIB Board and Gustavo Chiabrando, Claudia Banchio, Jorge Casale, Oscar Campetella, Cecilia Alvarez, Walter Berón, Paula Casati, Santiago Mora, Claudia Spampinato, Elina Welchen, Eduardo Zabaleta, Juan Carlos Díaz Ricci, Susana Pasquaré, Gabriela Salvador, Eduardo Cánepa, María Celeste Ruete, Laura Delgui and Ignacio Cebrián

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Universidad Nacional de Cuyo

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Ministerio de Cultura de la provincia de Mendoza

Bodega Trapiche

SAIB 2012 CONGRESS

Monday, October 29th	Tuesday, October 30th	Wednesday, October 31st	Thursday, November 1st
	9:00 - 11:00 **Symposia*** *Room A*: IUBMB Symposium *Room B:* COB Cell Motility Symposium	9:00 - 11:00 **Symposia*** *Room A:* IUBMB Symposium *Room B*: Lipids Symposium	9:00 – 11:00 **Symposia** *Room A*: Plant Bioch.& Mol. Biol. Symposium *Room B*: Microbiology Symposium*
	11:00-11:30 Coffee break	11:00-11:30 Coffee break	11:00-11:30 Coffee break
	11:30 - 12:30 *Room A:* **PABMB Lecture*** Joseph S. Takahashi	11:30 - 12:30 *Room A:* **COB Lecture*** Roberto Mayor	11:30 - 12:30 *Room A:* **Plenary Lecture*** Pieter C. Dorrestein
14:00-15:30 Registration	12:30 - 15:00 **Posters & Lunch** CB (P01/26) MI (P01/19) BT (P01/07) SB (P01/05) LI (P01/08) PL (P01/17) NS (P01/08) ST (P01/05)	12:30 - 15:00 **Posters & Lunch** CB (P27/54) MI (P20/35) EN (P01/08) BT (P08/14) LI (P09/16) PL (P18/31) SB (P06/10) ST (P06/13)	12:30 - 15:00 **Posters & Lunch** CB (P55/78) MI (P36/57) EN (P09/12) BT (P15/21) LI (P17/24) PL (P32/49) NS (P09/13) ST (P14/20)
15:30-16:30 *Room A:* **Opening Ceremony**	15:00-16:00 *Room A:* **Plenary Lecture*** Christophe Lamaze	15:00-16:00 *Room A:* **Short Lectures*** Amira Klip Gregg Gundersen	15:00-16:00 *Room A:* **Teaching & Communication in Science** Diego Golombek
16:30 – 17:30 *Room A:* **Plenary Lecture** Adrián Paenza	16:00-18:00 **Oral Communications** *Room A:* CB (C01/08) *Room B*: MI (C01/08) *Room C:* PL (C01/08)	16:00-18:00 **Oral Communications** *Room A:* CB (C09/15) and EN (C01) *Room B*: BT (C01/04) and ST (C01-04) *Room C* PL (C09/12) and SB (C01/04)	16:00-18:00 **Oral Communications** *Room A:* CB (C16/21); ST (C05); SB (C05) *Room B*: LI (C01/07); NS (C01) *Room C* MI (C09/16)
17:30-18:00 Coffee break			
18:00 - 20:00 *Room A:* **IUBMB Symposium***	18:00-18:30 Coffee break	18:00-18:30 Coffee break	18:00-18:30 Coffee break
20:00-21:00 *Room A:* **AME Plenary Lecture*** Adrián Krainer	18:30-19:30 *Room A:* **Sols Lecture** Miguel Blázquez	18:30-19:30 *Room A:* **Hector Torres Lecture*** Sergio Grinstein	18:30-19:30 *Room A:* **EMBO Lecture*** Reinhard Jahn
21:00 Cocktail		19:30 *Room A:* SAIB Assembly	21:30 Closing Dinner

*Activities in English.

PROGRAM

MONDAY, October 29, 2012

14:00-15:30 **REGISTRATION**

15:30-16:30 ***OPENING CEREMONY***

Luis S. Mayorga
SAIB President
IHEM-CONICET, Facultad de Ciencias Médicas,
Universidad Nacional de Cuyo, Mendoza, Argentina

16:30-17:30 ***PLENARY LECTURE***

Adrián Paenza
Departamento de Matemáticas, Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, Buenos Aires, Argentina
"Challenging your intuition"
Chairperson: Luis S. Mayorga

17:30-18:00 ***COFFEE BREAK***

18:00-20:00 ***"IUBMB" SYMPOSIUM***

Chairpersons: Claudia Tomes and Silvia Belmonte

18:00-18:40 ***Josep Rizo***
Department of Biophysics, University of Texas Southwestern Medical Center
Dallas, Texas, USA
"Reconstituting basic steps of synaptic vesicle fusion"

18:40-19:20 ***Alan Morgan***
Department of Cellular and Molecular Physiology, University of Liverpool,
Liverpool, United Kingdom
"*Caenorhabditis elegans dnj-14*: a model of human neurodegenerative disease"

19:20-20:00 ***Nicolas Vitale***
Institut des Neurosciences Cellulaires et Intégratives UPR-3212 CNRS,
Strasbourg, France
"Fusogenic lipids in membrane fusion: the case of phosphatidic acid"

20:00-21:00 ***"AME" LECTURE***

Adrián R. Krainer
Cold Spring Harbor Laboratory, Cold Spring Harbor,
New York, USA
"Antisense modulation of alternative splicing: From the bench to the clinic"
Chairperson: José Luis Bocco

21:00 ***COCKTAIL***

TUESDAY, October 30, 2012

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

Room A

"IUBMB" SYMPOSIUM
Chairpersons: Hugo Maccioni and Cecilia Alvarez

09:00-09:40 ***Bruno Goud***
Department of Cell Biology, Institut Curie,
Paris, France
"Regulation of myosin motors by Rab GTP-ases: focus on myosin II and myosin Va"

09:40-10:20 ***Thierry Galli***
INSERM ERL U950, Institut Jacques Monod,
Paris, France
"Targeting and regulation of vesicular SNAREs in membrane Traffic - TI-VAMP/VAMP7's case"

10:20-11:00 ***Alfredo Cáceres***
Laboratorio Neurobiología, INIMEC-CONICET, Universidad Nacional de Córdoba,
Córdoba, Argentina
"Participation of LIMK1, PKD1 and BARS in Golgi outpost formation"

Room B

"COB" CELL MOTILITY SYMPOSIUM
Chairpersons: Carlos Arregui and José Daniotti

09:00-09:30 ***Olivier Pertz***
Department of Biomedicine, University of Basel,
Basel, Switzerland
"Spatio/temporal RHO GTPase signaling to the cytoskeleton during neurite outgrowth"

09:30-10:00 ***Maddy Parsons***

Randall Division of Cell and Molecular Biophysics, King's College London, Guys Campus, London, UK

"Integrin-specific signalling in the regulation of cell motility"

10:00-10:30 ***Georgina N. Montagna***

Max Planck Institute, Berlin, Germany

"Role of small heat shock protein 20 in intra-dermal migration of malaria parasite transmission stage"

10:30-11:00 ***Sylvie Dufour***

Institut Curie-CNRS-UMR 144, Paris, France

"Adhesion receptor crosstalk during the enteric neural crest cell migration in the embryonic gut"

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

11:30-12:30 ***"PABMB" LECTURE***

Joseph S. Takahashi

Howard Hughes Medical Institute, Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, USA

"The *clock* gene: from centimorgans to Ångstroms"

Chairperson: Beatriz L. Caputto

12:30-15:00 ***POSTERS & LUNCH***

Cell Biology (CB P01/26)	Microbiology (MI P01/19)
Biotechnology (BT P01/07)	Structural Biology (SB P01/05)
Lipids (LI P01/08)	Plant Bioch. & Mol. Biol. (PL P01/17)
Neuroscience (NS P01/08)	Signal Transduction (ST P01/05)

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

Christophe Lamaze

Laboratoire Trafic, Signalisation et Ciblage Intracellulaires, UMR 144 Curie/CNRS, Institut Curie, Paris, France

"Membrane dynamics and mechanics in intracellular signaling"

Chairperson: María Isabel Colombo

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

Room A

CELL BIOLOGY (C01/08)

Chairpersons: Ricardo Boland and César Casale

16:00-16:15
CB-C01
THE PALMITOYLTRANSFERASE SWF1 IS A BONA FIDE ZINC BINDING PROTEIN
González Montoro A, Quiroga R, Valdez Taubas JE.

16:15-16:30
CB-C02
CNBP UNFOLDS GUANINE CUADRUPLEX IN PROTO-ONCOGENES PROMOTERS
Challier E, Calcaterra NB, Armas P.

16:30-16:45
CB-C03
NA+/ CA2+ AND NA+/CA2+-K+ EXCHANGERS: BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION IN HUMAN PLATELETS
Bistué Millón MB, Elso – Berberian G, Asteggiano CG.

16:45-17:00
CB-C04
MOLECULAR STUDIES IN EXT1 AND EXT2 GENES IN MULTIPLE OSTEOCHONDROMATOSIS PATIENTS (EXT1/EXT2-CDG)
Delgado MA, Martinez Domenech EG, Dodelson de Kremer R, Asteggiano CG.

17:00-17:15
CB-C05
C/EBPß AND EGR-1 COORDINATELY REGULATE *ChkA* EXPRESSION DURING RA-INDUCED NEURONAL DIFFERENTIATION
Domizi P, Banchio C.

17:15-17:30
CB-C06
THE RELATIONSHIP BETWEEN *Coxiella burnetii* AND Rho GTPases OF HOST CELL DURING INFECTION
Salinas Ojeda R, Aguilera M, Rosales E, Carminati S, Berón W.

17:30-17:45
CB-C07
BIOCHEMICAL CHARACTERIZATION OF MITOCHONDRIAL COMPLEX III INHIBITION BY NITRIC OXIDE
Iglesias DE, Bombicino SS, Boveris A, Valdez LB.

17:45-18:00
CB-C08
HETEROCHROMATIN LOCALIZATION OF p19INK4d IS ASSOCIATED WITH SENESCENCE TRIGGERED BY GENOTOXICS
Sonzogni SV, Castillo DS, Cánepa ET.

Room B

MICROBIOLOGY (C01/08)

Chairpersons: Patricia Romano and Andrea Smania

16:00-16:15
MI-C01
STAPHYLOCOCCAL α-TOXIN INDUCES ACTIVATION AND DEGRADATION OF C-JUN AND JUND TRANSCRIPTION FACTORS
Moyano AJ, Racca AC, Andreoli V, Sola C, Panzetta-Dutari G, Bocco JL.

16:15-16:30
MI-C02
A MITOCHONDRIAL CYCLOPHILIN FROM *Trypanosoma cruzi* AND ITS ROLE IN PROGRAMMED CELL DEATH
Bustos P, Perrone A, Cámara M, Postan M, Moreno S, Bua J.

16:30-16:45
MI-C03
A CONSORTIUM OF REGULATORY SYSTEMS CONTROLS THE EXPRESSION OF A *Salmonella*-SPECIFIC EFFLUX COMPLEX
Cerminati S, Checa SK, Soncini FC.

16:45-17:00
MI-C04
CHARACTERIZATION OF THE *Serratia marcescens* MUTANT STRAIN IN THE GENE THAT ENCODES ShlA CYTOL
Di Venanzio G, Stepanenko T, García Véscovi E.

17:00-17:15
MI-C05
THE TWO-COMPONENT SYSTEMS PrrBA AND NtrYX REGULATE THE ADAPTATION OF *Brucella* TO LOW-OXYGEN TENSION
Carrica M, Fernandez I, Paris G, Goldbaum F.

17:15-17:30
MI-C06
***Brucella abortus* PrrB/A: A CONSERVED REDOX-RESPONSIVE TWO-COMPONENT SYSTEM**
Fernández I, Carrica MC, Sieira R, Paris G, Goldbaum FA.

17:30-17:45
MI-C07
TWO BINDING SITES INVOLVED IN BACTERIAL ADP-GLUCOSE PYROPHOSPHORYLASES ACTIVATION
Asención Diez M, Aleanzi M, Ballicora MA, Iglesias AA.

17:45-18:00
MI-C08
CHARACTERIZATION OF THE *Mesorhizobium loti* MAFF303099 TYPE-III SECRETION SYSTEM (T3SS)
Sánchez CM, Lepek VC.

Room C

PLANT BIOCHEMISTRY & MOLECULAR BIOLOGY (C01/08)

Chairpersons: Juan C. Díaz Ricci and María E. Alvarez

16:00-16:15
PL-C01.
EXPRESSION OF TMV AN CP MODULE THE RNA DECAY PATHWAY IN PLANT AND CONTRIBUTES TO VIRAL SYMPTOMS
Conti G, Rodriguez MC, Zavallo D, Manacorda CA, Asurmendi S.

16:15-16:30
PL-C02.
CHARACTERIZATION OF *Arabidopsis thaliana* E3-UBIQUITIN-LIGASE SINA-L7
Peralta DA, Gomez-Casati DF, Busi MV.

16:30-16:45
PL-C03.
THE CATIONIC LIPID DI-C14-AMIDINE STIMULATES DEFENSES AGAINST MICROBIAL PATHOGEN IN ARABIDOPSIS
Cambiagno DA, Ruysschaert JM, Lonez C, Álvarez ME.

16.45-17:00
PL-C04.
A DEFINED AQUAPORIN STOICHIOMETRY IN ORDER TO UNDERSTAND WATER PERMEABILITY AND PH SENSITIVITY
Yaneff A, Sigaut L, Marquez M, Alleva K, Pietrasanta L, Amodeo G.

17:00-17:15
PL-C05.
REGULATION OF ASF1 PROTEINS BY CELL CYCLE PROGRESSION AND UV-B RADIATION IN PLANTS
Lario L, Ramirez-Parra E, Gutierrez C, Spampinato CP, Casati P.

17:15-17:30
PL-C06.
ROLE OF AtHsCB IN PLANT IRON METABOLISM
Leaden L, Busi MV, Gomez-Casati DF.

17:30-17:45
PL-C07.
COX17, COPPER AND STRESS: A NEW MENAGE A TROIS IN *Arabidopsis thaliana*
Garcia L, Colombatti F, Welchen E, Gonzalez DH.

17:45-18:00
PL-C08.
ROLE OF TCP15 IN THE REGULATION OF CARPEL DEVELOPMENT AND CYTOKININ RESPONSES IN *Arabidopsis thaliana*
Uberti-Manassero NG, Gonzalez DH.

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

18:30-19:30 ***"SOLS" LECTURE***

Miguel A. Blázquez
Instituto de Biología Molecular y Celular de Plantas (CSIC)
Universidad Politécnica de Valencia, España
"Plant DELLA proteins as coordinators of growth in time and space"
Chairperson: Carlos Andreo

WEDNESDAY, October 31, 2012

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

Room A

IUBMB SYMPOSIUM
Chairpersons: Sebastián Asurmendi and Marcela Michaut

09:00-09:40 ***Benjamin Podbilewicz***
Department of Biology, Technion, Haifa, Israel
"Mechanisms of cell-cell fusion"

09:40-10:20 ***Richard S. Nelson***
Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma, USA
"Deciphering the role of plant cell cytoskeleton and membranes on virus accumulation and movement"

10:20-11:00 ***Andrea Gamarnik***
Fundación Instituto Leloir-CONICET, Buenos Aires, Argentina.
"Replication and dynamics of dengue virus RNA"

Room B

LIPIDS SYMPOSIUM
Chairpersons: María González Baró and Mario Guido

09:00-09:30 ***Silvia Sookoian***
Departmento de Hepatología Clínica y Molecular, Instituto de Investigaciones Médicas IDIM-CONICET, Buenos Aires, Argentina.
"Lipids and the liver: the systemic consequences of human fatty liver disease"

09:30-10:00 ***Carla B. Green***
Department of Neuroscience, UT Southwestern Medical Center, Dallas, Texas, USA.
"Loss of nocturnin, a circadian deadenylase, confers resistance to diet-induced obesity"

10:00-10:30 ***Martin E. Young***

Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA.

"The cardiomyocyte circadian clock influences myocardial lipid metabolism"

10:30-11:00 ***Mauricio Díaz-Muñoz***

Instituto de Neurobiología, Campus UNAM-Juriquilla, Querétaro, México

"Liver lipidic handling and food restriction: link between molecular clock and metabolic activity"

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

11:30-12:30 ***"COB" LECTURE***

Roberto Mayor

Department of Cell and Developmental Biology, University College London, London, UK.

"Collective cell migration: union is strength"

Chairperson: Nora B. Calcaterra

12:30-15:00 ***POSTERS & LUNCH***

Cell Biology (CB P27/54)	Microbiology (MI P20/35)
Enzymology (EN P01/08)	Biotechnology (BT P08/14)
Lipids (LI P09/16)	Plant Bioch. & Mol. Biol. (PL P18/31)
Structural Biology (SB P06/10)	Signal Transduction (ST P06/13)

15:00-16:00 ***SHORT LECTURES***

Chairpersons: Alfredo Cáceres and Gustavo Chiabrando

15:00-15:30 ***Amira Klip***

The Hospital for Sick Children and The University of Toronto, Toronto, Canada.

"Insulin signals governing translocation of glucose transporter GLUT4 in muscle cells"

15:30-16:00 ***Gregg G. Gundersen***

Department of Pathology and Cell Biology, Columbia University, New York, USA.

"Cell polarity from the inside-out: how the nucleus organizes the cytoplasm"

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

Room A

CELL BIOLOGY (CB C09/15) & ENZYMOLOGY (EN-C01)

Chairpersons: Eduardo Cánepa and Luis López

16:00-16:15
CB-C09.
ACTIVATION OF THE ALDOSE REDUCTASE BY DIRECT INTERACTION WITH TUBULIN
Rivelli JF, Peretti SO, Santander VS, Previtali G, Primo E, Casale CH.

16:15-16:30
CB-C10.
THE ACTIN CYTOSKELETON AND A RhoA-CONTROLLED PATHWAY PARTICIPATED IN STARVATION MEDIATED AUTOPHAGY
Aguilera MO, Berón W, Colombo MI.

16:30-16:45
CB-C11
THE ENDOSOMES AND THE GOLGI COMPLEX ARE INVOLVED IN THE INFECTIOUS BURSAL DISEASE VIRUS LIFE CYCLE
Delgui LR, Rodríguez JF, Colombo MI.

16:45-17:00
CB-C12
HEMIN INDUCES AUTOPHAGY IN LEUKEMIC ERYTHROBLAST CELL LINE
Fader CM, Vergara AN, Moor F, Salassa N, Colombo MI.

17:00-17:15
CB-C13
GEOMETRIC FEATURES OF TRANSMEMBRANE DOMAINS ARE MAJOR DETERMINANTS OF GOLGI LOCALIZATION
Quiroga R, Trenchi A, González Montoro A, Valdez Taubas JE, Maccioni HJF.

17:15-17:30
CB-C14
EFFECT OF A RNA-BINDING PROTEIN (RBP) ON THE DYNAMICS OF THE MOLECULAR CIRCADIAN CLOCK
Nieto PS, Revelli JA, Garbarino-Pico E, Guido ME, Tamarit F.

17:30-17:45
CB-C15
DIFLUOROMETHYLORNITHINE (DFMO) IS A NEW INHIBITOR OF AUTOPHAGY AND *Trypanosoma cruzi* INFECTION
Vanrell MC, Cueto JA, Barclay JJ, Colombo MI, Carillo C, Gottlieb RA, Romano PS.

17:45-18:00
EN-C01
MOLECULAR THERMODYNAMICS FOR CELL BIOLOGY AS TAUGHT WITH BOXES
Mayorga LS, López MJ, Becker W.

Room B

BIOTECHNOLOGY (BT-C01/04) & SIGNAL TRANSDUCTION (ST-C01/04)

Chairpersons: Ana V. Rodriguez and Paula Portela

16:00-16:15
BT-C01.
BIOTECHNOLOGICAL STRATEGIES FOR ATRAZINE PHYTOREMEDIATION IN HUMID PAMPA
Merini LJ, Massot F, Calabró López RA, Giulietti AM.

16:15-16:30
BT-C02.
SCREENING OF BACTERIAL OXIDASES
Portillo HG, Glodowsky AP, Britos CN, Trelles JA.

16:30-16:45
BT-C03.
BIOSYNTHESIS OF ANTIVIRAL COMPOUNDS USING IMMOBILIZED THERMOPHILIC BACTERIA
De Benedetti EC, Rivero CW, Lozano ME, Trelles JA.

16.45-17:00
BT-C04.
FLOXURIDINE BIOTRANSFORMATION IN ONE POT REACTION USING LACTIC ACID BACTERIA
Cappa VA, Britos CN, Vallejo M, Marguet EM, Trelles JA.

17:00-17:15
ST-C01.
OXER1, A LEUKOTRIENE RECEPTOR, IS PRESENT AND ACTIVE IN STEROIDOGENIC CELLS
Cooke M, Cornejo Maciel F.

17:15-17:30
ST-C02.
CONTRIBUTION OF PROLINE RESIDUES OF DESK IN THE SENSING AND TRANSMISSION OF COLD STIMULUS
Porrini L, Mansilla MC, De Mendoza D.

17:30-17:45
ST-C03.
IDENTIFICATION OF YEAST PKA SUBUNITS TRANSCRIPTION REGULATORS USING TWO-COLOR CELL ARRAY SCREENING
Pautasso C, Cañonero L, Zaremberg V, Rossi S.

17:45-18:00
ST-C04.
DEEP INSIDE THE NUCLEI: DECODING THE ROLE OF ARGININE DEIMINASE DURING ENCYSTATION OF *Giardia Lamblia*
Vranych CV, Merino MC, Mayol G, Touz MC, Rópolo AS.

Room C

PLANT BIOCHEMISTRY & MOLECULAR BIOLOGY (PL-C09/12) and STRUCTURAL BIOLOGY (SB-C01/04)

Chairpersons: Mariana Martín and Rodolfo Rasia

16:00-16:15
PL-C09.
NUCLEAR IMPORT AND DIMERIZATION OF TOMATO ASR1, A WATER STRESS-INDUCIBLE PROTEIN EXCLUSIVE TO PLANTS
Ricardi MM, González RM, Estévez JM, Iusem ND.

16:15-16:30
PL-C10.
RRNA DEGRADATION THROUGH A RIBOPHAGY-LIKE MECHANISMS IS NECESSARY FOR CELLULAR HOMEOSTASIS IN PLANTS
Macintosh G, Hillwig M, Morriss S, Floyd B, Bassham D.

16:30-16:45
PL-C11.
STRUCTURAL FEATURES DEFINING THE *IN VIVO* FUNCTION OF THE miRNA PROCESSING PROTEIN HYL1
Burdisso P, Milia FJ, Schapire AL, Bologna NG, Palatnik JF, Rasia RM.

16:45-17:00
PL-C12.
THE AtIPB GENE INDUCES RESPONSE MECHANISMS RELATED WITH PATHOGEN ATTACK IN ARABIDOPSIS PLANTS
Colombatti F, Andrade A, Garcia L, Alemano S, Gonzalez DH, Welchen E.

17:00-17:15
SB-C01.
SEARCHING FOR THE MOLECULAR BASIS OF *IN VIVO* MEMBRANE PROTEIN FOLDING IN *Aspergillus nidulans*
Sanguinetti M, Iriarte A, Amillis S, Marín M, Musto H, Ramón A.

17:15-17:30
SB-C02.
CHARACTERIZATION OF TWO TRANSCRIPTIONAL REGULATORY SEQUENCES OF INTRON5 OF THE RUNX1 Gene
Alarcon R, Fernandez V, Rebolledo B, Gutierrez S.

17:30-17:45
SB-C03.
A NEW PHOSPHATASE FROM AN ANTARCTIC BACTERIUM: MOLECULAR BASIS OF COLD ADAPTATION MECHANISM
Aran M, Smal C, Gallo M, Pellizza L, Cicero D.

17:45-18:00
SB-C04
NEW INSIGHTS INTO THE MECHANISM OF ACTIVATION OF THE HCV NS3 PROTEASE BY THE NS4A COFACTOR
Gallo M, Eliseo T, Bazzo R, Paci M, Summa V, Cicero DO.

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

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

Sergio Grinstein
Cell Biology Program, Hospital for Sick Children,
Toronto, Canada
"Imaging phagocytosis: receptors, signaling and the cytoskeleton"
Chairperson: Hugo D. Luján

19:30 **SAIB GENERAL ASSEMBLY**

THURSDAY, November 1, 2012

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

Room A

PLANT BIOCHEMISTRY & MOLECULAR BIOLOGY SYMPOSIUM
Chairpersons: Diego Gómez Casati and Ricardo Wolosiuk

09:00-09:30 ***Rodrigo A. Gutiérrez***
Departmento de Genética Molecular y Microbiología,
Pontificia Universidad Catolica de Chile, Santiago, Chile
"Nitrogen regulatory networks controlling plant root growth"

09:30-10:00 ***Jesus Vicente-Carbajosa***
Centre for Plant Genomics & Biotechnology, Universidad Politecnica de Madrid-INIA, Campus de Montegancedo, Madrid, España
"Conserved gene networks in the seed and beyond"

10:00-10:30 ***Fernando L. Pieckenstain***
IIB-INTECH, Universidad Nacional de San Martín, CONICET,
Chascomús, Argentina.
"Polyamines and polyaminooxidases in plant defense against pathogenic microorganisms"

10:30-11:00 ***Jorgelina Ottado***
IBR-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas,
Universidad Nacional de Rosario, Santa Fe, Argentina.
"Characterization of Hpa1 from *Xanthomonas axonopodis* pv. *Citri* in plant-pathogen interactions"

Room B

MICROBIOLOGY SYMPOSIUM

Chairpersons: Cecilia Mansilla and Carlos Argaraña

09:00-09:30 ***Soeren Molin***
Department of Systems Biology, Technical University of Denmark, Denmark.
"Evolutionary dynamics of *Pseudomonas aeruginosa* in cystic fibrosis airways"

09:30-10:00 ***Pablo Aguilar***
Institut Pasteur de Montevideo, Montevideo, Uruguay.
"Eisosomes and plasma membrane organization"

10:00-10:30 ***Gabriela Gago***
Instituto de Biología Molecular y Celular de Rosario. IBR-CONICET, Rosario, Argentina.
"Transcriptional regulation of lipid biosynthesis in mycobacteria"

10:30-11:00 ***Hugo Luján***
Laboratorio de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Católica de Córdoba, Córdoba, Argentina.
"Development of an oral vaccine platform based on the protective properties of surface proteins of the intestinal parasite *Giardia lamblia*"

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

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

Pieter C. Dorrestein
Skaggs School of Pharmacy and Pharmaceutical Sciences and Departments of Pharmacology, Chemistry and Biochemistry, UC, San Diego, USA.
"New ways to see, the creation of microbial Rosetta stones"
Chairperson: Hugo Gramajo

12:30-15:00 ***POSTERS & LUNCH***

Cell Biology (CB P55/78)	Microbiology (MI P36/57)
Enzymology (EN P09/12)	Biotechnology (BT P15/21)
Lipids (LI P17/24)	Plant Bioch. & Mol. Biol. (PL P32/49)
Neuroscience (NS P09/13)	Signal Transduction (ST P14/20)

15:00-16:00 ***"PABMB" TEACHING AND COMMUNICATION IN SCIENCE***

Diego Golombek
Departmento de Ciencia y Tecnología, Universidad Nacional de Quilmes-CONICET, Buenos Aires, Argentina.

"Life outside the lab: public communication of science as part of our job"

Chairperson: María Teresa Damiani

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

Room A

CELL BIOLOGY (CB-C16/21), SIGNAL TRANSDUCTION (ST-C05) and STRUCTURAL BIOLOGY (SB-C05)

Chairpersons: Alberto Díaz Añel and Fernando Irazoqui

16:00-16:15
CB-C16
***Tripanosoma cruzi* BDF3 BINDS ACETYLATED α TUBULIN AND MIGRATES TO THE FLAGELLUM DURING METACYCLOGENESIS**
Alonso VL, Villanova GV, Ritagliati C, Motta CM, Cribb P, Serra EC.

16:15-16:30
CB-C17
A Rab-GEF CASCADE DURING HUMAN SPERM EXOCYTOSIS
Bustos MA, Lucchesi O, Ruete MC, Tomes CN.

16:30-16:45
CB-C18
VACUOLAR PROTEIN SORTING RECEPTOR: NEW MOLECULAR EVIDENCE OF A SORTING PATHWAY IN *Giardia lamblia*
Miras SL, Rivero MR, Feliziani C, Zamponi N, Quiroga R, Rópolo AS, Touz MC.

16:45-17:00
CB-C19
EPSIN–LIKE PROTEIN: A NOVEL CLATHRIN ASSOCIATED PROTEIN IN *Giardia lamblia*
Feliziani C, Zamponi N, Miras SL, Lanfredi-Rangel A, Touz MC.

17:00-17:15
CB-C20
POSTNATAL ENDOSULFAN EXPOSURE ALTERS ENDOCRINE SIGNALLING AND ENDOMETRIAL PROLIFERATION IN THE RAT
Varayoud J, Milesi MM, Alarcón R, Rivera OE, Muñoz-de-Toro M, Luque EH.

17:15-17:30
CB-C21
RETINOIC ACID REDUCES MIGRATION OF HUMAN BREAST CANCER CELLS: ROLE OF RETINOIC ACID RECEPTOR BETA
Flamini MI, Gauna G, Vargas Roig L.

17:30-17:45
ST-C05
AR, ER, MnSOD AND COXIV IN 17β-ESTRADIOL AND TESTOSTERONE ANTIAPOPTOTIC EFFECTS IN C2C12 CELLS
Pronsato L, La Colla A, Ronda AC, Milanesi L, Vasconsuelo A, Boland R.

17:45-18:00
SB-C05
ENHANCED TOLERANCE OF *Escherichia coli* TO THERMAL AND OXIDATIVE STRESS
Rimmaudo L, Aran M, Maselli G, Mora García S, Wolosiuk RA.

Room B

LIPIDS (LI C01/07) & NEUROSCIENCES (NS C01)

Chairpersons: Susana J. Pasquaré and Norma Sterin de Speziale

16:00-16:15
LI-C01
UNRAVELING KEY AMINO ACIDS FOR *Bacillus SP.* ACYL-LIPID DESATURASES ACTIVITY
Sastre DE, Uttaro AD, De Mendoza D, Altabe SG.

16:15-16:30
LI-C02
ROLE OF NF-κB IN COX2 EXPRESSION IN RENAL CELLS UNDER HYPERTONIC STRESS
Casali CI, Weber K, Faggionato D, Messinger D, Fernández Tome MC.

16:30-16:45
LI-C03
BEHAVIOR OF SPHINGOMYELINS WITH VERY LONG CHAIN FATTY ACIDS IN BILAYERS AND MONOLAYERS
Peñalva DA, Fanani ML, Maggio B, Aveldaño MI, Antollini SS.

16.45-17:00
LI-C04
DIVERSE GLICEROLIPID SYNTHESIZING ENZYMES CONTRIBUTE TO THE DAILY RHYTHMS IN PHOSPHOLIPID SYNTHESIS
Acosta Rodríguez VA, Marquez S, Salvador GA, Pasquaré SJ, Gorné LD, Garbarino Pico E, Giusto NM, Guido ME.

17:00-17:15
LI-C05
IMPLICATION OF SPHINGOSINE KINASE IN MDCK CELL TRANSITION FROM POLARIZED TO DIFFERENTIATED PHENOTYPE
Santacreu BJ, Sterin-Speziale NB, Favale NO.

17:15-17:30
LI-C06.
NUCLEAR LIPID DROPLETS ARE DYNAMIC STRUCTURES
Lagrutta LC, Layerenza JP, Montero Villegas S, Sisti MS, García de Bravo MM, Ves-Losada A.

17:30-17:45
LI-C07
LIPID CHANGES IN RAT SPERMATOZOA DURING ISOLATION AND FUNCTIONAL ACTIVATION *IN VITRO*
Oresti GM, Peñalva DA, Luquez JM, Antollini SS, Aveldaño MI.

17:45-18:00
NS-C01
PROTEIN MALNUTRITION DIMINISHES CEREBRAL CORTEX BDNF EXPRESSION AND EVOKES ANXIETY-LIKE BEHAVIOURS
Belluscio LM, Berardino BG, Ceruti JM, Cánepa ET.

Room C

MICROBIOLOGY (MI C09/16)

Chairpersons: Paula Vincent and José Luis Barra

16:00-16:15
MI-C09
IDENTIFICATION AND CHARACTERIZATION OF PHOSPHATIDIC ACID PHOSPHATASE ENZYMES IN *Streptomyces coelicolor*
Comba S, Menendez-Bravo S, Arabolaza A, Gramajo H.

16:15-16:30
MI-C10
ANTIGENICITY AND PROTECTIVE CAPACITY OF NOVEL VACCINE CANDIDATES FOR *Trypanosoma cruzi*
Ziliani M, Alba-Soto CD, Sanchez DO, Tekiel V.

16:30-16:45
MI-C11
BtaE: A POLAR ADHESIN INVOLVED IN BINDING OF *Brucella suis* TO HOST CELLS AND VIRULENCE IN MOUSE
Ruiz V, Posadas DM, Estein S, Van der Henst C, Abdian P L, Sieira R, De Bolle X, Zorreguieta A.

16.45-17:00
MI-C12
BB3576 DIGUANILATE CYCLASE PROTEIN REGULATES MOTILITY AND BIOFILM IN *Bordetella bronchiseptica*
Sisti F, Fernández J.

17:00-17:15
MI-C13
SEQUENCE ANALYSIS OF *fur* GENE FROM *H. pylori*-INFECTED PATIENTS WITH IRON DEFICIENCY
Chamorro N, Serrano C, Burce E, Venegas A, Harris P.

17:15-17:30
MI-C14.
CONDITIONED MEDIUM FROM VERO-TRYPOMASTIGOTES MODIFY INTEGRIN β1 EXPRESSION IN MICE BONE MARROW CELLS
Fuchs AG, Gonzalez MN, Riarte AR, Ruiz AM.

17:30-17:45
MI-C15
***Cotton leafroll dwarf virus* PO PROTEIN IS A SILENCING SUPPRESSOR WHICH INHIBITS LOCAL RNA SILENCING**
Delfosse VC, Agrofoglio YC, Casse MF, Hopp HE, Bonacic Kresic I, Ziegler-Graff V, Distéfano AJ.

17:45-18:00
MI-C16
ANTIVIRAL AND IMMUNOMODULATORY ACTIVITIES OF SYNTHETIC STIGMASTANE ANALOGS
Michelini FM, Ramirez JA, Molinari A, Galigniana M, Galagovsky LR, Alché LE.

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

18:30-19:30 ***"EMBO" LECTURE***

Reinhard Jahn
Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany
"SNARE proteins in neuronal exocytosis - interplay between proteins and lipids"
Chairperson: Luis S. Mayorga

21:30 ***CLOSING DINNER***

L01.
Plenary Lecture
CHALLENGING YOUR INTUITION
Paenza A.
Departamento de Matemáticas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

Is Math what they told us it was? Should we give it a second chance? Are we really aware of the Math that is around us? My experience in trying to send the message - Large Numbers - Monty Hall - Fair Division - The Random Feature in your CD or MP3 player - The chef, the pizza and how to cut it - Hair in our heads - How do I know that there are two people in the room who share their birthdays?

L02.
"AME" Lecture
ANTISENSE MODULATION OF ALTERNATIVE SPLICING: FROM THE BENCH TO THE CLINIC
Krainer AR.
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.

Spinal muscular atrophy (SMA) is a motor neuron disease, caused by loss-of-function mutations in the *Survival motor neuron 1 (SMN1)* gene. Patients retain one or more copies of the nearly identical, but splicing-defective *SMN2* gene. A single-nucleotide C6T transition in *SMN2* exon 7 leads to predominant skipping of the exon. As a result, *SMN2* only expresses a limited amount of full-length, functional SMN protein; the main product is an exon-7-skipped isoform, SMN7, which is unstable. The small amount of full-length SMN expressed from *SMN2* is essential for survival of SMA patients, but only partially compensates for the loss of *SMN1.* We previously developed a 2'MOE P=S antisense oligonucleotide (ASO-10-27 or ISIS-SMN_{Rx}) complementary to a potent splicing silencer in intron 7. This ASO efficiently promotes *SMN2* exon 7 inclusion and restores SMN protein levels in various tissues of SMA mouse models when delivered to the target tissues. I will discuss our recent data comparing CNS versus systemic delivery of ISIS-SMN_{Rx} in a severe SMA mouse model that survives only about 10 days. Unexpectedly, our results indicate that SMA is not motor-neuron cell-autonomous, and suggest that correction of *SMN2* splicing in peripheral tissues is necessary and sufficient for phenotypic rescue, at least in the context of the mouse model. I will also discuss the ongoing clinical trials of ISIS-SMN_{Rx}.

L03.
"PABMB" Lecture
THE *Clock* GENE: FROM CENTIMORGANS TO ÅNGSTROMS
Takahashi JS.
Howard Hughes Medical Institute, Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390-9111, USA.

The molecular mechanism of circadian clocks in mammals is generated by a set of genes forming a transcriptional autoregulatory feedback loop. The "core clock genes" include: *Clock, Bmal1, Per1, Per2, Cry1* and *Cry2.* The discovery of "clock genes" led to the realization that circadian gene expression is widespread throughout the body and that the clock is cell autonomous. The cellular autonomy of circadian clocks has raised a number of questions concerning synchronization and coherence of rhythms at the cellular level as well as circadian organization at the systems level.
Recently, we have focused on the biochemical mechanisms of the core circadian transcriptional regulators and have used structural biology and genomics to study the CLOCK: BAMAL1 complex and its genomic targets. Using x-ray crystallography, we have solved the three-dimensional structure of the CLOCK: BMAL1 heterodimeric complex. I will describe insights gained from this work. In addition, we have interrogated on a genome-wide level the cis-acting regulatory elements (cistrome) of the entire CLOCK: BMAL1 transcriptional feedback loop. This has revealed a global circadian regulation of transcription factor occupancy, RNA polymerase II recruitment and initiation, nascent transcription and chromatin remodelling.

L04.
Plenary Lecture
MEMBRANE DYNAMICS AND MECHANICS IN INTRACELLULAR SIGNALING
Lamaze C.
Laboratoire Trafic, Signalisation et Ciblage Intracellulaires, UMR 144 Curie/CNRS, Institut Curie, 26 rue d'Ulm, 75005 Paris, France.

Fifteen years ago, we could establish the first control of EGF signaling through clathrin-dependent endocytosis of its receptor, EGF-R. This work has opened a new field of research, at the crossroads of trafficking and signaling, that is intensively investigated today. In addition to the classical clathrin-dependent endocytosis, several so-called clathrin-independent endocytic pathways have been identified during the last ten years. We propose that these pathways can selectively control signaling activities of receptors and effectors through endocytosis and endosomal compartmentalization. I will address these new aspects of membrane trafficking through the activation of the JAK/STAT signaling pathway by interferons.
Cells perceive their microenvironment not only through signaling receptors, but also through physical and mechanical cues, such as extracellular matrix stiffness, confined adhesiveness and shear pressure of blood. Cells translate these stimuli by mechanotransduction into biochemical signals controlling multiple cellular functions. We have recently elucidated a novel aspect of mechanotransduction through a unique combination of cell biology and membrane physics experiments. We have investigated the mechanical role of caveolae, those characteristic plasma membrane invaginations that are particularly abundant in cells that experience mechanical stress. We could show that caveolae are mechano-sensors that play a major role in the homeostasis of the membrane tension of the cell membrane. We further showed that mechanosensing through caveolae involves the JAK/STAT signaling pathway. Caveolae are therefore mechanosensors and mechanotransducers, which constitute a physiological membrane reservoir to quickly accommodate sudden and acute mechanical stresses.

L05.
"SOLS" Lecture
PLANT DELLA PROTEINS AS COORDINATORS OF GROWTH IN TIME AND SPACE
Blázquez MA.
Instituto de Biología Molecular y Celular de Plantas (CSIC) Univ. Politécnica de Valencia, Spain.

Plant morphogenesis relies on specific patterns of cell division and expansion. However, little is known about the mechanisms that coordinate the timing and the direction of an organ's growth. In Arabidopsis and other plants, DELLA proteins are key repressors of growth. We have now found that DELLAs act as a relay between the signals that trigger growth in a specific time of the day, and the orientation of cortical microtubules in epidermal cells, which ultimately influence the direction of cell expansion. First, DELLA protein levels are regulated by the circadian clock, which promotes their degradation at the end of the night thus allowing maximal cell expansion rate at dawn; and second, physical interaction between DELLA proteins and the prefoldin complex, a cytosolic chaperone required for tubulin folding, results in the DELLA-dependent accumulation of the complex in the nucleus. As a consequence of the mislocalization of prefoldin, the availability of α/β-tubulin heterodimers is severely compromised and microtubules become disorganized. This model is confirmed by the observation that the daily rhythm of plant growth in natural conditions is accompanied by coordinated oscillation of DELLA accumulation, prefoldin subcellular localization, and cortical microtubule reorientation.

L06.
"COB" Lecture
COLLECTIVE CELL MIGRATION: UNION IS STRENGTH
Mayor R.
University College London; UK.

The Neural Crest forms in the neural folds at the border of the neural plate and gives rise to a huge variety of cells, tissues and organs. One of the astonishing characteristic of neural crest cells is that they are able to migrate very long distances in the embryo. The neural crest has been called the "explorer of the embryo" as it is one of the embryonic cell types that migrate most during development, eventually colonizing almost every tissue.
In this talk I will discuss our recent finding about neural crest migration. We have shown that neural crest cells, classically described as mesenchymal cells, migrate in large clusters and that interactions with neighbor cells are essential to control directional migration. We have found two kind of cellular interactions: repulsive interactions corresponding to contact inhibition of locomotion and mutual attraction between cells, that we called co-attraction. I will present our cellular and molecular data that identify collective cell migration as a feature of neural crest and the molecules responsible for such behavior. Finally, I will discuss some mathematical modeling that integrates different kinds of cell interactions to explain collective neural crest migration.

L07.
Short Lecture
INSULIN SIGNALS GOVERNING TRANSLOCATION OF GLUCOSE TRANSPORTER GLUT4 IN MUSCLE CELLS
Klip A.
The Hospital for Sick Children and The University of Toronto, Canada.

Skeletal muscle is the major site for glucose disposal following a meal. Glucose enters skeletal muscle through the GLUT4 glucose transporter. Insulin causes a net gain in surface GLUT4 units in muscle, brought about through a redistribution of GLUT4 from intracellular compartments to the plasma membrane PM. This process fails in obesity, causing insulin resistance leading to type 2 diabetes. GLUT4 is intracellularly retained through an idle cycle between a specialized compartment and recycling endosomes. Insulin-derived signals cause escape from cycling and a net gain in vesicles with GLUT4 that fuse with the plasma membrane via the SNARE proteins VAMP2 and syntaxin4. We currently study the insulin signals that govern GLUT4 translocation, using L6 muscle cells stably expressing myc-tagged GLUT4, RNAi silencing, transfection of mutants, and analysis of GLUT4 distribution by confocal and TIRF microscopy. We will show that insulin signals bifurcate downstream of PI3-kinase to activate Akt and Rac. Downstream of Akt lies the Rab-GAP AS160, its target Rabs 8A and 13, and their respective effectors myosin Va and MICAL-L2. These, along with Myosin 1c and cortical actin filaments, are required for GLUT4 translocation. Finally, I will indicate the steps that are defective in conditions causing insulin resistance.
Supported by the Canadian Institutes of Health Research.

L08.
Short Lecture
CELL POLARITY FROM THE INSIDE-OUT: HOW THE NUCLEUS ORGANIZES THE CYTOPLASM
Gundersen GG, Chang W, Folker E.
Department of Pathology & Cell Biology, Columbia University, New York, NY. USA.

In cells polarizing for migration, the nucleus is moved rearward by retrogradely moving actin cables that couple to the nucleus through novel structures termed transmembrane actin-associated nuclear (TAN) lines, which are assemblies of outer and inner nuclear membrane proteins nesprin-2G and SUN2 (Gomes, E *et al*, Cell, 2005; Luxton, G *et al*. Science, 2010). Disruption of TAN lines prevents nuclear movement and decreases cell migration, suggesting that rearward nuclear position is important for cell migration. We explored how nuclear positioning may affect cell migration by knocking down other nuclear membrane proteins and found that emerin and SUN1 knockdown disrupted rearward nuclear movement and instead caused chaotic nuclear movement. Myosin IIB knockdown also caused chaotic nuclear movement and emerin, SUN1 and myosin IIB knockdown all disrupted the directionality of actin flow suggesting that emerin and SUN1 affect nuclear movement by affecting actin flow, perhaps by regulating myosin IIB. Consistent with this, we found that emerin specifically interacted with myosin IIB and that the perinuclear localization of myosin IIB was dependent on emerin. Possible mechanisms for how emerin and SUN1 may contribute to myosin II activation and how perinuclear myosin II activation organizes actin flow will be discussed. Overall, our results suggest that the nucleus contains separate systems that function in the mechanical positioning of the nucleus (TAN lines) and in "reading out" the position of the nucleus by affecting the directionality of actin flow and the activation of myosin in the cell.

L09.
"HECTOR TORRES" Lecture
IMAGING PHAGOCYTOSIS: RECEPTORS, SIGNALING AND THE CYTOSKELETON
Grinstein S.
Cell Biology Program, Hospital for Sick Children, Toronto M5G 1X8, Canada.

Engulfment and elimination of microorganisms by macrophages, neutrophils and dendritic cells is an essential component of the innate immune response. This process, known as phagocytosis, involves extensive remodeling of the membrane and of the actin cytoskeleton. These responses are rapid, transient and highly localized, complicating their analysis by conventional biochemical means. We used digital imaging of live cells to analyze the spatio-temporal features of signal transduction during phagosome formation. During the course of particle engulfment, phosphoinositide-specific probes revealed large, transient and highly localized changes. We also developed novel probes to track the distribution and dynamics of phosphatidylserine (PS) in live cells by non-invasive means. Because PS and inositides confer negative charge to the inner aspect of the plasma membrane, we developed genetically-encoded probes to measure surface potential in live cells. Expression of such probes in macrophages revealed acute changes in the surface charge of the cytosolic leaflet of the plasma membrane, which were restricted to sites of phagocytosis and maturing phagosomes. These changes were attributable to phospholipid metabolism, with comparatively little change in PS content. Importantly the local alterations in surface potential were accompanied by dissociation from the membrane of important signal transduction regulators, such as K-Ras and especially Rac1, which drives actin polymerization during pseudopod extension. We concluded that lipids and the charge they contribute to the membrane play a key role as determinants of protein targeting and activation during phagocytosis.

L10.
Plenary Lecture
NEW WAYS TO SEE, THE CREATION OF MICROBIAL ROSETTA STONES
Dorrestein PC.
Skaggs School of Pharmacy and Pharmaceutical Sciences and Departments of Pharmacology, Chemistry and Biochemistry, UC San Diego, CA, USA.

In this lecture I will describe the latest mass spectrometry based tools and genome mining approaches we have developed to study the metabolic exchange of microbes.[1-7] The goal for visualizing molecules microbes and bridging this with genome mining is to enable the functional translation to understand their molecular language. The presentation will highlight microbial imaging mass spectrometry, the development of mass spectrometry based genome mining tools, in conjunction with live colony mass spectrometry, molecular networking, molecular dendrograms, and ambient mass microscopy to study metabolic exchange and ecology of microbial communities for applications in agriculture, diagnostics and therapeutic discovery. Finally, perhaps most significantly, this lecture will introduce the development of a molecular "Genbank" and its search engines, co-developed with Nuno Bandeira that will merge natural product analysis with modern genome mining.

References:
1. Yang YL, Xu Y, Straight P, Dorrestein PC. *Nature Chemical Biology* (**2009**) 5,885-7.
2. Wei-Ting Liu, Yu-Liang Yang, Yuquan Xu, Lamsa A, Haste NM, Yang JY, Ng J, Gonzalez D, Ellermeier CD, Straight PD, Pevzner PA, Pogliano J, Nizet V, Pogliano K, Dorrestein PC. *Proc. Natl. Acad. Sci. USA*. (**2010**) 107,16286-90.
3. Watrous JD, Dorrestein PC. *Nature Reviews Microbiology* (**2011**), 9, 683-694.
4. Yu-Liang Yang, Yuquan Xu, Kersten R, Wei-Ting Liu, Meehan MJ, Moore BS, Bandeira N, Dorrestein PC. *Angewandte Chemie* (**2011**), 50, 5839-42.
5. Kersten RD, Yu-Liang Yang, Yuquan Xu, Cimermancic P, Sang-Jip Nam, Fenical W, Fischbach MA, Moore BS, Dorrestein PC. *Nature Chemical Biology* (**2011**), 7, 794802.
6. Phelan VV, Wei-Ting Liu, Pogliano K, Dorrestein PC. *Nature Chemical Biology* (**2012**), 8, 2635.
7. Watrous J, Roach P, Alexandrov T, Heath B, Yang JY, Kersten RD, van der Voort M, Pogliano K, Gross H, Raaijmakers J, Moore BS, Laskin J, Bandeirac N, Dorrestein PC. *Proc. Natl. Acad. Sci. USA.,* **(2012),** in press.

L11.
"PABMB" Teaching and Communication in Science
LIFE OUTSIDE THE LAB: PUBLIC COMMUNICATION OF SCIENCE AS PART OF OUR JOB
Golombek D.
Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes/CONICET, Argentina. E-mail: dgolombek@unq.edu.ar

Public communication of science (PUS) is an academic field on its own, although its main actors are scientists in all fields of research. Here we shall discuss the responsibilities of scientists to communicate their results to broad audiences, including the use of different media, styles and formats. The sometimes conflictive relation between scientists and journalists will also be discussed. In addition, topics such as the perception of scientists performing popularization activities by their own community (i.e., the "Sagan effect") and the "deficit model" which assumes a vertical information transmission from experts to the public are considered important conflicts in science communication. Another key issue concerning the role of researchers in PUS is the evaluation of such activities by scientific institutions, which has only recently been acknowledged in our country. We will examine different formats and topics with which researchers may (and should) participate in PUS activities, leading to a significant increase in science literacy in the region.

L12.
"EMBO" Lecture
SNARE PROTEINS IN NEURONAL EXOCYTOSIS INTERPLAY BETWEEN PROTEINS AND LIPIDS
Jahn R.
Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, 37077 Göttingen, Germany.

Neurotransmitter release from presynaptic nerve endings is mediated by Ca^{2+} -dependent exocytosis of synaptic vesicles. Exocytotic membrane fusion is carried out by the SNARE proteins synaptobrevin/VAMP, syntaxin 1, and SNAP-25. Upon membrane contact, the vesicular SNARE synaptobrevin forms complexes with the plasma membrane-resident SNAREs SNAP-25 and syntaxin 1. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion ("zipper" hypothesis of SNARE function). The final steps of SNARE assembly are controlled by several additional proteins including the calcium sensor synaptotagmin, complexin, and the SM protein Munc-18.

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and *in vitro* fusion reactions with native and artificial membranes. Our recent results lend strong support to the zipper hypothesis, showing that during SNARE complex formation the helical bundle extends into the membrane and that at least *in vitro* a single SNARE complex suffices to bring about effective fusion of bilayers. Furthermore, SNARE-"mimetics" in which the SNARE motifs are replaced by simpler and better controlled artificial interaction domains are capable of inducing fusion *in vitro* (collaboration with U. Diederichsen), lending further support to the zippering concept. Moreover, we have studied intermediate states of the SNARE-dependent fusion pathway involving techniques such as cryo-electron microscopy, resulting in novel insights into the structure of fusion intermediates.

IUBMB-S01.
RECONSTITUTING BASIC STEPS OF SYNAPTIC VESICLE FUSION
Ma C, Su L, Xu Y, Seven A, Rizo J.
Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA.

Neurotransmitter release depends critically on: the SNAREs syntaxin-1, synaptobrevin and SNAP-25, which form SNARE complexes that bridge the vesicle and plasma membranes; NSF/SNAPs, which disassemble SNARE complexes; Munc18-1, which binds to syntaxin-1 and, together with Munc13, orchestrates SNARE-complex assembly; and the Ca^{2+} sensor synaptotagmin-1. Previous attempts to reconstitute neurotransmitter release revealed efficient fusion of syntaxin-1/SNAP-25-liposomes with synaptobrevin-liposomes in the presence synaptotagmin-1/Ca^{2+}, in stark contrast with physiological data showing that Munc18-1 and Munc13 are essential for neurotransmitter release. We now solve this paradox, showing that Munc18-1 displaces SNAP-25 from syntaxin-1 and that syntaxin-1/Munc18-1-liposomes fuse efficiently with synaptobrevin-liposomes in a manner that requires SNAP-25, Munc13-1 and synaptotagmin-1/Ca^{2+}. Moreover, when starting with syntaxin-1/SNAP-25-liposomes, NSF/α-SNAP disassemble the syntaxin-1/SNAP-25 heterodimers, thus inhibiting fusion, and fusion then requires Munc18-1 and Munc13-1. These results suggest that the pathway to fusion does not proceed through syntaxin-1/SNAP-25 heterodimers and starts at the syntaxin-1/Munc18-1 complex; Munc18-1 and Munc13 then orchestrate membrane fusion together with the SNAREs, synaptotagmin-1 and Ca^{2+} in a manner that is not inhibited by NSF/SNAPs.

IUBMB-S02.
***Caenorhabditis elegans dnj-14:* A MODEL OF HUMAN NEURODEGENERATIVE DISEASE**
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Cysteine string protein (CSP) is a synaptic vesicle-localized member of the Hsp40/DnaJ family of molecular chaperones. Drosophila and mouse CSP mutants are characterized by impaired neurotransmission, pre-synaptic neurodegeneration and premature mortality. Mutations in the human *dnajc5* gene, which encodes CSPα, cause a rare human neurodegenerative disorder known as adult-onset neuronal ceroid lipofuscinosis (ANCL). Investigating CSP function in simple model organisms may therefore shed light on potential therapeutic approaches for ANCL and other neurodegenerative diseases. To this end, we have been studying the single CSP homologue in *C. elegans*: *dnj-14*. RNAi knock-down or deletion of *dnj-14* resulted in a shortened lifespan and an age-dependent reduction in locomotion. This correlated with an age-dependent impairment in neurotransmitter release, as determined via indirect aldicarb assays. Mutation of *dnj-14* also resulted in neurodegeneration in aged worms, as indicated by reduced expression of a pan-neuronal GFP marker. A pilot chemical screen identified a compound that not only rescued the short lifespan of *dnj-14* mutants, but also rescued a distinct *C. elegans* neurodegeneration model based on expression of mutant human tau. Future studies of the *dnj-14* model may therefore have applications not only for ANCL, but more generally for human neurodegenerative diseases.

IUBMB-S03.
FUSOGENIC LIPIDS IN MEMBRANE FUSION: THE CASE OF PHOSPHATIDIC ACID
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Cells have evolved specific intracellular compartments that permit local concentration of macromolecules. These macromolecules are transported from one part of the cell to another and eventually released into the extracellular space to participate in cell-to-cell communication. Neurons and neuroendocrine cells secrete neurotransmitters and hormones by exocytosis, a highly regulated process in which secretory vesicles fuse with the plasma membrane to release their contents in response to a calcium trigger. To date, many proteins that catalyze the formation, targeting and fusion of secretory vesicles have been identified. However, the lipid composition of vesicles and their target membrane is also critical and lipid modifications may be required at several stages of the exocytotic pathway. I will present compelling evidence for a key role phosphatidic acid (PA) in different membrane fusion process. Among various possibilities our data indicate that PA most likely acts by changing the membrane curvature needed for membrane fusion to occur. Finally I will present results that indicate that several GTPases and kinases control the spatial and temporal coordination of these lipid modifications to form efficient membrane fusion machinery.

IUBMB-S04.
REGULATION OF MYOSIN MOTORS BY Rab GTPases: FOCUS ON MYOSIN II AND MYOSIN Va
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Rab GTPases make up the largest family of small GTPases, with over 60 members in humans. They reversibly associate with membranes via hydrophobic generylgeranyl groups attached to their carboxy-terminus and undergo a guanine-nucleotide dependent molecular switch. When in their GTP bound, "on" conformation, Rab proteins recruit a diverse range of "effectors" that serve to organise the four main steps in membrane transport: vesicle budding, delivery, tethering, and fusion with the target compartment.
Growing evidence indicate that actin-based motors of the myosin family represent an important class of Rab effectors. We will illustrate in two examples how Rab proteins regulate the activity of myosins: i) the Golgi-associated Rab6 that recruits myosin II for the fission of Rab6 positive transport carriers (Misery-Lenkei *et al*., Nat Cell Biol 2010, 12: 645); and ii) a subset of endosomal Rab proteins that interact with myosin Va for regulating, in concert with the actin cytoskeleton, membrane organization and topology of post-Golgi compartments (Lindsay *et al*., Submitted for publication).

IUBMB-S05.
TARGETING AND REGULATION OF VESICULAR SNAREs IN MEMBRANE TRAFFIC TI-VAMP/VAMP7's CASE

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Vesicular (v)- and target (t)-SNAREs play essential role in intracellular membrane fusion through the formation of cytoplasmic alpha-helical bundles. SNARE proteins need to be targeted to their proper donor or acceptor membrane. Furthermore, several v-SNAREs have a Longin amino-terminal extension which, by promoting a closed conformation, plays an auto-inhibitory function, decreases membrane fusion efficiency. Here we address the molecular mechanisms of targeting and activation of the Longin v-SNARE TI-VAMP/VAMP7.
We found that the TI-VAMP/VAMP7 partner Varp, a Rab21 guanine nucleotide exchange factor, interacts with GolginA4 and the kinesin 1 Kif5A. Activated Rab21-GTP in turn binds to MACF1, an actin and microtubule regulator, which is itself a partner of GolginA4. These components are required for directed movement of TI-VAMP/VAMP7 vesicles from the cell center to the cell periphery. We further show that exocytosis mediated by TI-VAMP/VAMP7 is activated by tonic treatment with insulin and IGF-1 but not by depolarization and intracellular calcium rise. In search of a potential downstream mechanism, we found that TI-VAMP is phosphorylated by c-Src kinase on Longin domain's Tyrosine 45. Accordingly, a phospho-mimetic but not phospho-dead mutation of Tyrosine 45 activates both t-SNARE binding and exocytosis.
The molecular mechanisms uncovered here suggest an integrated view of the transport and exocytosis of TI-VAMP/VAMP7 vesicles.

IUBMB-S06.
PARTICIPATION OF LIMK1, PKD1 AND BARS IN GOLGI OUTPOST FORMATION

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The neuronal Golgi apparatus localizes to the perinuclear region and also extends into dendrites as tubulo-vesicular structures designated as Golgi outposts (GO ps). Interestingly, NMDAR are sorted at the ER from AMPAR, bypassing the somatic Golgi and merging instead from GOps, raising the possibility that may serve as platforms for local delivery of synaptic receptors. Disruption of the dynein/dynactin complex reduces the number of GOps and dendritic branches, suggesting a direct relationship between their abundance and dendritic complexity. However, the crucial question of how GOps are generated remains unanswered. Two possible scenarios have been proposed, one involving fragmentation of the somatic Golgi followed by transport into dendrites and other one involving local de novo production from dendritic ER. Live cell imaging of cultured hippocampal neurons reveals that GOps are generated from the somatic Golgi by a sequence of events involving: 1) Tubulation of a Golgi cisterna; 2) Elongation and penetration into a dendrite (deployment); 3) Tubule fission; and 4) Condensation of the fissioned tubule. Suppression of LIMK1, PKD1 or BARs reduce the number of GOps and induce Golgi deployment/tubulation without fission; by contrast, ectopic expression of these proteins or LPA treatment or reelin stimulate Golgi outpost formation, an effect dependent on RhoA-ROCK and dynamin.

IUBMB-S07.
MECHANISMS OF CELL-CELL FUSION

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Cells fuse during fertilization and formation of organs. For example, macrophages, eye lens cells, placental cells, and muscle human cells fuse. We have described the pathways of cell-cell fusions in the epidermis and vulva of *Caeorhabditis elegans* and designed genetic screens and molecular studies of candidate fusogens. We initially approached cell fusion by mutational analysis, obtaining many mutations in two genes that we found are necessary for the cell fusion process. We identified EFF-1 and AFF-1, two type I membrane glycoproteins essential and sufficient for cell fusion in *C. elegans*. EFF-1 and AFF-1 are members of the first family of eukaryotic cell fusion proteins (fusogens). EFF-1 and AFF-1 from nematodes can fuse heterologous insect and mammalian cells. These fusogens are required in both fusing cells and the process is via the universal intermediate of hemifusion. We recently solved the three-dimensional structure of EFF-1 protein, we study the fusogenic activities of this protein in cells and in reconstituted in vitro systems. We will describe that AFF-1 and EFF-1 can substitute authentic viral fusogenic proteins and mediate fusion of viral envelopes to cells.

IUBMB-S08.
DECIPHERING THE ROLE OF PLANT CELL CYTOSKELETON AND MEMBRANES ON VIRUS ACCUMULATION AND MOVEMENT

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As obligate organisms, viruses require host factors to successfully infect and spread in their hosts. Once plant viruses are introduced into plant cells, they must replicate their genomes, move intracellularly to the plasmodesmata, and then be transported through the plasmodesmata to adjacent cells. We have centered our research toward understanding the mechanism(s) of intracellular transport of virus and virus components with a goal of discovering methods to limit their intercellular spread and induction of disease. For multiple plant viruses, whether RNA-based or DNA-based, the cytoskeleton and membranes have been shown necessary to transport virus-induced cytoplasmic complexes and ectopically-expressed viral proteins within the cell. In our recent studies with *Tobacco mosaic virus* (TMV), an RNA-based virus, and *Cauliflower mosaic virus* (CaMV), a DNA-based virus, we determined that actin-associated proteins influence their intercellular spread. For TMV, we have mapped the domain within the viral protein component necessary for this actinomyosin (XI-2) interaction. For CaMV, we have identified a non-myosin actin interactor (CHUP1) that influences its movement. In addition to these actin-associated proteins, for TMV we have identified membrane-associated proteins that influence its spread. Models of intracellular transport for these disparate viruses will be discussed.

IUBMB-S09.
REPLICATION AND DYNAMICS OF DENGUE VIRUS RNA

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Dengue virus (DENV) is the single most significant arthropod-borne virus pathogen in humans. In spite of the urgent medical need to control dengue infections, vaccines are still unavailable, and many aspects of DENV biology and pathogenesis remain elusive. The viral genome is a single stranded, positive-sense, RNA molecule that encodes a single long open reading frame. The promoter for DENV RNA synthesis is a large stem-loop structure located at the 5' end of the genome. This structure specifically interacts with the viral RNA dependent RNA polymerase NS5 and promotes RNA synthesis at the 3' end of a circularized genome. The circular conformation of the viral genome is mediated by long range RNA-RNA interactions that span thousands of nucleotides. Experimental data was obtained that supports the idea that alternative conformations of the viral RNA are crucial for infectivity. To investigate different mechanisms of viral RNA remodeling, the activity and properties of the viral NS3 helicase was analyzed. NS3 contains ATPase and helicase activities. Using different NS3 variants, we found that, besides the RNA unwinding activity, DENV NS3 greatly accelerates annealing of complementary RNA strands with viral or non-viral sequences. We propose that NS3 could function regulating the folding or unfolding of DENV RNA structures that are important for viral replication.

COB-S01.
SPATIO-TEMPORAL RHO GTPase SIGNALING TO THE CYTOSKELETON DURING NEURITE OUT GROWTH
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Rho GTPases are key regulators of the cytoskeletal dynamics that allow complex morphogenetic events such as neurite outgrowth to occur. Classical loss of function experiments, using dominant negative (DN) constructs, have suggested that the Rho GTPases Rac1 and Cdc42 regulate neurite extension, whereas RhoA controls growth cone collapse and neurite retraction. We developed novel biosensors that allow to measure the spatio-temporal dynamics of RhoA, Rac1 and Cdc42 activity in single living cells. Surprisingly, we observe a much more complex scenario in which all three GTPases are activated in distinct, micrometer-sized domains in growth cone filopodia. During growth cone collapse, at least RhoA and Cdc42 are globally activated throughout the growth cone. This suggests a much more complicated modality of spatio-temporal signaling than previously anticipated. We hypothesize that in each dynamic subcellular domain, a specific Rho GTPase activates precise effector pathways to fine tune cytoskeletal dynamics in time and space. Using the neurite proteome, and a RNA interference screen, we provide novel insight about the complex molecular circuitry that regulates these complex spatio-temporal Rho GTPase signaling programs.

COB-S02.
INTEGRIN-SPECIFIC SIGNALLING IN THE REGULATION OF CELL MOTILITY
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Cell invasion through extracellular matrix (ECM) is a hallmark of the metastatic cascade. Cancer cells require adhesion to surrounding tissues for efficient migration to occur, which is mediated through the integrin family of receptors. Integrins are known to be upregulated in a number of human cancers, however specific roles for these ubiquitous receptors in mediating cell invasion are poorly understood. Here, we demonstrate that human breast carcinoma cells show distinct morphological phenotypes in 2D and 3D matrices following silencing of either β1 or β3 integrins. Surprisingly, knockdown of either integrin resulted in enhanced cell invasion into two different models of 3D ECM. Furthermore, β3kd cells exhibited this enhanced invasion only in 3D cultures containing fibroblasts, whereas cells lacking β1 showed constitutively higher invasive potential *in vitro* and *in vivo*. Mechanistically, in situ analysis using FRET biosensors revealed that enhanced invasion in cells lacking β1 integrins was directly coupled with reduced activation of focal adhesion kinase (FAK) and the small GTPase RhoA resulting in formation of enhanced dynamic protrusions and invasion. This data demonstrates a specific role for different integrins in the modulation of a FAK-RhoA-actomyosin signalling axis to regulate cell invasion through complex ECM environments.

COB-S03.
ROLE OF SMALL HEAT SHOCK PROTEIN 20 IN INTRA-DERMAL MIGRATION OF MALARIA PARASITE TRANSMISSION STAGE
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Substrate-dependent motility in eukaryotic cells depends on regulated turnover of microfilaments. The *Plasmodium* sporozoite is a highly motile single cell eukaryote that employs its own actin/myosin-based motor machinery for gliding locomotion, penetration of cellular barriers, and invasion. Suggestive evidence has accumulated over the years that small heat shock proteins (sHSPs) contribute to modulation of microfilaments, although their potential role in actin-based motility has largely been ignored.
In our study, we asked whether *HSP20* of the malarial parasite regulates motility of the extracellular stages of the parasite life cycle. Using experimental genetics we observed that lack of *HSP20* profoundly affects the turnover of sporozoite-substrate adhesion sites. This defect translates into aberrant cellular speed and trajectories *in vitro***.** Loss of *HSP20* function profoundly impaired malaria transmission to the mammalian host by *Anopheles* mosquitoes. Bypassing the dermis by intravenous injection of sporozoites fully restored parasite life cycle progression. Apparently, colonization of the liver and productive invasion of the target cell, occurs normally in the absence of *HSP20*, highlighting a specific role for extracellular motility.
Our data provide the first genetic evidence for a role of small heat shock proteins in eukaryotic cell traction and migration.

COB-S04.
ADHESION RECEPTOR CROSSTALK DURING THE ENTERIC NEURAL CREST CELL MIGRATION IN THE EMBRYONIC GUT
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The colonisation of the developing gut by enteric neural crest cells (ENCC) is a remarkable migratory process, giving rise to the enteric nervous system (ENS) that controls the peristaltic, the immune response and secretary activity of the gut wall. ENCC express a repertoire of adhesion receptors during this process, including various integrins and N-cadherin. We analysed the role of β1-integrins, N-cadherin and ECM composition in the regulation of ENCC migration and ENS ganglia network.
The conditional ablation of β1-integrins in ENCC affects their migration and increases their aggregation. These defects lead to a distal aganglionosis resembling the Hirschsprung disease in Humans. The migration defect of β1-null ENCC occurs during the invasion of the caecum, a region rich in fibronectin and tenascin-C. B1-integrins are required to overcome the inhibitory effect of tenascin-C and to stimulate the fibronectin-dependent migration of ENCC. The results of double mutant studies where ENCC were devoided in β1-integrins and N-cadherin highlight the complex regulation of cell-cell and cell-matrix adhesion and the requirement for a correct balance etween these two types of adhesion during ENS ontogenesis.
This points out that the collective ENCC migration requires enough levels of both cell-cell and cell-ECM adhesion and the finely tuned expression of both N-cadherin and β1-integrin.

LI-S01.
LIPIDS AND THE LIVER: THE SYSTEMIC CONSEQUENCES OF HUMAN FATTY LIVER DISEASE
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Nonalcoholic fatty liver disease (NAFLD) results from the abnormal accumulation of lipids within hepatocytes, mostly in the form of triglycerides. This disease is commonly associated with abdominal obesity, type 2 diabetes, hypertension and dyslipemia, the typical components of the metabolic syndrome (MS). There is indeed a general agreement about NAFLD is the hepatic manifestation of MS. People with MS are at risk for cardiovascular disease, including coronary heart disease and stroke. The importance of NAFLD and its relationship with the MS is now increasingly recognized as recent data suggest that NAFLD is linked to increased cardiovascular risk independently of the broad spectrum of risk factors of the MS. Indeed, it is hypothesized that NAFLD is not merely a marker of cardiovascular disease but may also be involved in its pathogenesis, and that both entities share common pathways. We observed that the liver plays an important role in the development of CV disease, as a "pro-inflammatory" and "pro-atherogenic" organ. In fact, we observed that steatohepatitis increase atherosclerotic and cardiovascular risk by local overexpression of mediators of atherogenesis, endothelial damage, and regulators of blood pressure. In addition, we showed that epigenetic changes in liver DNA modulate peripheral insulin resistance and intermediate phenotypes of the MS.

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LI-S02.
LOSS OF NOCTURNIN, A CIRCADIAN DEADENYLASE, CONFERS RESISTANCE TO DIET-INDUCED OBESITY
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Recent evidence has demonstrated that circadian clocks control the rhythmic expression of a large number of genes involved in metabolism and other aspects of circadian physiology. Our studies have focused on a rhythmically expressed gene called *Nocturnin* which encodes a deadenylase an enzyme that removes the poly(A) tails from mRNAs and has been implicated in the regulation of mRNA stability or translatability. This gene is an output of the clock with peak expression in the early night in a wide range of metabolically-relevant tissues. The targeted disruption of the *Nocturnin* gene in mice confers resistance to diet-induced obesity. Mice lacking Nocturnin remain lean on high fat diets, with lower body weight and reduced visceral fat. However, unlike lean lipodystrophic mouse models, these mice do not have fatty livers. The mutant mice do not exhibit increased activity, reduced food intake or a higher metabolic rate. However, through *in vivo* and *in vitro* studies, we have determined that the *Nocturnin* knockout mice have deficits in lipid metabolism and in glucose and insulin sensitivity. We propose that Nocturnin has a role in the absorption of dietary lipid, presumably by altering genes necessary for metabolism through circadian post-transcriptional modifications of targeted transcripts.

LI-S03.
THE CARDIOMYOCYTE CIRCADIAN CLOCK INFLUENCES MYOCARDIAL LIPID METABOLISM
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Maintenance of circadian alignment between an organism and its environment is essential to ensure metabolic homeostasis. Synchrony is achieved by cell autonomous circadian clocks. Despite a growing appreciation of the integral relation between clocks and metabolism, little is known regarding the direct influence of a peripheral clock on cellular fatty acid/lipid metabolism. To address this important issue, we have recently generated two distinct mouse models wherein the circadian clock is genetically disrupted in a metabolically active organ (i.e., the heart) *in vivo*. These models are the cardiomyocyte-specific clock mutant (CCM) and cardiomyocyte-specific Bmal1 knockout (CBK) mice. Using these complementary models, we have revealed that the cardiomyocyte circadian clock directly regulates: 1) a host of genes involved in fatty acid and lipid metabolism; 2) triglyceride turnover; and 3) transcriptional, metabolic, and functional responsiveness of the heart to fatty acids (both acute and chronic). Collectively, these data provide evidence for direct regulation of fatty acid/lipid metabolism by a peripheral clock, and reveal a potential mechanistic explanation for accelerated metabolic pathologies following prevalent circadian misalignment in Western society.

LI-S04.
LIVER LIPIDIC HANDLING AND FOOD RESTRICTION: LINK BETWEEN MOLECULAR CLOCK AND METABOLIC ACTIVITY
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Daytime restricted feeding (DRF) is an experimental model used to study the interactions between the timing system and the metabolic networks. This protocol also underlies the expression of the so called food entrained oscillator (FEO). In the liver, it has been reported that food access by 2 h per day during 3 weeks is a potent synchronizer of the clock genes and various parameters of the circadian physiology. Using this procedure, we will provide evidence that DRF promotes rheostatic adaptations in peroxisomal markers in the liver in association with a high lypolitic activity from the adipose tissue (increased free fatty acids release). In these conditions, all hepatic PPARS (α, β and γ) showed altered 24 h-rhythmicity. Some factor that could be underlying the changes of lipidic handling of the liver during the expression of the FEO are: redox state (NAD^+/NADH ratio), energy charge (proportion of adenine nucleotides), PGC1-α (transcriptional factor), sirtuins (deacetylace enzymes). Taken together, the modifications of lipidic metabolism can be considered as a part of a more generalized adaptive response by which the organism optimize the nutrients handling during the DRF and the FEO expression.

PL-S01.
NITROGEN REGULATORY NETWORKS CONTROLLING PLANT ROOT GROWTH

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Nitrogen (N)-based fertilizers increase agricultural productivity but have detrimental effects on the environment and human health. Research is genrating improved understanding of the signaling components plants use to sense N and regulate metabolism, physiology, and growth and development. However, we still need to integrate these regulatory factors into signal transduction pathways and connect them to downstream response pathways. We used systems approaches to identify gene regulatory networks involved in N responses using *Arabidopsis thaliana* as a plant model system. Using next generation sequencing, microarray technologies and integrative network bioinformatics tools we are dissecting nitrate-regulatory networks controlling root growth. We will discurss our current experimental efforts towards mapping gene networks leading to nitrate induced changes in root system architecture. We will also discuss our new bioinformatics tools to identify new components of the nitrogen response in *Arabidopsis thaliana.* Systems biology approaches is accelerating the identification of new components and N-regulatory networks linked to other plant processes. A holistic view of plant N nutrition should open avenues to translate this knowledge into effective strategies to improve N-use efficienty and enhance crop production systems for more sustainable agricultural practices.

PL-S02.
CONSERVED GENE NETWORKS IN THE SEED AND BEYOND

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Seeds represent an evolutionary advantage for plant dispersal and growth resumption under optimal environmental conditions. Seeds are highly organised structures comprising maternal, embryo and endosperm tissues that undergo independent and coordinated developmental and metabolic processes. Unravelling the control of gene expression in the seed is of great significance both to basic and applied research.
I will present an updated review on the identification of critical elements in seed-gene promoters and interacting transcription factors (TFs) controlling their expression. Among them the C-group bZIPs (Opaque2-like) are important regulators of seed gene expression, but also participate in responses to abiotic stress conditions in vegetative tissues. Interestingly, some seed-specific genes and their regulators are conserved in non-seed plants like bryophytes, suggesting that they could derive from an ancestral network relevant to the conquer and adaptation to the land habit, and in the course of evolution recruited to the seed.

PL-S03.
POLYAMINES AND POLYAMINOOXIDASES IN PLANT DEFENSE AGAINST PATHOGENIC MICROORGANISMS

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Polyamines, natural polycationic compounds essential for all living organisms, are involved in a variety of physiological processes. Polyamines are positively charged at physiological pH, thus interacting with negatively charged macromolecules such as nucleic acids and proteins. Plant polyamine metabolism is affected by a number of stresses, including pathogen infection. Recently, the use of model plants such as *Nicotiana tabacum* and *Arabidopsis thaliana*, as well as novel inhibitors of polyamine catabolism, increased our knowledge on the role of PAs in plant defense.
Diamino and polyaminooxidases, which catabolize polyamines and release hydrogen peroxide, were shown to contribute to tobacco resistance to pathogenic bacteria, fungi and viruses. Modifications of plant spermine levels by different experimental approaches demonstrated that this tetraamine contributes to *A. thaliana* resistance to the biotrophic bacterium *Pseudomonas viridiflava* and cucumber mosaic virus. Interestingly, *in planta* spermine accumulation led to a variety of transcriptional changes related to plant defense. Further elucidation of PA-mediated changes in the expression of defense-related genes represents an interesting challenge for molecular plant pathologists.

PL-S04.
CHARACTERIZATION OF Hpa1 FROM *Xanthomonas axonopodis* pv. *CITRI* IN PLANT-PATHOGEN INTERACTIONS

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Xanthomonas axonopodis pv. *citri* (Xac), the bacterium responsible for citrus canker, contains a gene in the *hrp* [for hypersensitive response (HR) and pathogenicity] cluster that encodes a harpin protein called Hpa1. Harpins are glycine-rich, cysteine-lacking, heat-stable proteins that trigger HR responses. Xac Hpa1 elicited HR in nonhost plants, whereas, in the host plant citrus, it elicited a weak defence response with no visible phenotype. Recombinant Hpa1 protein aggregated bacterial cells and co-infiltrations of Xac with Hpa1 in citrus leaves increased the number of cankers. To characterize the effect of Hpa1 during the disease, a XacΔhpa1 mutant was constructed, and infiltration of this mutant caused a smaller number of cankers. Also, the lack of *hpa1* hindered bacterial aggregation both in culture medium and *in planta*. Moreover, analysis of citrus leaves infiltrated with Hpa1 revealed alterations in mesophyll morphology. We expressed the N-terminal and C-terminal regions and found that, although both regions elicited HR in nonhost plants, only the N-terminal region produced increased virulence and bacterial aggregation, supporting the role of this region of the protein as the main active domain. Our results suggest that, Hpa1 main roles in citrus canker are to alter leaf mesophyll structure and to aggregate bacterial cells, and thus increasing virulence and pathogen fitness.

MI-S01.
EVOLUTIONARY DYNAMICS OF *Pseudomonas aeruginosa* IN CYSTIC FIBROSIS AIRWAYS
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P. aeruginosa is a versatile environmental organism with a broad metabolic repertoire, which is useful both in relation to changing environmental conditions and in connection with infections of plants, fungi and animals/humans. We have in recent years conducted a comprehensive study of the genomic and phenotypic dynamics of airway infecting *P. aeruginosa* lineages in a number of CF patients associated with the Copenhagen CF clinic in Denmark. Since 1973 bacterial isolates from lung expectorates of CF patients have been collected and stored in the clinic, and this strain collection represents bacterial evolution and adaptation covering up to 200,000 bacterial generations of growth in CF airways. The approach we have taken has been to combine full-genome sequencing of longitudinal isolates of *P. aeruginosa* from a number of chronically infected patients with global gene expression analysis and other types of phenotypic characterization. Interestingly, our data show that two dominant and transmissible lineages in the Copenhagen CF Clinic have followed very different evolutionary trajectories, suggesting that there could be several routes towards persistence of colonization of the CF airways. In one of these lineages a small number of early mutations in global regulatory genes seem to be highly important for the coloization success of this clone, and we are currently investigating the combined impacts of these mutations on the overall bacterial phenotype. The common evolutionary profile of the lineage based on analysis of a large number of patients is one of relative phenotypic and genetic homogeneity across the patients, suggesting that the early adaptive regulatory mutations found in all isolates from all infected patients resulted in optimal fitness followed by random neutral mutations with no significant phenotypic consequences.

MI-S02.
EISOSOMES AND PLASMA MEMBRANE ORGANIZATION
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Membrane compartmentalization allows the spatial segregation of different functions, such as signal transduction and protein trafficking, and ensures their fidelity and efficiency. Studies on the budding yeast *Saccharomyces cerevisiae* have revealed that fungal plasma membranes are organized in different subdomains. One of them termed eisosomes consists of punctate patches that are distinct from lipid rafts. Eisosomes constitute 300 nm long and 50 nm deep nanoscale furrow-like invaginations of the plasma membrane where proteins and lipids segregate. The core components of eisosomes, paralog proteins Pil1 and Lsp1, are membrane-sculpting BAR (BIN/Amphiphysin/Rvs) proteins. Purified recombinant Pil1 and Lsp1 tubulate liposomes and form tubules when expressed in mammalian cells. Structural homology modeling and site-directed mutagenesis indicate that Pil1 positively charged surface patches are needed for membrane binding and liposome tubulation. Sphingolipid signaling mediator proteins Slm1 and Slm2, have F-BAR domains and that these domains are needed for targeting to furrow-like plasma membrane invaginations. Unlike any other known BAR domain-containing protein, Pil1 and Lsp1 form stable and static curved domains *in vivo*. Eisosomes represent a fantastic model to address the mechanistic understanding of curvature-induced membrane nanodomains formation and signaling compartmentalization in living cells.

MI-S03.
DEVELOPMENT OF AN ORAL VACCINE PLATFORM BASED ON THE PROTECTIVE PROPERTIES OF SURFACE PROTEINS OF THE INTESTINAL PARASITE *Giardia lamblia*
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Despite the impact of world-wide vaccination programs, which have significantly reduced the incidence and mortality of infectious diseases, there is still a great necessity to develop novel, cheap and safe innovative vaccination strategies inducing long-lasting immunity. Promising approaches include innovative delivery systems for efficient antigen-presentation. Since most infectious agents invade the organism via mucosal surfaces, adaptive mucosal immunity plays a central role in protecting the host against infections. Oral administration of vaccines represent a very attractive option, notably because it is non invasive and suitable for mass vaccination. However, the main impediment for oral vaccine development has been that orally administered antigens are easily destroyed by the gastrointestinal tract or potentially capable of inducing immune tolerance. The intestinal parasitic protozoan *Giardia lamblia* expresses at its surface variant-specific surface proteins (VSPs) that are extremely resistant to the low pH of the stomach as well as to intestinal proteases, allowing the parasite to survive in the harsh environmental conditions of the small intestine. These VSPs are able to induce potent mucosal and systemic immunity against this diarrhea-causing parasite upon immunization via the oral route. In addition, It has been reported that retrovirus-based virus-like particles (VLPs) given by injection are efficient immunogens to induce both cellular and humoral responses, particularly inducing potent neutralizing antibody responses. We thus hypothesized that the expression onto VLPs of *Giardia* VSPs should shield these particles for oral administration. This should result in efficient cellular immune responses against epitopes presented inside the particles and neutralizing antibody response against heterologous envelope protein pseudotyped onto the particles together with the shielding VSPs. To obtain a proof of principle and, simultaneously, to develop a potential vaccine candidate, we used Influenza Hemagglutinin (HA) as a vaccinal antigen, for which we have already established all the procedures to monitor cellular and humoral anti-HA immune responses, including challenge experiments with live virus. We produced our vaccines composed of VSP-HA chimeric proteins or HA-expressing VLPs covered with VSPs and HA and the corresponding controls. Our results clearly demonstrated that *Giardia* VSP can protect vaccinal antigens in the gastrointestinal track for oral administration of vaccines, generating strong T and B cell-mediated protective responses. The development of this universal platform for oral delivery of vaccines should have a broad application to different infectious diseases

MI-S04.
TRANSCRIPTIONAL REGULATION OF LIPID BIOSYNTHESIS IN MYCOBACTERIA
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Mycolic acids (MA) are major components of the cell envelope of mycobacteria and play an important role in its architecture and interaction with the environment. Biosynthesis of MA involves two fatty acid synthase systems, FAS-I and FAS-II, which should work in a coordinate manner to keep lipid homeostasis tightly regulated. Our pioneer studies demonstrated that in mycobacteria, the expression of the *fasII* operon is regulated at the transcriptional level by MabR; which binds to an operator sequence present in the *fasII* promoter region, modulating in this way the biosynthesis of MA. Interestingly, overexpression of MabR not only affects the expression of *fasII* genes but also of *fas*, providing strong evidences of the existence of a sophisticated regulatory network involved in the regulation of the two mycobacterial FAS systems at the transcriptional level. Here, we present the *in vivo* and *in vitro* characterization of FasR, a new regulatory protein that specifically binds p*fas* to regulate the *de novo* fatty acid biosynthesis in mycobacteria. We also present evidences that long-chain acyl-CoAs are the effector molecules that coordinate the expression of the two FAS systems at the transcriptional level. A better understanding of this complex process of regulation of lipid homeostasis in mycobacteria will greatly contribute to the development of new strategies to control this disease.

CB-C01.
THE PALMITOYLTRANSFERASE SWF1 IS A BONA FIDE ZINC BINDING PROTEIN

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S-acylation of proteins is catalysed by a family of palmitoyltransferases characterised by the DHHC cysteine rich domain. Swf1 is a member of this family, responsible for the modification of SNAREs and glycosyltransferases in yeast. We carried out a random mutagenesis assay designed to uncover essential amino acids in Swf1 and identified 22 novel loss-of-function mutations, most of which are localised within the DHHC domain. Homology modelling of the tertiary structure of Swf1 DHHC-CRD shows that it could contain two C3H zinc binding pockets, in a structure formed by three beta hairpins. The screen revealed that mutation of each of the eight amino acids predicted to be involved in zinc coordination results in inactive Swf1. All of these mutations render Swf1 less stable, suggesting a structural role for Swf1 zinc fingers. Sequence conservation studies of the amino acids that form each zinc binding pocket indicate that upon mutation of one of them the negative selection on the others is lost, in agreement with a function which requires all four of them. Finally we show directly that recombinant Swf1 DHHC-CRD domain is able to bind zinc. Conservation and complementation studies suggest that the DHHC domain of palmitoyltransferases which have lost the amino acids that coordinate zinc may still acquire a similar structure by alternative stabilization mechanisms.

CB-C02.
CNBP UNFOLDS GUANINE CUADRUPLEX IN PROTO-ONCOGENES PROMOTERS

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Cellular nucleic acid binding protein (CNBP) is a conserved nucleic acid chaperone protein associated to cell proliferation and survival processes. CNBP binds to a G-rich sequence present in the *c-MYC* promoter up-regulating its expression. Recently, we have shown that CNBP modulates *in vitro* the folding of guanine quadruplex (G4) structures present in the *c-MYC* promoter. G4s are nucleic acid secondary structures formed by the stacking of two or more planar layers of four guanines interacting by Hoogsteen hydrogen bonds and stabilized by monovalent cations. G4s have been found with a high frequency in the control regions of human proto-oncogenes. Here we evaluated the effect of CNBP on G4s present in the promoter of several proto-oncogenes by Circular Dichroism (CD) and Polymerase Stop Assays (PSA). CD spectra of RET, HRAS and BCL2 G4s did not change in presence of CNBP; however, it decreased the characteristic parallel G4 positive signal observed for *c-MYC*, VEGF, *c-KIT*, HIF-1a, KRAS and PDGF G4s. In agreement, PSA data indicate that CNBP destabilizes structured G4 in the latter proto-oncogenes. Data suggest a relationship between the CNBP effect and the intrinsic stability of the G4s, which may depend on the length of their central loop. Further characterization of the role of CNBP on proto-oncogene expression is being carried out using cell culture lines overexpressing CNBP.

CB-C03.
NA+/ CA2+ AND NA+/CA2+-K+ EXCHANGERS: BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION IN HUMAN PLATELETS

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Congenital Disorders of Glycosylation (CDG) are human genetic diseases due to defects in the synthesis of N-, O-glycoproteins, and even in the synthesis of glycolipids. The clinical features range from a severe multisystem to mild phenotype. Thrombus-hemorrhagic events are frequently observed in these patients. In platelets tight regulation of Ca2+ signaling is necessary to prevent inappropriate thrombus formation. The Na+/ Ca2+ (NCX) and Na+/Ca2+-K+ (NCKX) exchangers play a crucial role in controlling cytosolic [Ca2+]. The aim of this work is to contribute to the characterization of these exchangers in a human megakaryocytic cell line (DAMI cells) and human platelets. Our results demonstrate (i) the presence of NCX and the NCKX proteins in DAMI cells and human platelets by western blot, (ii) that both types of exchangers are functional by measurements of the Ca2+ [^{45}Ca] uptake, (iii) the presence of two likely N-glycosylation sites in NCX protein and different N- and O-glycosylation sites for NCKX1 in the hydrophilic N-terminal segment "by bio-informatics analysis", and (iv) that exchanger proteins are heavily glycosylated, by lectin affinity chromatography and immunoprecipitation analysis. Future studies will elucidate the functional role of glycosylation in the targeting and activity of NCX and NCKX exchangers in human platelets and CDG patients.
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CB-C04.
MOLECULAR STUDIES IN EXT1 AND EXT2 GENES IN MULTIPLE OSTEOCHONDROMATOSIS PATIENTS (EXT1/EXT2-CDG)

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Mutations in two tumors suppressor genes, EXT1 (8q24) and EXT2 (11p11-p13), have been identified in multiple osteochondromatosis (EXT1/EXT2-CDG) an autosomal dominant disorder in O-glycosylation pathway. These genes encode glycosyltransferases (ext1 and ext2) involved in heparan sulfate biosynthesis. Mutations in EXT disturbs the binding of growth factors in chondrocytes and loss of heterozygosity (LOH) in mosaic cells produces osteochondroma formation. Aim: To contribute to the knowledge of the molecular bases of EXT1/EXT2-CDG. Clinical diagnosis was achieved in 33 patients. Genomic DNA from peripheral blood cells and tissue samples were obtained. The EXT1 and EXT2 exons were studied by PCR, Sanger sequencing and Multiple Ligation Probe Amplification (MLPA) studies. The EXT1 promoter was genotyped. Western blot of ext proteins were analyzed in osteochondromas tissues samples. Our results demonstrate: (a) 69% mutations in EXT1or EXT2 patients (b) 20% were deletions identified by MLPA (c) no mutation detected in 31% of the patients (c) absence of loss of heterozygocity (d) differential expression of ext1 or ext2 in patients with EXT mutations as well as in patients without mutations detected. Disease causing mutations in 31% patients remain unknown. It led us to investigate in to a putative EXT3 locus or in a transcriptional regulatory mechanism in EXT genes.
CONICET, FONCyT, UCC.

CB-C05.
C/EBPß AND EGR-1 COORDINATELY REGULATE *Chka* EXPRESSION DURING RA-INDUCED NEURONAL DIFFERENTIATION

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Neuronal differentiation is characterized by neuritogenesis and neurite outgrowth, processes which are dependent on membrane biosynthesis. We previously demonstrated that during retinoic acid (RA) induced differentiation of neuroblastoma cells, the augmented phosphatidylcholine (PC) synthesis is supported by the sequential expression of two enzymes of the Kennedy pathway: choline kinase alpha (CKa) and CDP:phosphocholine cytydilyltransferase alpha (CCTa). We are interested in elucidating the mechanism by which RA induces the expression of *Chka* gene. Using promoter reporter assay, electromobility gel shift assay, analysis of point mutations and overexpression of different transcription factors we demonstrated that the region between -953 and -901 bp of *Chka* promoter is essential for RA induction. This region contains C/EBP and Egr binding sites which are implicated in *Chka* transcriptional induction. We demonstrated that C/EBPß overexpression promote *Chka* expression and stimulates neuronal differentiation even in the absence of RA, and we also observed that Egr is necessary for *Chka* response to RA. In summary, these results suggest that *Chka* regulation by RA is a complex mechanism in which the transcription factors C/EBPß and Egr participate together to induce expression of *Chka*.

CB-C06.
THE RELATIONSHIP BETWEEN *Coxiella burnetii* AND Rho GTPases OF HOST CELL DURING INFECTION

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Phagocytosis is an important host defense mechanism against pathogens. In this process the actin cytoskeleton and GTPases of Rho family play important roles. *Coxiella burnetii (Cb)* is an intracellular pathogen that enters into host cells by a mechanism poorly characterized. We have evidence that GTPases of Rho family are involved in the interaction *Cb*-host cells. In this work we investigate the roles of RhoA, Rac1 and Cdc42 in the *Cb* internalization. In order to do so, HeLa cells were treated with Toxin B, an inhibitor of Rho GTPases, and infected with *Cb*. We observed that Toxin B diminished *Cb* uptake. Similar results were observed in HeLa cells overexpressing the dominant negative mutants of the Rho GTPases. These results suggest that these GTPases are important for *Cb* internalization. Since RhoA showed the strongest effect, we decided to examine the involvement of RhoA effectors ROCK and mDia1 on *Cb* entry. By using Y27632, an inhibitor of ROCK, we observed in treated cells a decrease in *Cb* uptake. On the other hand, cells transfected with pEGFP encoding the negative mutant mDia1-ΔN1 (RhoA binding domain) showed a lower level of internalization regarding those transfected with pEGFPmDia1-full length (FL) or -ΔN3 (actin polymerization domain). These results suggest that the effectors of RhoA, ROCK and mDia1, participate in the phagocytosis of *Cb*.

CB-C07.
BIOCHEMICAL CHARACTERIZATION OF MITOCHONDRIAL COMPLEX III INHIBITION BY NITRIC OXIDE

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Little is known about how nitric oxide (NO) interacts with the NO-reactive component of mitochondrial complex III. The aim of this work was to study the inhibitory effect of NO on electron transfer between cyt. *b* and cyt. *c* using beef heart inside-out particles (ETPH-Mg^{2+}). Succinate-cytochrome *c* reductase activity (222‡4 nmol/min.mg protein) was inhibited (51%) by 500 µM GSNO; this activity was also reduced (36%) when ETPH-Mg^{2+} were incubated in the presence of L-arginine and mtNOS cofactors, suggesting that this effect is caused by mtNOS-produced NO. In mitochondrial membranes, ~240 nM NO reduced cyt. b_{562} by 70%. The effective [NO] was assessed using a NO-sensitive electrode: 500 µM GSNO releases 240 nM NO to the reaction medium when the assay is achieved during 2 min. NO produced an increase in $O_2^{\cdot-}$ and H_2O_2 production rates with a maximal effect at 500 µM GSNO (1.3±0.1 nmol $O_2^{\cdot-}$/min.mg protein; 0.64±0.05 nmol H_2O_2/min.mg protein). The $O_2^{\cdot-}/H_2O_2$ ratio was 2.0 in accordance to the stoichiometry of the $O_2^{\cdot-}$ dismutation reaction. ETPH-Mg^{2+} incubated in the presence of succinate showed an EPR signal at g~1.99, compatible with a stable semiquinone (UQH•), which was increased by NO. The interaction of NO with complex III leads to electron transfer inhibition, in an $[O_2]$ independent manner, with a $[UQH_{\cdot}]_{ss}$ enhancement, which in turn, generates an increase in $O_2^{\cdot-}$ and H_2O_2 production rates

CB-C08.
HETEROCHROMATIN LOCALIZATION OF p19INK4d IS ASSOCIATED WITH SENESCENCE TRIGGERED BY GENOTOXICS

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Cellular senescence is a permanent cell cycle arrest triggered by exogenous or endogenous stress. The DNA Damage Response (DDR) and global induction of heterochromatin (SAHF, senescence-associated heterochromatic foci) have been involved in the irreversibility of senescence. SAHF are proposed to enforce cellular senescence by suppressing the transcription of genes involved in proliferation.
Previous work showed that senescent stimuli upregulates p19INK4d (p19) at both its mRNA and protein levels. We observed a significant increase in the percentage of cells undergoing senescence when p19 is stably overexpressed compared to wild type or p19 deficient counterparts. In this work, we evaluated the mechanism involved in p19 induction and the potential role of p19 in replicative and genotoxic-induced senescence. Reporter gene assays using the p19 promoter suggest that its induction is mediated by E2F1 in genotoxic but not replicative senescence. A senescence stimulus caused p19 translocation to the nucleus where it binds to chromatin enriched in HP1, suggesting its preferential interaction with heterochromatin. Moreover, p19 overexpressing cells exhibit reduced MNase chromatin accessibility. In vivo correlation was observed since mice tissue display an age-dependent increase in p19. We propose that p19 is involved in senescence playing a role in global heterochromatin arrangement.

CB-C09.
ACTIVATION OF THE ALDOSE REDUCTASE BY DIRECT INTERACTION WITH TUBULIN

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Previously we demonstrated that high glucose concentrations induces polimerization of microtubules, formation of acetylated tubulin/Na^+,K^+-ATPase (NKA) complex and consequently inhibition of enzymatic activity. The increase in the content of microtubules is due to increased levels of sorbitol formed by the activation of the aldose reductase (AR). This enzyme forms a complex with tubulin, which result in the increase of AR activity. In this work we study the effect of the tubulin on the AR activity . For this, we purified tubulin from rat brain, and AR by expression of AKR1B1 (human AR) gen in E. coli BL21a strain. With the purified proteins we determine the interaction between both of them and the effect on the enzymatic activity. The results indicate that: i) both proteins interact in direct form, ii) tubulin increases the AR activity more than seven times, iii) tubulin has not effect when it is polymerized before interaction with AR, and iv) both tyrosine and nitrotyrosine are able to prevent in vitro the association between tubulin and AR and consequently the enzymatic activation. These results suggest that AR activity is regulated by direct interaction with tubulin and that the enzymatic activity is involved in the tubulin polymerization and regulation of NKA activity when the cells are subjected to high concentrations of glucose.

CB-C10.
THE ACTIN CYTOSKELETON AND A RhoA-CONTROLLED PATHWAY PARTICIPATED IN STARVATION MEDIATED AUTOPHAGY

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Autophagy is process in which a double membrane vesicle sequesters cytoplasmic material destined to degradation. In cells exists a basal level of autophagy that could be enhanced by different stimulus. Initially, autophagosome formation requires membrane remodeling to generate a vesicle which subsequently matures in a process that involves fusion with other compartments. In the current work we have demonstrated that actin is necessary for starvation-mediated autophagy. When the actin cytoskeleton was depolymerized, the increase in autophagic vacuoles in response to the starvation stimulus was abolished without affecting the maturation of the autophagosomes. Interestingly, actin filaments colocalized with Atg14, Beclin 1 and PtdIns3P-rich structures stained with the double FYVE domain or the protein DFCP1. In addition, we have found that a RhoA-controled pathway has a regulatory function on starvation-mediated autophagy. Overexpression of RhoA mutants or depletion of the protein, as well as inhibition of some downstream effectors, hampered starvation-mediated autophagy. Also, similar to actin, RhoA colocalized with Beclin1 suggesting that both protein are involved in the same stage. Taken together, our data indicate that the actin cytoskeleton and the RhoA and its effectors, have a key role at very early stages of autophagosome formation linked to the PtdIns3P generation step

CB-C11.
THE ENDOSOMES AND THE GOLGI COMPLEX ARE INVOLVED IN THE INFECTIOUS BURSAL DISEASE VIRUS LIFE CYCLE

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Infectious bursal disease virus (IBDV) is a dsRNA virus that causes severe disease in chickens. We have defined the cellular localization of replication complexes of IBDV based on colocalization analysis of two main viral components, the proteins VP3 and VP1. Our results indicate that VP3 and VP1 localized to vesicular structures with features of early and late/lysosomal endocytic compartments located in the juxtanuclear region of infected cells. Employing well-established Golgi complex markers we found that VP3-containing vesicles were closely associated to the Golgi apparatus. Depolymerizing the microtubule network with nocodazole, which reorganizes the Golgi complex into mini-stacks, and Brefeldin A-treatment, that causes the dispersion of the Golgi complex, caused a profound change in the VP3 localization depicting a punctate distribution scattered throughout the cytoplasm, remaining strongly associated to the Golgi mini-stacks. Analysis of intracellular viral protein levels and viral infective particles in BfA-treated infected cells indicates that these parameters were markedly affected, suggesting a role for the Golgi complex in the replication of IBDV. These results constitute the first study toward elucidating the subcellular localization of IBDV replication complexes and in establishing a link between the endo/lysosomal and the secretory pathways with the process.

CB-C12.
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. Autophagy has been associated with several physiologic processes as erythroid maturation. LC3 is a protein present in autophagosomal membrane, therefore is considerate as a bonafide marker of this structure. Our results indicate that hemine (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 in K562 cells incubated with hemin, an enlargement GFP-Lamp1 positive vesicles labeled with mitotracker. We have also shown in erythroblastic leukemic cells co-expressing RFP-LC3 and GFPRab11 (a multivesicular bodies marker) and incubated in the presence of hemin enlarged vesicles labeled with both markers. We have performed western blot analysis 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 GFP-LC3. Taken together, our results suggest that hemin could induce an autophagic response in K562 cells, probably allowing an efficient and faster maturation.

CB-C13.
GEOMETRIC FEATURES OF TRANSMEMBRANE DOMAINS ARE MAJOR DETERMINANTS OF GOLGI LOCALIZATION

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Subcellular localization of bitopic transmembrane (TM) proteins is achieved through a variety of mechanisms, of which the most studied is direct or indirect interaction with coatomers using citoplasmic small linear motifs (SLIMs). However, vesicular transport from the Golgi to the plasma membrane is mostly carried out in vesicles that apparently form without the need of a coatomer. Additionally, no SLIMs have been described to determine Golgi export, and only one SLIM has been proposed to participate in Golgi retention, though our bioinformatic queries have estimated that it is only present in 12% of Golgi resident proteins. We have constructed a dataset of proteins with known subcellular locations and studied their TM domains. Geometric properties of TM domains seem to determine Golgi retention or Golgi export, independently of any SLIMs present in cytoplasmic tails of integral membrane proteins. Additionally, using the Saccharomyces cerevisiae SNAREs Sft1 (Golgi apparatus) and Sso1 (plasma membrane) as model proteins, we modified the length and amino acid composition of their TMDs and determined their subcellular localization both in yeast and in mammalian cells. We find that short TMDs with voluminous exoplasmic hemi-TMDs confer golgi residence while longer TMDs with less voluminous exoplasmic hemi-TMDs confer plasma membrane residence to these proteins.

CB-C14.
EFFECT OF A RNA-BINDING PROTEIN (RBP) ON THE DYNAMICS OF THE MOLECULAR CIRCADIAN CLOCK

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Most living beings exhibit physiological and behavioural circadian rhythms with an endogenous period of about 24 h, which can be synchronized and anticipate to periodic cues. At molecular level, the circadian timekeeping mechanism is driven by a set of genes, called clock genes which interact in oscillatory transcriptional-translational networks within cells. The majority of mammalian cells have a circadian molecular clock and they are coordinated by a master circadian pacemaker in the mammalian brain called the suprachiasmatic nucleus (SCN).
Numerous observations have revealed the importance of post-transcriptional regulation on circadian gene expression (Garbarino-Pico and Green 2007) and recent studies have demonstrated that several clock genes are post-translationally regulated by RBPs (reviewed in Lowrey and Takahashi 2011 and Garbarino-Pico et al., 2011) .
In the present work we show numerical results obtained with a mathematical model for the circadian molecular clock, which includes the regulation of Per mRNA translation by a RBP. We found that depending on the values of the RBP-associated parameters, the period and amplitude of the oscillator are affected. The magnitude of those changes depend on whether the repression of transcription is assumed as a cooperative or non-cooperative event.

CB-C15.
DIFLUOROMETHYLORNITHINE (DFMO) IS A NEW INHIBITOR OF AUTOPHAGY AND *Trypanosoma cruzi* INFECTION

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Autophagy is a cell process that in normal conditions serves to recycle cytoplasmic components and aged or damaged organelles. The autophagic pathway has been implicated in many physiological and pathological situations, even during the course of infection by intracellular pathogens. Recently it was demonstrated that the polyamine spermidine is a physiological inducer of autophagy. Otherwise, autophagy induction significantly increases host cell colonization by *T. cruzi*, the etiological agent of Chagas disease. In this work we have analyzed the effect of polyamine depletion on the autophagic response of the host cell and on *T. cruzi* infectivity. Our data show that depleting intracellular polyamines by inhibiting the biosynthetic enzyme ornithine decarboxylase with DFMO suppressed the induction of autophagy associated with a decrease in protein abundance of LC3 and Atg5, two proteins required for autophagosome formation. As a consequence of inhibiting host cell autophagy, DFMO impaired *T. cruzi* colonization, indicating that polyamines and autophagy facilitate parasite infection. While other autophagy inhibitors are nonspecific and potentially toxic, DFMO is an FDA approved drug that may have value in limiting autophagy and spread of the infection in Chagas disease and possibly other pathological settings.

CB-C16.
***Trypanosoma cruzi* BDF3 BINDS ACETYLATED α TUBULIN AND MIGRATES TO THE FLAGELLUM DURING METACYCLOGENESIS**

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Bromodomains are highly conserved acetyl-lysine binding domains found in nuclear proteins. The *Trypanosoma cruzi* genome encodes five Bromodomain Factors. Bromodomain Factor 3 (*Tc*BDF3) cytolocalization was determined using purified antibodies by western blot and immunofluorescence in all life cycle stages of *T. cruzi*. In epimastigotes and amastigotes it was detected at the cytoplasm, the flagellum and the flagelar pocket and in trypomastigotes only at the flagellum. Subcellular localization of *Tc*BDF3 was also determined by digitonin extraction, ultrastructural immunocytochemistry and expression of *Tc*BDF3 fused to Cyan Fluorescent Protein (CFP). The expression of a truncated version of the protein having only the bromodomain leads to growth retardation and lower yields of trypomastigotes in in vitro metacyclogenesis experiments. Acetylated a tubulin has been found in the axoneme of flagella and cilia of different organisms. To study the possible interaction between *Tc*BDF3 and tubulin we performed colocalization assays in isolated cytoskeletons and flagella from *T. cruzi* epimastigotes and trypomastigotes. Interaction between the two proteins was confirmed by coimmunoprecipitation and far western blot assays with synthetic peptides and recombinant TcBDF3. The results suggest that *Tc*BDF3 may be involved in the remodeling of the cytoskeleton during the metacyclogenesis process.

CB-C17.
A Rab-GEF CASCADE DURING HUMAN SPERM EXOCYTOSIS

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Rab3 and Rab27 are the two major "secretory Rabs" implicated in calcium-regulated exocytosis in a variety of cells. Understanding how their actions are coordinated is a challenge in the field. Sperm contains a single, large dense-core granule that is released by regulated exocytosis (termed the acrosome reaction, AR) during fertilization. The acrosomal contents are released using the same conserved fusion machinery utilized by neurons and neuroendocrine cells. Two Rab proteins, Rab3 and Rab27, are required for sperm exocytosis. The levels of GTP-bound Rab27 and Rab3 increase upon initiation of exocytosis. With a fluorescence microscopy-based method developed in the laboratory, we found that challenging with an AR inducer increases the population of cells exhibiting GTP-bound Rabs in the acrosomal region of human sperm. Interestingly, introducing recombinant Rab27A loaded with GTP-γ-S into streptolysin-O permeabilized sperm elicits a remarkable increase in the number of cells evincing GTP-bound Rab3A. In the converse condition, recombinant Rab3A does not modify the percentage of Rab27-GTP-containing cells. Furthermore, Rab27A-GTP recruits a Rab3 GDP/GTP exchange factor (GEF) activity from human sperm extracts. Our findings suggest that Rab27/Rab3A constitute a Rab-GEF cascade, the first ever described in dense-core granule exocytosis.

CB-C18.
VACUOLAR PROTEIN SORTING RECEPTOR: NEW MOLECULAR EVIDENCE OF A SORTING PATHWAY IN *Giardia lamblia*

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In *Giardia*, lysosome-like peripheral vacuoles (PVs) need to specifically coordinate their endosomal and lysosomal functions to be able to successfully perform endocytosis, protein degradation and protein delivery, but how cargo, ligands and molecular components generate specific routes to the PVs remains poorly understood. Recently, we found that delivering membrane Cathepsin C and the soluble acid phosphatase (AcPh) to the PVs is adaptin (AP1)-dependent. However, the receptor that links AcPh and AP1 was never described. We have studied protein-binding to AcPh by using H6-tagged AcPh, and found that a membrane protein interacted with AcPh. This protein, named GlVps (for Giardia lamblia Vacuolar protein sorting), mainly localized to the ER-nuclear envelope and in some PVs, probably function as the sorting receptor for AcPh. The tyrosine-binding motif found in the C-terminal cytoplasmic tail domain of GlVps was essential for its exit from the endoplasmic reticulum and transport to the vacuoles, with this motif being necessary for the interaction with the medium subunit of AP1. Thus, the mechanism by which soluble proteins, such as AcPh, reach the peripheral vacuoles in *Giardia* appears to be very similar to the mechanism of lysosomal protein-sorting in more evolved eukaryotic cells.

CB-C19.
EPSIN–LIKE PROTEIN: A NOVEL CLATHRIN ASSOCIATED PROTEIN IN *Giardia lamblia*

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The epsin N-terminal homology (ENTH) domain is an evolutionarily conserved protein module, which is found in proteins involved in clathrin-mediated trafficking. By searching in the GDB, we found that GlENTHp (Giardia lamblia ENTH protein) contains an ENTH domain that shares the 3D structure with human epsin ENTH domain, displaying an α-helical structure composed of 7 α-helices. This domain is present in epsin or epsin-related (epsinR) proteins, which are involved in endocytosis and protein trafficking from the Golgi to the lysosomes, respectively. Both, epsin and epsinR possess clathrin-binding motifs, but only epsin incorporates an ubiquitin-interaction motif. Using Giardia's specific anti-clathrin and anti-ubiquitin Abs, we determined that GlENTHp interacts with both proteins. Subcellular localization showed that this protein is located mainly in the cytosol and occasionally in the nuclei. Biochemical analysis showed that GlENTHp binds PI(3,4,5)P_3 and PI(4)P, linked to the plasma membrane and Golgi, respectively. Moreover, protein-protein interaction experiments showed that GlENTHp physically interacts with αAP-2 (involved in endocytosis) and with αAP-1 (implicated in the Golgi-to-lysosome trafficking). Altogether, these results suggest that GlENTHp participates in the machinery for clathrin-mediated membrane budding, functioning as a dual epsin-epsinR protein.

CB-C20.
POSTNATAL ENDOSULFAN EXPOSURE ALTERS ENDOCRINE SIGNALLING AND ENDOMETRIAL PROLIFERATION IN THE RAT

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Previously we showed that an early postnatal exposure to the pesticide endosulfan reduces fertility in a rat model. Here, we evaluated the molecular changes in the subepithelial stroma of the endometrium associated with subfertility. Newborn female rats received vehicle or endosulfan (600 ug/kg/day) on postnatal day 1, 3, 5, and 7. To address the molecular effects of endosulfan exposure on the pregnant uterus, we evaluated the implantation-associated protein expression and endometrial proliferation on day 5 of pregnancy (preimplantation uterus) using immunohistochemistry. The animals received 60 mg/kg of bromodeoxiuridine (BrdU) and uterine tissues were harvested 2 hour latter. The endosulfan-treated rats showed an impaired proliferation of subepithelial stromal cells associated with a silencing of Hoxa10 protein. This reduction was accompanied by an increase of the co-repressor SMRT (silencing mediator for retinoic acid and thyroid hormone receptor). Furthermore, an up-regulation of estrogen receptor alpha and progesterone receptor expression was observed. The subfertility detected after postnatal exposure to endosulfan is associated with a modified uterine environment during the preimplantation period. Alterations in the endocrine signalling and endometrial proliferation could explain, at least in part, the effects of early exposure to endosulfan on the implantation process.

CB-C21.
RETINOIC ACID REDUCES MIGRATION OF HUMAN BREAST CANCER CELLS: ROLE OF RETINOIC ACID RECEPTOR BETA

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Breast cancer is the most common malignancy in women producing 98% of deaths when distant metastases occur. Retinoic acid receptor ß (RARß) expression is loss in 50% of invasive breast carcinoma compared with normal tissue and it has been associated with lymph node metastasis. Our hypothesis is that RARß protein is involved in metastasis process. Objectives: 1) To verify in MCF7 cells (RARß gene methylated and silenced) if the re-expression of the gene, after treatment with retinoic acid (RA), inhibits cell migration. 2) To determine in T47D cells (RARß expressed) if the silencing of this gene increases cell migration. 3) To identify the possible molecular mechanisms by which RA/RARß exerts its effect. Methods: MTT and migration assays, WB, RNA interference and immunofluorescence. Results: Administration of RA in MCF7 cells induces RARß gene expression that is greatest after 72 hours with 1uM concentration. We note that the higher concentration of RA and higher expression of RARß yields a 60% inhibition of cell migration ($p < 0.01$) and significantly decreases the expression of proteins that stimulate the migration (c-Src, moesin and HSP27). When RARß gene silencing is performed, the RA fails to inhibit migration significantly. Conclusion: RARß is necessary to inhibit breast cancer cells migration induced by RA modulating the expression of proteins involved in cell migration/invasion.

SB-C01.

SEARCHING FOR THE MOLECULAR BASIS OF *IN VIVO* MEMBRANE PROTEIN FOLDING IN *Aspergillus nidulans*

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Our group has developed an *in vivo* model to contribute to the understanding of how codon usage and translation kinetics can determine membrane protein folding *in vivo*. Making use of a GFP-tagged version of UreA, the urea transporter of the ascomycete *Aspergillus nidulans,* we can perform site-directed mutagenesis and analyze its effects on functionality and subcellular localization. Through an evolutionary analysis we have identified conserved "frequent" or "rare" codons in UreA orthologues from the eight sequenced *Aspergilli.* When two rare codons located in the N-terminal portion of the protein are changed into synonymous, frequent ones, the resulting strain shows impaired ability to grow on urea at 37ºC, but not at 25ºC. ^{14}C-urea transport assays support these results. Western blot and epifluorescence microscopy show a lower amount of protein in the membrane of the mutant strain, which is apparently due to a decrease in UreA synthesis. Preliminary results on conformational flexibility studies also show some differences in the trypsin digestion products of wild-type and mutant UreA. In order to determine differences in local translation kinetics, in vitro assays are currently being optimized. We speculate that the two conserved, rare codons could play a role in establishing a pause which may be important in the first steps of UreA synthesis and sorting to the membrane.

SB-C02.

CHARACTERIZATION OF TWO TRANSCRIPTIONAL REGULATORY SEQUENCES IN INTRON5 OF THE RUNX1 Gene

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The RUNX1 transcription factor is a critical hematopoietic regulator. Its gene is a frequent target of leukemia-causing translocations. One of the most commonly translocations found in myeloid leukemia patients is the t(8;21), where the mapped breakpoints are grouped together in three regions of the intron5, denominated breakpoint cluster regions (BCRs). These BCRs co-localize with chromatin structural elements such as DNaseI hypersentive sites (DHS), which are associated with the presence of regulatory elements such as promoters, enhancers and silencers. We perform a bioinformatics analysis of intron5 to search for potential regulatory modules. Based on evolutionary conservation nine potential enhancer regions named: α, β, γ, δ, ε, κ, λ, π and σ were identified. Next, we evaluate the functionality of two of the potential enhancers regions, δ and ε, in HeLa, myeloid HL-60 and Lymphoid Jurkat cells using a luciferase gene reporter in pGL3 promoter vector. Our results show that both regions repressed luciferase expression in the hematopoietic cell lines HL-60 and Jurkat. However, region δ activates luciferase transcription in HeLa cells. Moreover, both regions are associated with H3K4mel and H3K27ac which are marks characteristic of enhancer modules. Our results suggest that regions δ and ε in intron5 of RUNX1 are functional enhancers.

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SB-C03.

A NEW PHOSPHATASE FROM AN ANTARCTIC BACTERIUM: MOLECULAR BASIS OF COLD ADAPTATION MECHANISM

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A new bacterial strain designated as JUB59 was isolated from the Antarctic sea surface and grouped into the Flavobacteriaceae family. The bacterium was identified as a new species within the genus Bizionia and named *Bizionia argentinensis* (BA). Its genome was recently sequenced and represents an important source for the discovery of new proteins with biological activity at low temperatures. In order to structurally characterize a group of proteins of unknown function of BA, we carried out a method based on the selection of protein targets by NMR. By this method we were able to select proteins that given its solubility and folding became good candidates for structural determinations. Here, we present the solution structure of one of the selected targets, the protein BA42, revealing a previously unknown sequence/structure pair. By comparing the 3D structure of BA42 with existing databases, we characterize the phosphatase activity *in vitro*. This activity was dependent on divalent ions, particularly Mg^{2+}. We also identified the key residues involved in the active site: E5 and K37. A smaller number of interactions and a more flexible structure of the active site, compared with the mesophilic counterpart, may explain the molecular basis of the cold adaptation mechanism.

SB-C04.

NEW INSIGHTS INTO THE MECHANISM OF ACTIVATION OF THE HCV NS3 PROTEASE BY THE NS4A COFACTOR

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Hepatitis C virus (HCV) NS3 protein is a multifunctional enzyme with a serine protease (NS3p) located in the N-terminal one-third. The NS4A protein binds to the N-terminal NS3p domain to stimulate the proteolytic activity. As it is an essential component for HCV replication, the NS3p-4A has emerged as a prime target for antiviral intervention. The mechanism of activation of NS3p by NS4A is not yet clearly understood. Here, we have solved the structure of a P2-P4 macrocyclic inhibitor-NS3p complex by NMR. The binary complex interacts stably with a peptide corresponding to protease activation domain of NS4A. This fact opened the possibility to compare features of the catalytic machinery with and without the cofactor. Our results propose a separation between two events triggered in NS3 by action of NS4A: N-terminal domain and active site protease stabilizations. In solution an activated complex is formed, even in the absence of a well structured and complete N-terminal ß-barrel. This observation points to a situation in solution very different from that observed in the crystalline structures in which in the presence of NS4A a stable and ordered N-terminal domain is achieved. The NS3-4A interaction we observe has fundamental functional implications because it represents the *in vivo* conformation of the complex in an early stage, before a tight association of NS3-4A with the membrane.

SB-C05.
ENHANCED TOLERANCE OF *Escherichia coli* TO THERMAL AND OXIDATIVE STRESS

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2-Cys peroxiredoxins (2-Cys Prx) are peroxidases devoid of prosthetic groups that mediate in defence against oxidative stress and peroxide activation of signaling pathways. The associated activities -peroxidase and chaperone- have been assigned to multiple cytoprotective functions. At heat shock temperatures, 2-Cys Prx assembles into oligomeric structures, which possess higher chaperone activity than the decamer found at lower temperatures.

To gain insight into the ability of 2-Cys Prx to withstand thermal and oxidative stress, we screened a library of mutants prepared by directed evolution of *Escherichia coli* 2-Cys Prx (AhpC) for tolerance to temperature and oxidants. Thus, we isolated a mutant of *E.coli* AhpC that allowed the survival of the host cells at 53°C, i.e. 8 °C higher than the wild type protein. We also observed that *E. coli* cells were more tolerant to thermal and oxidative stress when transformed with Picrophilus torridus AhpC, from an archea that grows in extreme acidic and hot habitats. Congruent with these results, both mutant and archea AhpC were thermostable while wild type AhpC denaturated irreversible upon increasing temperature.

The approach of directed evolution opens the possibility to improve *E.coli* AhpC for increased tolerance to thermal and oxidative stress that could be adapted to the development of organisms more resistant to extreme environments.

PL-C01.
EXPRESSION OF TMV MP AND CP MODULE THE RNA DECAY PATHWAY IN PLANTA AND CONTRIBUTES TO VIRAL SYMPTOMS

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Plant viral disease symptoms are associated to hosts metabolic and physiological alterations. Here we use a transgenic system to study the effect of expressing individual TMV proteins on host gene expression (Nicotiana tabacum). We studied the effect of capsid (CPT42W) and movement protein (MP) and both CP and MP co-expression (MPxCPT42W). None of them, neither individually nor co-expressed exhibited silencing suppression activity, although plants that co-expressed them showed altered levels of miRNAs and developmental changes resembling symptoms. In this work we showed that GFP transient expression in MPxCPT42W is enhanced, suggesting a modulation of transgene silencing. Interestingly, this effect persisted when a set of viral suppressors were co-expressed, indicating that RNA decay pathway could be the target system altered by TMV proteins. We found that the expression level of RNA decay pathway genes was altered in MPxCPT42W plants and we focused in RNA exosome related genes, Rrp41 and Rrp43. We silenced their expression by means of VIGS in N. benthamiana and observed morphological defects that resemble TMV symptoms. We studied the expression level of several miRNAs and RNA decay related genes in these plants. Altogether, the data suggest that the RNA exosome decay pathway is modulated by TMV infection and could be another cause for the development of symptoms in the virus disease.

PL-C02.
CHARACTERIZATION OF *Arabidopsis thaliana* E3-UBIQUITIN-LIGASE SINA-L7

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Arabidopsis Seven in absentia-like 7 (SINA-L7) is a putative Zinc finger (SIAH-like) protein belonging to the Seven in absentia family composed by at least 35 proteins. It is expressed in various growth stages and almost all plant tissues. Its homolog was first identified in Drosophila associated with the development of photoreceptors, cell cycle arrest and protein polyubiquitination. Recently, about 50 SIAH-1 (human homolog) substrate proteins have been described associated with proteasome-mediated degradation. Due to the importance of the study of protein degradation by means of polyubiquitination pathway, we set out to characterize SINA-L7 by studying its expression by qPCR in various plant tissues of Arabidopsis. Results show that SINA-L7 is highly expressed in tissues with active cell division. In addition, we found that the expression of SINA-L7 follows a circadian rhythm and that UV exposure doubles its expression. Using an ubiquitination assay we found that SINA-L7 has E3 ubiquitin ligase activity and that residue K124 is involved in such process. Therefore our findings show that SINA-L7 is E3 ubiquitin ligase that would be involved in protein labeling by polyubiquitination.

PL-C03.
THE CATIONIC LIPID DI-C14-AMIDINE STIMULATES DEFENSES AGAINST MICROBIAL PATHOGEN IN ARABIDOPSIS

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Cationic liposomes are able to stimulate cell-mediated immune responses in animal models. Among them, di-C14-amidine (C14) activates the secretion of cytokines and chemokines on dendritic cells through the Toll Like Receptor (TLR)- 4 pathway. Although plants use TLR-like Pathogen Recognition Receptors (PRR) to recognize microbes at extracellular level, the effect of cationic lipids on plant immunity has not been evaluated so far. We here assessed this capacity for C14 by evaluating the salicylic acid- (SA) and jasmonic acid- (JA) dependent defense pathways in Arabidopsis. A single application of the lipid produced sustained activation of SA-sensitive genes in wild type plants. In contrast, reduced or transient induction of these genes is observed in the triple mutant lacking the PRR receptors FLS2, EFR and CERK1, suggesting that C14 stimulates PRR protein/s. At the intracellular level, the effect of C14 seems to be transmitted through the Toll-interleukin Receptor-like- (TIR), but not the Coiled-Coil- (CC) dependent cascade. Moreover, preliminary results showed that C14 immunizes plants against a virulent isolate of *Pseudomonas syringae*. Taken together these results indicate that C14 induces endogenous plant defense pathway/s providing resistance to biotrophic bacterial pathogens, pointing out this lipid as a new elicitor for the identification of novel plant immunity components.

PL-C04.
A DEFINED AQUAPORIN STOICHIOMETRY IN ORDER TO UNDERSTAND WATER PERMEABILITY AND PH SENSITIVITY

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Objectives: Plant plasma membrane (PM) aquaporins are classified in PIP1 and PIP2 subgroups, all showing the ability to block water permeation after cytosolic acidification. While PIP2 is found as a homotetramer structure, PIP1 requires to be co-expressed with PIP2 to reach the plasma membrane and thus enhance the osmotic water permeability (Pf). Our working hypothesis is that FaPIP1;1, interacts with FaPIP2;1 in a defined stoichiometry providing heterotetrameric units able to modify both Pf and pH sensitivity. **Methods:** Water transport and PIPs pH regulation were studied in Xenopus oocyte employing a N228DFaPIP2;1 mutant. All EYFP-tagged PIPs were localized by fluorescent confocal microscopy. **Results:** FaPIP1;1 cannot reach the PM unless it is co-expressed with native FaPIP2;1 or N228D FaPIP2;1. N228DFaPIP2;1 can only achieve PM if it is co-expressed with FaPIP1;1. The pH sensitivity remains constant when different ratios of FaPIP1;1 and FaPIP2;1 or N228D are co-expressed, but differ from the pH sensitivity of FaPIP2;1 expressed alone. The maximum Pf achieved is constant when different ratios of FaPIP1;1 and FaPIP2;1 cRNA are injected but variable when FaPIP2;1 is replaced by N228D. **Conclusion:** Results establish a possible stoichiometry for FaPIP1;1-FaPIP2;1 that defines the pH sensitivity of the membrane water transport.

PL-C05.
REGULATION OF ASF1 PROTEINS BY CELL CYCLE PROGRESSION AND UV-B RADIATION IN PLANTS

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ASF1 is a key histone H3/H4 chaperone that participates in a variety of DNA and chromatin-related processes, including DNA repair, in which chromatin assembly and disassembly is of primary relevance. Information concerning the role of ASF1 proteins in post-UV response in higher plants is currently limited. An initial analysis of in vivo localization of ASF1A and ASF1B indicates that both proteins are mainly expressed in proliferative tissues. Analysis of promoter sequences identified ASF1A and ASF1B as targets of the E2F transcription factors. These observations were experimentally validated, in vitro and *in vivo*. These data suggest a regulation of ASF1A and ASF1B during cell cycle progression. In addition, we found that ASF1A and ASF1B are associated with the UV-B induced DNA damage response. Transcript levels of ASF1A and ASF1B were ASF1B increased following UV-B treatment and RNAi silenced plants for both genes showed increased sensitivity to UV-B compared to wild type plants. Finally, by coimmunoprecipitation analysis we found that ASF1, physically interacts with N- terminal acetylated histones H3 and H4, and with acetyltransferases of the HAM subfamily, which are known to be involved in cell cycle control and DNA repair, among other functions. Together, here we provide evidence that ASF1A and ASF1B are regulated by cell cycle progression and DNA repair after UV-B irradiation.

PL-C06.
ROLE OF AtHscB IN PLANT IRON METABOLISM

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Iron is an essential nutrition element in all organisms. It is fundamental to the metabolism of mitochondria and chloroplasts. Iron-sulfur (Fe-S) clusters are produced inside both organelles and the assembly and maturation of Fe-S proteins require a complex cellular machinery. In our laboratory we have characterized different genes and proteins that are postulated to be involved in the (Fe-S) cluster biosynthesis in Arabidopsis thaliana. In this work we evaluate the effect of the overexpression of AtHscB (co-chaperone, involved in the Fe-S cluster biogenesis) on iron metabolism in A. thaliana plants. Two transgenic lines overexpressing AtHscB were analyzed. We measure the transcript level of several genes that are regulated by iron by qPCR. Results show that the mRNA levels of ferrochelate reductase (FRO2) and a metal transporter (IRT1) (involved in iron transport) are increased about 11- and 21-fold, respectively. By contrast, the transcript level of ferritin1 (AtFer1) was decreased 5-fold. Furthermore, the histochemical detection of iron deposits using the Perls method showed an increased of iron content in roots. Our results suggest an alteration of iron metabolism, content and distribution in the transgenic lines, in addition, the induction of FRO2 and IRT1 in the s-HscB lines is in agreement with the sensing of iron deficiency.

PL-C07.
COX17, COPPER AND STRESS: A NEW MENAGE A TROIS IN *Arabidopsis thaliana*

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A series of accessory proteins is essential to deliver and insert copper into cytochrome c oxidase (CcO). However, recent findings suggest that these accessory proteins have additional functions in *Arabidopsis thaliana*. In order to understand the functions of COX17-1 and COX17-2, homologues of yeast copper chaperones involved in CcO assembly, we silenced their expression in *A. thaliana* using artificial microRNAs. Copper homeostasis is altered in these plants, as evidenced by their higher copper content and the repression of the low-copper-responsive MIR398 gene. In addition, these silenced plants showed accumulation of reactive oxygen species, higher electron transfer rate (ETR) and lower dark respiration rate when grown under different stress conditions (high salt concentration, high light). Transcript levels of genes involved in the alternative respiratory pathway (AOX1a and NDB2), as well as other stress-responsive genes (BCS1, CAT3, APX1 and ASO) were lower in silenced plants. We conclude that COX17-1 and COX17-2 function in copper homeostasis and plant stress responses. Our results suggest the existence of a link between copper metabolism, mitochondrial function and stress responses in plants.

PL-C08.
ROLE OF TCP15 IN THE REGULATION OF CARPEL DEVELOPMENT AND CYTOKININ RESPONSES IN *Arabidopsis thaliana*

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TCP-domain proteins constitute a family of transcription factors specific of plants. They can be divided into two classes, I and II. The precise function and mechanism of action of many class I TCP proteins is not well understood. In order to elucidate this, we transformed Arabidopsis plants with the coding region of AtTCP15 fused to the EAR repressor domain, under the control of its own promoter (p15::TCP15-EAR). We obtained plants with altered gynoecia that showed an overgowth of the replum, forming narrow structures ended in stigmatic papillae. Application of cytokinin to p15::TCP15-EAR plants generated an overproduction of stigmatic papillae along the medial zone and a pronounced arrest in carpel elongation. Production of similar structures was observed after treatment of a knockout mutant in AtTCP15. Interestingly, plants ectopically expressing AtTCP15 showed defects in carpel fusion under normal conditions and abnormal proliferation of carpel-like structures in the gynoecium when treated with cytokinin. According to this, several cytokinin response regulators (ARRs) showed changes in expression in plants with altered AtTCP15 levels function. Taken together, these results suggest an important role of AtTCP15 in regulating cell proliferation and diferentiation in the gynoecium through the modulation of cytokinin response pathways.

PL-C09.

NUCLEAR IMPORT AND DIMERIZATION OF TOMATO ASR1, A WATER STRESS-INDUCIBLE PROTEIN EXCLUSIVE TO PLANTS

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The ASR (for ABA/water stress/ripening) protein family, first described in tomato as nuclear and involved in adaptation to dry climates, is widespread in the plant kingdom including crops of high agronomic relevance. We show both nuclear and cytosolic localization for ASR1 (the most studied member) in histological plant samples by immunodetection, as typically found in small proteins readily diffusing through nuclear pores. Indeed, a nuclear localization was expected based on sorting prediction softwares, which also highlight a monopartite nuclear localization signal (NLS) in the primary sequence. However, here we prove that such an "NLS" of ASR1 from tomato is dispensable and non-functional, being the transport of the protein to the nucleus due to simple diffusion across nuclear pores. We attribute such a targeting deficiency to misplacing in that cryptic NLS of two conserved contiguous lysine residues. Based on previous in vitro experiments regarding quaternary structure, we also performed live cell imaging assays through confocal microscopy to explore dimer formation in planta. We found homodimers in both the cytosol and the nucleus and demonstrated that assembly of both subunits together can occur in the cytosol, giving rise to translocation of preformed dimers. The presence of dimers was further corroborated by means of *in vivo* crosslinking of nuclei followed by SDS-PAGE.

PL-C10.

RRNA DEGRADATION THROUGH A RIBOPHAGY-LIKE MECHANISMS IS NECESSARY FOR CELLULAR HOMEOSTASIS IN PLANTS

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The RNase T2 family is found in all eukaryotes. Phylogenetic and gene expression analyses have shown that some plant RNase T2 enzymes (belonging to Class II) may carry out a housekeeping function. To test this hypothesis we functionally characterized RNS2, the only Class II RNase T2 in Arabidopsis. RNS2 has a putative ER-retention signal, and microscopy and subcellular fractionation studies showed that it is localized in the endoplasmic reticulum and the vacuole. Mutants lacking RNS2 activity accumulate RNA intracellularly, mainly in the vacuole, and in vivo-labeling experiments showed that both 18S and 28S rRNA subunits have a longer half-life in mutants than in WT plants. Microarray analyses identified a set of genes differentially expressed in rns2 mutants, and comparisons with public expression data determined that most genes are also regulated by sugars, starvation, and the redox state of the cell. Moreover, mutants with reduced or absent RNS2 display constitutive autophagy. These phenotypes and the subcellular localization of the enzyme indicate it is necessary for normal decay of rRNA and that this enzyme participates in a ribophagy-like mechanism that targets ribosomes for recycling under normal growth conditions. Lack of proper rRNA recycling in the mutants alters cellular homeostasis, which results in induction of macroautophagy as a compensatory mechanism.

PL-C11.

STRUCTURAL FEATURES DEFINING THE *IN VIVO* FUNCTION OF THE miRNA PROCESSING PROTEIN HYL1

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HYL1 is a protein involved in microRNA (miRNA) biogenesis that contains two double-stranded RNA binding domains (dsRBDs). HYL1 enhances the efficiency and precision of the RNase III protein DCL1 and participates in miRNA strand selection. We have previously demonstrated that the first domain (dsRBD1) is the major contributor to RNA binding and we defined three main binding regions (R1, R2 and R3) based on the NMR mapping of the interaction. With the aim of gaining insight on the contribution of each region to HYL1 activity, we constructed five dsRBD1 mutants (one located in R1, three in R2 and one in R3) and we transformed *hyl1* plants to evaluate phenotype rescue. We found that regions R1 and R3 are the most relevant for the function of HYL1 *in vivo*. These plants have a lower phenotype recovery percentage and RT-qPCR assays showed miRNA and pre-miRNA levels similar those of *hyl1* deficient plants. To better understand the underlying reasons of the differential behavior of HYL1 mutants, we further biophysically characterized the mutant domains. The ^{15}N-HSQC spectra show that all mutant domains are well folded and that the low efficiency in processing of R1 mutants may be the result of a decrease in the protein stability whereas R3 mutants may have a lower substrate binding affinity. Our results highlight the multiplicity of factors that determine *in vivo* protein activity.

PL-C12.

THE AtIPB GENE INDUCES RESPONSE MECHANISMS RELATED TO PATHOGEN ATTACK IN ARABIDOPSIS PLANTS

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In this work, we analyze the effect of AtIPB (Increased Plant Biomass), a gene that encodes a nuclear protein with unknown functions in plants. To determine the role of AtIPB, we generated overexpressing (OE) plants and obtained an insertional mutant in the coding region of this gene. Under normal growth conditions, OE plants exhibited increased biomass (hence the gene name), higher ROS levels and increased content of scavengers like ascorbic acid and glutathione. At the transcriptional level, higher levels of Pathogen Related (PR) transcripts (PR1 and PR5) were observed. We also evaluated the connection between hormones and biotic stress responses in our plants. Through mass spectrometry, we determined that OE plants contained higher salicylic acid (SA) levels than wild-type plants. Moreover, OE and mutant seedlings showed different transcriptional dose-response curves when grown on MS medium containing 0.5 mM SA. In order to evaluate the effect of AtIPB in plant responses to biotic stress, we are performing survival assays of different pathogens on these plants. The results suggest that AtIPB would be a candidate to obtain plants better prepared to overcome biotic stress growing conditions.

BT-C01.
BIOTECHNOLOGICAL STRATEGIES FOR ATRAZINE PHYTOREMEDIATION IN HUMID PAMPA

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As one of the main world atrazine (ATZ) consumers (7000 ton.y^{-1}), the need of developing biotechnological strategies to mitigate the environmental impact of ATZ application associated to intensive agricultural practices, is evident. In this way, a pilot scale open field experiment was performed to assess the phytoremediation performance of the recently reported atrazine tolerant ryegrass species (Lolium multiflorum) in a cornfield. Accordingly, a 16 ha field was selected in Ramallo city vicinity (30°40'35,2'' S – 60°09'21,0'' W), as representative of the intensive agricultural practices. Soil was sampled before sowing and 36 plots of 100 m^2 were settled to evenly cover the plot. Then, corn crop was sowed and 2 L.ha^{-1} of Atrazine500 (Dow) applied postemergence. After harvest, ryegrass was sowed at 22 Kg.ha^{-1} rate in a paired plots fashion. Core soil samples were monthly collected and separated in different depths in order to assess either the horizontal or the vertical ATZ movement. Soil samples were extracted and ATZ and metabolites content analyzed by HPLC-UV. Parallel, soil agronomical properties were assessed. Results indicate that ryegrass implantation improves soil ATZ degradation and agronomical properties. Concluding, ryegrass-intercropping strategy fits as biotechnological strategy for minimizing ATZ environmental impact associated to intensive agronomical practices.

BT-C02.
SCREENING OF BACTERIAL OXIDASES

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Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are multi-copper-containing enzymes which catalyze the oxidation of a wide range of phenolic and non phenolic aromatic compounds. These enzymes can be used for several applications, such as bio-bleaching in pulp and paper, dye decolorization, detoxification of recalcitrant pollutants, organics synthesis as biocatalysts and bioremediation. In contrast to fungal laccases, only a few bacterial laccases have been studied. Bacterial laccases are much more stable in extreme conditions and could be overproduced with high yields.
In this work a screening of bacterial laccase producers over a collection of more than 100 microorganisms was done, including mesophilic, thermophilic and psycrophilic bacteria. Laccase activity was determinated spectrophotometrically using ABTS (2-2´-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) as substrate. Different pH value (4 to 7) does not affect activity and stirring improves between 4 to 12-fold ABTS oxidation. For meso- and psycrophiles, the best temperature reaction was 45ºC. However, for thermophilic bacteria the optimal temperature was 60ºC. These optimization improved 10-fold ABTS oxidation compared to *E. coli* and reached 0.309 EU/ml.

BT-C03.
BIOSYNTHESIS OF ANTIVIRAL COMPOUNDS USING IMMOBILIZED THERMOPHILIC BACTERIA

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Biocatalysis is widely used in replacement of many chemical reactions which requires several steps of protection and deprotection and the use of toxic solvents, obtaining racemic mixtures as final products. Particularly, thermophilic microorganisms are very useful in industrial bioprocesses due to the high stability of their enzymes. The application of immobilization techniques improve biocatalyst reusability, simplify product recovery and allow bioprocess scale-up.
In this work, we describe the use of thermophilic bacteria as biocatalysts for the biosynthesis of nucleoside analogues with antiviral or antitumoral activity. Different parameters such amount of microorganisms, substrate ratio, temperature and culture time were evaluated. 2,6-diaminopurine and 6-chloropurine ribosides were obtained with conversion rates of 83% and 68%, respectively at short reaction times. The conversion values for 2,6-diaminopurine and 6-chloropurine-2´-deoxyribosides were 90%. Besides, this biocatalyst was able to biosynthesize 6-bromopurine and 6-iodopurine nucleosides with high yields.
Finally, the biocatalyst immobilized in agarose matrix was active for more than 200 h obtained 23.4 g of nucleoside analogues.

BT-C04.
FLOXURIDINE BIOTRANSFORMATION IN ONE POT REACTION USING LACTIC ACID BACTERIA

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Synthesis of pharmaceutical compounds by bioprocess is a promising strategy that plays a central role in the development of Industrial Biotechnology. The use of microorganisms as biocatalysts is an alternative to traditional multistep chemical methods for nucleosides analogues synthesis. These molecules have relevant pharmacological applications in cancer or antiviral therapies. Floxuridine is a nucleoside analogue with proved antitumoral activity.
In this work, we performed a biocatalytic screening between more than 25 strains of lactic acid bacteria (LAB) to obtain floxuridine. The most active LAB was selected and used to optimize reaction parameters. The best reaction conditions for floxuridine biotransformation were 30ºC, 25 mM Tris-HCl buffer, pH 7, 6 mM thymidine and 2 mM 5-fluoruracil. Floxuridine yield was 94% in 1 h reaction for *Lactobacillus animalis*. This strain was stabilized by adsorption and entrapment techniques in different supports. L. animalis was successfully immobilized in DEAE-Sepharose and calcium alginate derivatives; these biocatalysts were able to yield 83% and 66%, respectively. In view of these results, we developed a scaling up bioprocess for both immobilized systems in batch and semicontinuos operation.

EN-C01.
MOLECULAR THERMODYNAMICS FOR CELL BIOLOGY AS TAUGHT WITH BOXES

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Thermodynamic principles are basic to an understanding of the complex fluxes of energy and information that are required to keep cells alive. These microscopic machines are non-equilibrium systems at the micron scale that are maintained in pseudo-steady-state conditions by very sophisticated processes. Therefore, several non-standard concepts need to be taught to rationalize why these very ordered systems proliferate actively all over our planet despite the second law of the thermodynamics. We propose a model consisting of boxes with different shapes containing small balls that are in constant movement because a stream of air is blowing from below. This is a simple macroscopic system that can be easily visualized by students and that can be understood as mimicking the behavior of a set of molecules exchanging energy. With such boxes, the basic concepts of entropy, enthalpy, and free energy can be taught while maintaining a molecular understanding of the concepts and stressing the stochastic nature of the thermodynamic laws. In addition, time-related concepts such as reaction rates and activation energy can be readily visualized. Moreover, the boxes provide an intuitive way to introduce the role of information and Maxwell's demons operating under non-equilibrium conditions on the cellular organization.

NS-C01.
PROTEIN MALNUTRITION DIMINISHES CEREBRAL CORTEX BDNF EXPRESSION AND EVOKES ANXIETY-LIKE BEHAVIOURS

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Undernutrition and malnutrition continue to be some of the most common conditions that affect the development of children living in developing countries. The aim of this work was to study the effects of early protein malnutrition over the development of the pup nervous system.
Pregnant CF-1 mice were fed with normal or low protein diet during pregnancy and lactation. Several developmental milestones were analyzed from P1 to P21. Pups from the low protein group exhibited a delay in righting reflex and grip strength progress, and in eye and ear opening and fur growth. We studied the expression of several neurotrophins and immediate early genes by RT-PCR and western blot and found a reduction in BDNF expression in the cortex of 3 week old pups from the low protein group. The presence of chromatin modifications was assessed by western blot, and we found an increase in the presence of acetylated histone 4 in the cortex of malnourished pups. Pups from both groups were fed with standard lab chow after weaning and subjected to the elevated plus maze (EPM) and open field tests in order to study the presence of anxiety-like behaviours in the young adults. The offspring from low protein group exhibited a more anxious behaviour in the EPM. Our results indicate that pups subjected to early protein malnutrition suffered a developmental delay and presented a more anxious behaviour.

LI-C01.
UNRAVELING KEY AMINO ACIDS FOR *Bacillus SP.* ACYL-LIPID DESATURASES ACTIVITY

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Fatty acid desaturases are enzymes that introduce double bonds with high stereo and regioselectivity into fatty acyl chains to produce unsaturated fatty acids (UFAs). UFAs are essential structural components of the cell membranes. The aim of this study was to indentify amino acid residues essential for the enzymatic activity of these enzymes. A sequence comparison was performed among various acyl-lipid desaturases from bacilli species. Eleven site-directed mutants in completely conserved residues were generated using Δ5 desaturase gene from *Bacillus cereus*. The mutants were functionally characterized by heterologous expression in a *B. subtilis* (*des*-) strain, revealing that nine residues (G90, W104, R149, L157, H228, P257, L275, Y282 and L284) were obligatorily required for the desaturase activity, likely for preserving the structural integrity of the active site pocket. In addition, to locate the structural determinants of activity/specificity from *Bacillus* desaturases a series of chimeric enzymes were constructed by domain swapping between *B. cereus* and *B. subtilis* Δ5 desaturases. Our results indicated that the N-terminal region and the two first His-rich boxes of the *B. cereus* desaturase were responsible for the differences detected in activity/specificity of both enzymes. This study provides novel information on structure-function relationships of the membrane-bound desaturases.

LI-C02.
ROLE OF NF-κB IN COX2 EXPRESSION IN RENAL CELLS UNDER HYPERTONIC STRESS

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Renal medullary cells are exposed to variable and high concentrations of NaCl as part of the urinary concentrating system. In such hypertonic conditions, renal cells still survive and function by activating the transcription of various osmoprotective genes, among them, cyclooxygenase 2 (COX2). It was demonstrated that NF-κB transcription factor is involved in COX2 expression in different cell types but not in renal cells. The present work evaluates whether NF-κB regulates COX2 expression in renal epithelial cells under hypertonicity. MDCK cell cultures were grown during 1.5, 3, 6, 12 and 24 h in isotonic (298 mOsm/Kg H2O) and NaCl-hypertonic (520 mOsm/Kg H2O) media in the absence or presence of NF-κB inhibitors, PDTC and parthenolide. RT-PCR and westernblot (WB) analysis showed the increase in the expression of COX2 and NF-kB after hypertonic challenge. Cellular fractionation followed by WB, and microscopy showed the translocation of NF-κB from the cytosol to the nucleus suggesting the activation of the transcription factor. The NaCl-induction of COX2 mRNA and protein was prevented by PDTC and parthenolide, showing that NF-κB is involved in COX2 expression. Taken together these results suggest that the up-regulation of the osmoprotective gene COX2 in cells submitted to high-NaCl medium occurs due to the NF-κB activation.

LI-C03.
BEHAVIOR OF SPHINGOMYELINS WITH VERY LONG CHAIN FATTY ACIDS IN BILAYERS AND MONOLAYERS

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Very long-chain (C24 to C36) polyunsaturated fatty acids (VLCPUFA) are important acyl groups of sphingomyelin (SM) and ceramides of mammalian spermatozoa. Our project aims at establishing peculiarities of their biophysical properties and modes of interaction with other lipids in sperm membranes. In this study, rat testicular SM were separated into fractions enriched in nonhydroxy and 2-hydroxy VLCPUFA (N-V and H-V SM, respectively), and individual molecular species of SM with different N-V and H-V by combining different chromatographic techniques. In liposomes prepared from each of these fractions and species, thermotropic properties were studied by fluorescence spectroscopy. Two probes that localize at different depths in the bilayer (DPH, Laurdan) were used to study the influence of number of double bonds, fatty acid chain length, and presence of the 2-OH group. In ternary systems with phosphatidylcholine and cholesterol, N-V and H-V SMs did not segregate together with cholesterol, as saturated SMs do. In Langmuir monolayers, surface pressure, surface potential, and.reflectivity of p-polarized light were measured to determine average molecular area (degree of packing) and thickness in membranes. SM species containing N-V and H-V contrasted in all biophysical properties measured with those of the most common saturated (e.g., 16:0) SM.

LI-C04.
DIVERSE GLYCEROLIPID SYNTHESIZING ENZYMES CONTRIBUTE TO THE DAILY RHYTHMS IN PHOSPHOLIPID SYNTHESIS

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Circadian clocks regulate biochemical processes including lipid metabolism and their disruption may lead to metabolic disorders such as obesity, diabetes, etc. We have previously shown that the 32P-phospholipid synthesis oscillates daily in synchronized fibroblasts; however, little is known about the temporal regulation of glycerophospholipid (GPL) synthesis. We found a circadian change in the incorporation of 3H-glycerol into total GPLs in arrested NIH3T3 cells synchronized with a 2 h-serum shock with lowest levels at 28 and 56 h. A daily variation was also seen in the activity of GPL-synthesizing and -remodeling enzymes phosphatidate phosphohydrolase 1 (PAP1) and lysophospholipid acyltransferases (LPLAT), respectively with distinct and opposite profiles. We further investigated the temporal regulation of phosphatidylcholine (PC) synthesis through the Kennedy pathway with Choline Kinase (ChoK) and CTP:phosphocholine cytidylyltranferase (CCT) as key enzymes. PC labeling exhibited a daily variation, and maximum levels were accompanied by a brief increase in CCT activity peaking at 6 and 35 h together with the oscillation of ChoK mRNA (a isoform) and activity. Our results demonstrate that synchronized fibroblasts undergo a temporal regulation in the synthesis and remodeling of GPLs and particularly of PC involving concerted changes in specific enzyme activities and/or mRNA expression.

LI-C05.
IMPLICATION OF SPHINGOSINE KINASE IN MDCK CELL TRANSITION FROM POLARIZED TO DIFFERENTIATED PHENOTYPE

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We have demonstrated that sphingolipid biosynthesis is essential for hypertonicity-induced MDCK cell differentiation. Sphingolipids regulates several aspects of cell behavior, being sphingosine 1-phosphate (S1P) one of most important. Thus, we study the importance of S1P synthesis in the acquisition of the polarized-differentiated MDCK cell phenotype. In epithelial cell polarization/differentiation transition, cell-cell adhesion is an early event characterized by the establishment of adherents junctions. To evaluate the importance of S1P in this process, confluent MDCK cells were subjected to hypertonicity and concomitantly treated or not (control) with different concentrations of D-threo-dihydrosphingosine (Sphingosine Kinase inhibitor). After 48 h, cell phenotype was visualized by fluorescence microscopy of actin cytoskeleton and cell-cell adhesion structures (E-Cadherin, ß-catenin and a-catenin). As inhibitor concentration rise the cell-cell adhesions were impaired, and the characteristic polarized phenotype of the cells was lost. First, cells appeared lengthened, and thereafter acquired a fibroblast-like phenotype. Interestingly, ß-catenin suffered redistribution from plasmatic membrane to cytoplasmic and nuclear localization. In the present work we demonstrate that SK activity is essential in the MDCK cell differentiation process induced by hypertonic stress.

LI-C06.
NUCLEAR LIPID DROPLETS ARE DYNAMIC STRUCTURES

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The cell nucleus (N) is a highly compartmentalized organelle characterized by several dynamic domains. We have demonstrated that nuclear neutral lipids are organized and stored in lipid droplets (nLD), composed of few and small droplets randomly distributed. The nLD could thus be involved in nuclear-lipid homeostasis and serve as an endonuclear buffering system that can rapidly provides or incorporates lipids involved in signaling paths and transcription factors in the nucleus.
The aim of this work was to determine if nLD are a dynamic domain. With this purpose HepG2 were incubated with 18:1n-9 as an external stimulus since it increases cytosolic lipid droplet (cLD) number and size. Both LD (c and n) were stained with BODIPY493/503 and N with DAPI and viability controls were done. Due to 18:1n-9 treatment cellular shape was modified and both cLD and nLD increased (number and size). nLD increments were smaller than cLD (4 and 10 times respectively). If 18:1n-9 was excluded from the incubation mixture, LD increments were reverted. Under all conditions tested, nLD corresponded to a small pool (3-5%) with respect to total (nLD + cLD).
In conclusion, nLD are dynamic structures; their size and number can vary in response to external signals that influence TAG pools by a reversible mechanism.

LI-C07.
LIPID CHANGES IN RAT SPERMATOZOA DURING ISOLATION AND FUNCTIONAL ACTIVATION *IN VITRO*

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Hydrolysis of glycerophospholipids (GPL) and sphingomyelin (SM) are calcium-dependent reactions that are significantly stimulated in rat epididymal spermatozoa during incubations leading to capacitation (Cap) and particularly after acrosomal reaction (AR). In comparison with gametes whose contact during isolation with tissue divalent cations was avoided (C1), or kept to a minimum (C2), those used as controls in these incubations (C3) already had their glycerophospholipids (GPL) and sphingomyelin (SM) partially hydrolyzed. Using C1 as controls, the C3 gametes had less GPL per cell and more free fatty acids. The latter were released with cholesterol from cells to media during albumin-dependent capacitation. Significant decreases in membrane GPL occurred gradually in the order C1 > C2 > C3 > Cap > AR. Diminution in the major choline GPL specifically targeted phosphatidylcholine in the presence of a virtually unchanged amount of plasmenylcholine, the acrosome-reacted cells thus becoming relatively rich in plasmalogens. The unique SM with very long chain PUFA located on sperm heads was intact in C1 cells and maximally hydrolyzed into ceramides after completion of AR. Using fluorescent probes, liposomes prepared from the 5 conditions showed that C1 gametes had the minimun degree of order of the lipid bilayer, this increasing significantly as Cap and AR proceeded.

MI-C01.

STAPHYLOCOCCAL α-TOXIN INDUCES ACTIVATION AND DEGRADATION OF C-JUN AND JUND TRANSCRIPTION FACTORS

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Staphylococcus aureus displays a large set of virulence factors, among which the pore-forming α-toxin has been shown to be crucial in the establishment of severe and often fatal infections such as necrotizing pneumonia. However, its molecular mechanisms are not well understood. In the present study we evaluated the role of c-Jun and JunD, two transcription factors of the AP1 complex which are key regulators of cell fate upon diverse stimuli, including microbial infections. Western blot analysis as well as immunofluorescence studies showed a strong a-toxin-induced phosphorylation of c-Jun and JunD with a concomitant decrease of their protein levels. The use of pharmacological inhibitors showed that this phosphorylation was dependent on the JNK signaling pathway. On the other hand, inhibition of p38 and ERK increased the c-Jun and JunD protein levels. Furthermore, pretreatment with the MG132 proteasome inhibitor showed that this pathway contributed to the a-toxin-dependent degradation of c-Jun and JunD. In addition, Real-Time PCR revealed that c-Jun was also downregulated at transcriptional level. Finally, knockdown of c-Jun by siRNA showed that this protein contributed to the host cell survival against a-toxin. These results shed light on the nuclear response orchestrated by c-Jun and JunD during cell stress triggered by staphylococcal a-toxin.

MI-C02.

A MITOCHONDRIAL CYCLOPHILIN FROM *Trypanosoma cruzi* AND ITS ROLE IN PROGRAMMED CELL DEATH

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Cyclophilins (CyP) are enzymes involved in protein folding and are target of Cyclosporin A (CsA). In *T. cruzi*, we described the CyP gene family. One very interesting CyP in mammals is CyPD, involved in the mitochondrial permeability transition pore opening, which plays an important role in programmed cell death (PCD). *T. cruzi* has a CyPD homologue gene, *Tc*CyP21, which is expressed in the three stages of the parasite and localises in the mitochondrion. To further characterise this protein, we stimulated oxidative stress in epimastigotes using 5mM H_2O_2 or 1.25μM ß-lapachone. In these conditions, mitochondrial membrane potential decreased, cytochrome *c* translocated into the citosol, cleavage of a PARP-like protein was observed and DNA degradation occurred. All these typical apoptotic features were prevented by pre-incubation with 1μM CsA. To study the specific role of *Tc*CyP21, epimastigotes were transfected with pTEXOmni-*Tc*CyP21-GFP. Changes in morphology or growth rates were not observed. However, further experiments are undergoing.
This is the first evidence that a homologue of CyPD, which is involved in PCD events, is expressed in a protozoan parasite.
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MI-C03.

A CONSORTIUM OF REGULATORY SYSTEMS CONTROLS THE EXPRESSION OF A *Salmonella*-SPECIFIC EFFLUX COMPLEX

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CBA efflux systems are tripartite protein complexes that direct export of metal ions, xenobiotics or drugs either from the cytoplasm, the inner membrane or the periplasm into the extracellular space. GesABC is one of the five *Salmonella* CBA efflux pumps whose transcription is controlled by the Au-sensor GolS. Among the three transcriptional units controlled by GolS, gesABC has the most divergent GolS-dependent operator, resulting in its delayed expression in the presence of Au ions. Besides its essential role in Au-resistance, the GesABC system can also mediate the transport of different drugs in a strain deleted in the main drug transporter AcrAB, suggesting additional roles for cell detoxification. Using bioinformatics we identified putative cis-acting regulatory sequences recognized by global as well as stress-induced transcriptional regulatory systems that would control the expression of the efflux pump under different conditions. Their effective control of gesABC expression was verified by reporter expression on strains deleted in different regulatory systems. Specific recognition by these systems was confirmed by *in vitro* assays. Our results indicate that a regulatory systems' consortium controls the expression of gesABC, providing the proper supply of the efflux complex according to the quality and severity of different stress conditions that the pathogen encounters.

MI-C04.

CHARACTERIZATION OF THE *Serratia marcescens* MUTANT STRAIN IN THE GENE THAT ENCODES ShlA CYTOL

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Serratia marcescens ShlA hemolysin is one of the major virulence factors of this opportunistic pathogen. ShlA presents cytotoxic activity in several cellular lines and we have demonstrated that ShlA expression is controlled by the Rcs system. A *S. marcescens* mutant strain with impeded activation and secretion of ShlA was unable to induce autophagy from outside the CHO cells. However, intracelullary, this strain was found inside a LC3-positive vacuole. An increase of intracellular CFUs was detected at late times in the invasion process in either T24 or CHO epithelial cells, when compared to wild type strain. Nevertheless and in contrast to the wt strain, no mutant bacteria were detected in the culture supernatant when gentamicin was eliminated from the assay at late times p.i. In addition, intracellular CFUs of the mutant strain were highly diminished as early as 120 min p.i. in Bafilomycin A1 or NH4Cl treated cells, as it was also determined for the wt strain. In summary, our results reveal that: a) ShlA expression is responsible for the autophagic phenotype induced by *Serratia* in epithelial cells from the extracellular space, b) at late times p.i., ShlA is required to mediate the exit and dissemination of *Serratia* from the invaded cell, and c) ShlA is not implicated in early *Serratia* internalization and replication processes that would require an acidic environment to take place.

MI-C05.
THE TWO-COMPONENT SYSTEMS PrrBA AND NtrYX REGULATE THE ADAPTATION OF *Brucella* TO LOW-OXYGEN TENSION
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Brucella is the causative agent of the zoonotic disease brucellosis, which is endemic in many parts of the world. The success of *Brucella* as pathogen resides in its ability to adapt to the harsh environmental conditions found in mammalians host. One of its main adaptations is the induction of the expression of different genes involved in respiration at low oxygen tension. We describe a regulatory network involved in this adaptation. We show that in *Brucella abortus* the two-component system PrrBA regulates the expression of the two-component system NtrYX, which is involved in redox sensing, and the transcriptional regulators FnrR and NnrA, which are involved in oxygen and nitric oxide sensing, respectively. We also show that PrrBA and NtrYX coordinately regulate the expression of denitrification and high-affinity cytochrome oxidase genes. Strikingly, a double mutant strain in *prrB* and *ntrY* genes is severely impaired in growth and virulence, while the *ntrY* and *prrB* single mutant strains are not. The regulatory network proposed could contribute to shed light into the mechanisms used by *Brucella* for a successful adaptation to its replicative niche inside mammalians cells.

MI-C06.
***Brucella abortus* PrrB/A: A CONSERVED REDOX-RESPONSIVE TWO-COMPONENT SYSTEM**
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Brucella abortus is an intracellular bacterium that needs to adapt to an oxygen limited environment in order to achieve a successful infection. This prompted us to search putative systems that mediate the adaptation to the microaerobic conditions encountered by this pathogen.
We identified in *B. abortus* genome the homologues to a conserved two-component system called PrrB/A. In other microorganisms, it has been shown that PrrB senses the redox status and, through PrrA, generates a global response which involves expression of high–affinity cytochrome oxidases and induction of enzymes that allow the use of alternative electron-accepting sources. We performed biochemical studies which indicate that *B. abortus* PrrB is a histidine kinase more active under reductive conditions that phosphorylates PrrA, its cognate response regulator, as reported in other bacteria.
We also demonstrated, using qRT–PCR, that under microaerobiosis PrrB is necessary to achieve the induction of high–affinity cytochrome oxidases and denitrification enzymes. By EMSA we found that PrrA directly regulates the nitrate reductase operon by binding to its promoter, and that phosphorylation of PrrA increases the affinity for this DNA fragment.
Altogether, these results show that the homologues to PrrB/A in *B. abortus* form a functional two-component system involved in the adaptation to microaerobic conditions.

MI-C07.
TWO BINDING SITES INVOLVED IN BACTERIAL ADP-GLUCOSE PYROPHOSPHORYLASES ACTIVATION
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Bacterial glycogen synthesis is allosterically regulated at the level of ADPGlc pyrophosphorylase (ADPGlcPPase) by intracellular metabolites. In *Agrobacterium tumefaciens* the enzyme is activated by pyruvate (Pyr) and fructose-6P (F6P) while in *Escherichia coli* by fructose-1,6-bisP (FBP). Previously, the *Eco*-ADPGlcPPase activation kinetics were extensively characterized and two amino acid residues were identified as critical for allosteric response since mutants Q74A and W113A are insensitive to FBP. Crosstalk analysis between Pyr and F6P activation in wild-type *Atu*-ADPGlcPPase, together with studies on the behavior of Q67A and W106A mutant enzymes (homologous to *Eco* mutants) were conducted. Noteworthy, W106A lost sensitivity to F6P although was still activated by Pyr. Results allowed us to propose two binding sites involved in allosteric regulation. Also, kinetic properties of chimeric proteins made by switching the N-term and C-term portions of *Eco* and *Atu* enzymes support this hypothesis. We further studied *Eco*-ADPGlcPPase activation, determining that Pyr has a synergistic role in FBP activation. Also, combination of Pyr with another effector in the *Streptomyces coelicolor* enzyme added sustain to the two sites model for regulation in bacterial ADPGlcPPases. One of these sites is related with Pyr, behaving as fine modulator that governs activity and/or sensitivity to other effectors.

MI-C08.
CHARACTERIZATION OF THE *Mesorhizobium loti* MAFF303099 TYPE-III SECRETION SYSTEM (T3SS)
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T3SS has a relevant role in the symbiosis of rhizobia with legumes. The effectors translocated by this system were described to favour the nodulation efficiency in some legumes or conversely affect negatively the symbiosis in others.
M. loti MAFF303099 has a functional type III secretory system involved in the nodulation process on *Lotus* spp. Four putative *M. loti* T3SS effectors (Mlr6358, Mlr6331, Mlr6361, and Mlr6316) have been described in our laboratory, and it has been demonstrated that the N-terminal regions of them direct the secretion of a translational fusion to a reporter peptide through T3SS.
By using single, double, and triple mutants, we demonstrated the positive and negative participation of some of these proteins in the determination of competitiveness on *Lotus* spp. Low competitiveness values correlated with low nodulation efficiency for a mutant deficient in three of the putative *M. loti* effectors. Our data suggest that the net effect of *M. loti* T3SS function, on symbiotic process with Lotus, results from a balance between positive and negative effects.

MI-C09.

IDENTIFICATION AND CHARACTERIZATION OF PHOSPHATIDIC ACID PHOSPHATASE ENZYMES IN *Streptomyces coelicolor*

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Phosphatidic acid phosphatase (PAP) catalyzes the dephosphorylation of phosphatidate yielding diacylglycerol (DAG), the lipid precursor for triacylglycerol (TAG) biosynthesis. Considering the increasing interest of bacterial TAG as a potential source of raw material for biofuel production, we have focused our studies on the identification and physiological characterization of the putative PAP enzymes present in the TAG producing bacterium *Streptomyces coelicolor*. We have identified two *S. coelicolor* genes, named *lppa* and *lppß*, encoding for functional PAP proteins. Heterologous expression of *lppa* and *lppß* genes in *E. coli* resulted in higher levels of DAG in this bacterium. In addition, the expression of these genes in yeast complemented the temperature-sensitive growth phenotype of the PAP deficient strain GHY58 (*dpp1lpp1pah1*). In *S. coelicolor*, the simultaneous mutation of both genes provoked a drastic reduction in *de novo* TAG biosynthesis as well as in total TAG content. Consistently, overexpression of Lppa and Lppß in the wild type strain of *S. coelicolor*, led to a significant increase in TAG production. The present study describes for the first time the identification of PAP enzymes in bacteria, completing the whole set of enzymes required for *de novo* TAG biosynthesis and providing further insights on the genetic basis for prokaryotic oiliness.

MI-C10.

ANTIGENICITY AND PROTECTIVE CAPACITY OF NOVEL VACCINE CANDIDATES FOR *Trypanosoma cruzi*

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We have previously identified a group of 22 novel vaccine candidates for Chagas' disease by screening an epimastigote-subtracted trypomastigote cDNA expression library. Of these, we selected 3 genes for further studies: G2, a hypothetical protein (TcCLB.507003.70), A12, a putative lysosomal membrane glycoprotein (TcCLB.510825.30), and A11, a TcTASV-C surface antigen (TcCLB.511675.3). In this work we analyzed the antigenicity of these proteins in the course of experimental and natural infection with *T. cruzi* and evaluated the protective capacity of TcTASV-C. Sera obtained from humans and infected animals reacted against the recombinant proteins, suggesting that these genes are expressed in the parasite stages that infect the definitive host and validate them as vaccine candidates. To test the protective capacity of TcTASV-C, we employed a prime and boost vaccination schedule (2 doses of DNA + GM-CSF/mouse and 2 doses of protein + alum/ mouse; TcTASV-C: n=6; controls: n=6). Fifteen days after the last dose, animals were challenged with the highly virulent *T. cruzi* RA strain (lineage VI). Vaccinated animals presented lower levels of both circulating parasites and mortality (50% vs 100% at 30 d.p.i.) than controls, suggesting that TcTASV-C induces a partially protective response.

MI-C11.

BtaE: A POLAR ADHESIN INVOLVED IN BINDING OF *Brucella suis* TO HOST CELLS AND VIRULENCE IN MOUSE

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Increasing evidence indicates that adhesion of *Brucella* spp. to host cells is an important step to establish infection. We have previously shown that an unipolar type I monomeric autotransporter (BmaC) mediates the binding of *Brucella suis* to host cells through cell-associated fibronectin. Genome analysis shows that the *B .suis* genome encodes several additional potential adhesins. In this work we show that a protein from the Trimeric Autotransporter Family named as BtaE is involved in the binding of *B. suis* to hyaluronic acid and fibronectin. The *B. suis btaE* mutant was: impaired in the ability to bind to host cells, outcompeted by the wild type strain in co-infections experiments, and showed an attenuated phenotype in the mouse model. Similar to BmaC, the BtaE adhesin was only observed at one of the cell poles. In *Brucella* and other α-proteobacteria, the two daughter cells generated by asymmetric division are differentiated bacteria displaying different pole markers. Using pole markers we observed that BtaE is associated with the new pole. Furthermore, BmaC was also found to be localized to the same (new) pole, suggesting that *Brucella* adhesins are located at the new pole of the bacteria at a particular stage of the cell cycle.

MI-C12.

BB3576 DIGUANILATE CYCLASE PROTEIN REGULATES MOTILITY AND BIOFILM IN *Bordetella bronchiseptica*

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Cyclic diguanylate (c-di-GMP) is a ubiquitous second messenger that regulates diverse cellular functions, including motility, biofilm formation, and virulence in bacteria. Components of this regulatory network include GGDEF domain-containing proteins that synthesis c-di-GMP. In a previous work we demonstrated that heterologous expression of a *Pseudomonas aeruginosa* GGDEF protein modifies biofilm formation capacity and motility in *B. bronchiseptica* (Bb), a pathogenic bacterium that causes respiratory infections in a wide variety of hosts.

In this work, we analyzed the expression of BB3576, a putative GGDEF protein of *B. bronchiseptica*. To this end, bb3576 gene from Bb was amplified and cloned in the replicative plasmid pBBMCR5 under a constitutive promoter and transformed in Bb. Biofilm formation and motility were evaluated in the recombinant bacteria to detect possible changes in c-di-GMP concentrations. As found in other organisms that contain high levels of c-di-GMP, we observed that Bb was able to form biofilm and reduce its motility only in the case bacteria express GGDEF domain-containing protein. Interestingly, high *bb3576* mRNA expression levels were detected by real time PCR in avirulent phase, when motility is present. These results demonstrate the presence of a functional GGDEF protein in Bb and suggest a putative motility regulation function for Bb3576.

MI-C13.
SEQUENCE ANALYSIS OF *fur* GENE FROM *Helicobacter pylori*-INFECTED PATIENTS WITH IRON DEFICIENCY
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Objectives: Infection with *Helicobacter pylori* has been related to iron deficiency (ID) in the host. *fur H. Pylori* mutant strains have enhanced capability to capture Fe, contributing to iron deprivation. We cloned *fur* genes from *H. pylori* strains from patients with and without ID and analyzed their sequences to establish if there was any nucleotide or amino acid differences regarding the iron status of the host. **Methodology:** DNA from *H. pylori* strains was isolated from 10 infected patients. Iron status of the host was determined by sera markers such as levels of ferritin, transferrin, serum iron and Total iron binding capacity. Primers for amplification of the *fur* gene were designed from different sequences of *H. Pylori* strains available at GenBank. **Results:** From all *fur* promoter analyzed sequences, only a single change was observed at the -10 box in a patient with ID. Other changes found were located at a non critical region within the promoter region. Regarding the Fur codogenic region, a single mutation at the initial ATG codon in an ID sample was observed. For the remaining samples there were no changes at the residues required for Fur dimerization or DNA binding capability. **Conclusions:** We suggest that mutations on the *fur* gene do not occur very often in ID patients and the reported changes seem not to heavily affect the function of Fur.
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MI-C14.
CONDITIONED MEDIUM FROM VERO-TRYPOMASTIGOTES MODIFY INTEGRIN β1 EXPRESSION IN MICE BONE MARROW CELLS
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Chronic Chagas' disease produced miocarditis. Authologus bone marrow cells (BMC) is one of the most important hopes for chronic miocarditis treatment. However, BMC quality could be dependig to the host pathophysiological modifications. Our previous works demonstrated that β1 integrin (β1) expression is down regulated in BMC from chronically infected mice when they are exposed to PMA (SAP 2009). In order to investigate if this phenomenon is mediated directly by *T.cruzi* or by the immune system we studied the effect of 10% conditioned medium (cm) from trypomastigotes-Vero cells infection (MDT) on BMC obtained from healthy C57/B1.mice; ex vivo cells were incubated for 48 h with cm and 18 h with PMA or LPS. β1 expression was study by FACS with anti-β1-biotine and avidine-FITC. Results were expressed in fluorescence (fuo)/1% events. Background was obtained with cells labelled only with avidine-FITC. Protein electrophoresis of cm was performed in order to investigate differences in protein profile. β1 expression decreased in BMC - MDT plus PMA or LPS compared with the BMC incubated with cm from Vero cells (MDV) in 4 duplicate independent experiments and its correlated with cells size. (PMA) MDV:7±2.20; MDT 3.04±3.03; (LPS) MDV: 10.42±6.06, MDT 3.96±3.26 fluo/ 1% of recorded events, $p<0.05$ analyzed by T test one tail. Electrophoresis has done one band 25 kDa, only in cm from MDT. The *ex vivo* experiments were correlated to those obtained from *in vivo*. In this work we found that β1 expression was differently modulated by the non immunological host cells infected with *T.cruzi*. BMC populations modified for β1 expression were monocuclear cells.

MI-C15.
***Cotton leafroll dwarf virus* PO PROTEIN IS A SILENCING SUPPRESSOR WHICH INHIBITS LOCAL RNA SILENCING**
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Cotton leafroll dwarf virus (CLRDV) is a member of the *Polerovirus* genus from the *Luteoviridae* family that infects cotton, causing cotton-blue disease, and is transmitted by the *Aphid gossypii.* The P0 proteins of several poleoviruses were shown to possess a suppressor activity of post-trasncriptional gene silencing (PTGS). Here we examined the silencing suppressor activity of $P0^{CLRDV}$. Agrobacterium-mediated co-infiltration of sense GFP with $P0^{CLRDV}$ in *N. benthamiana* line 16c. Which already has an integrated GFP transgene, led to the suppression of local PTGS, giving bright green infiltration spots at 5 days post-infiltration (dpi). However, $P0^{CLRDV}$ was not able to interfere with systemic spread of PTGS and the plants showed vein proximal silencing of GFP in systemic leaves at 14 dpi. In order to investigate the effect of $P0^{CLRDV}$ on RNA silencing triggered by double stranded RNA, we performed experiments infiltrating leaves of *N. benthamiana* with $P0^{CLRDV}$, GFP and a dsRNA inducer GFFG deriving from the GFP gene, observing a suppressor phenotype. Silencing suppressor activity was also observed, in both systems, when $P0^{CLRDV}$ was expressed in the viral context by using a full-length infectious cDNA CLRDV clone. Nothern blot analysis of GFP transcript levels and GFP-specific smal terfering RNAs (21-24 nt) confirmed the visual observation that $P0^{CLRDV}$ mediate silencing suppression.

MI-C16.
ANTIVIRAL AND IMMUNOMODULATORY ACTIVITIES OF SYNTHETIC STIGMASTANE ANALOGS
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The stigmastane analog (22S,23S)-22,23-dihydroxystigmast-4-en-3-one (compound 1) displays antiviral activity *in vitro* against Herpes Simplex Virus Type 1 (HSV-1). The compound also significantly diminishes proinflammatory cytokines production in inflammatory cells. The 3-keto group and the double bond in Δ^4 in the steroid A ring provide the molecule with structural similarities to that of corticosteroids. In order to improve the immunomodulatory activity of this molecule, we designed new stigmastane analogs, keeping the (22S,23S)-22,23-dihydroxylated side chain of the steroidal structure, responsible of the antiviral activity, and introducing an additional double bond in Δ^1 and/or a fluorine atom in the molecule, structural features known to improve the anti-inflammatory activity of steroidal drugs. The presence of the $\Delta^{1\text{-}4\ ceto}$ in the stigmastane structure reduced anti HSV-1 activity and did not improve the immunomodulatory effect of the compound. However, a fluorine atom in C7 enhanced both antiviral and immunosuppressive activities of the new compounds, since they were more effective than compound 1 to reduce virus yields in epithelial cells infected with HSV-1 and cytokine production in infammatory cells, as determined by virus yield reduction assays and ELISA, respectively. Radioligand binding assays and reporter gene induction assays showed that the stigmastane analogs did not exhibit affinity for the glucocorticoids and mineralocorticoid receptors. The compounds would be exerting their effect through a mechanism of action different from that of commercial anti-inflammatory steroids drugs.

ST-C01.
OXER1, A LEUKOTRIENE RECEPTOR, IS PRESENT AND ACTIVE IN STEROIDOGENIC CELLS

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Hormonal regulation of steroidogenesis involves arachidonic acid (AA) metabolization through a 5-lipoxygenase (5-LOX) pathway. The products, especially 5-HpETE, are involved in the activation of the steroidogenic acute regulatory (StAR) protein promoter. However, the mechanism of action of these compounds is still unknown. The oxoeicosanoid receptor OXER1 is a G protein coupled receptor of the leukotriene family, with high affinity and response to 5-lipoxygenase AA derivatives. Thus, we tested the presence and functionality of the OXER1 in steroidogenic cells. RT-PCR and western blot analysis demonstrated the presence of the mRNA and protein of the receptor in the human H295R adrenocortical cells. The treatment of MA-10 cells, murine Leydig cell line with 8Br-cAMP together with docosahexaenoic acid (an antagonist of the receptor) partially reduced StAR induction and steroidogenesis. 5-oxo-ETE -the agonist with highest affinity and potency on the receptor- increased cAMP-dependent steroid production in a dose-dependent fashion. This agonist also increased StAR mRNA and protein levels in both cell lines. These results led us to conclude that AA might modulate StAR induction and steroidogenesis through the autocrine or paracrine action of 5-LOX products on the membrane receptor OXER1.

ST-C02.
CONTRIBUTION OF PROLINE RESIDUES OF DESK IN THE SENSING AND TRANSMISSION OF COLD STIMULUS

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Bacillus subtilis regulates the expression of the *des* gene coding for the Δ5 acyl lipid desaturase by a two-component system composed of a membrane-associated kinase, DesK, and a soluble transcriptional regulator, DesR, which stringently controls transcription of the des gene. This pathway is activated in response to a decrease in membrane fluidity provoked by a temperature downshift. However, the molecular detail of how the input signal is sensed by the transmembrane segments (TMS) of DesK and transmitted to the cytoplasmic catalytic domain is completely unknown. In order to answer this fundamental issue, we decided to identify residues critical for cold sensing. DesK has five proline residues in its TMS which are conserved in several membrane-bound thermosensors. To investigate whether these residues play an essential role in the function of DesK, we mutated each proline individually to alanine. All DesKPA mutants were unable to activate *des* transcription upon a temperature downshift, whereas phosphatase activity was retained. By error prone PCR mutagenesis on *desKPA* alleles we obtained additional mutations that restored the wild type behavior. These results strongly suggest that proline residues of DesK TMS play an important role in sensing and transmission of cold stimulus by kinking the helices in order to bring cytoplasmic domains into optimal positions for catalysis.

ST-C03.
IDENTIFICATION OF YEAST PKA SUBUNITS TRANSCRIPTION REGULATORS USING TWO-COLOR CELL ARRAY SCREENING

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Transcriptional regulation is a key mechanism that controls the fate and response of cells to diverse signals. Therefore the identification of regulators which mediate these signals is a crucial step in elucidating how cell fate is regulated. We present here a detailed analysis of TPKs and BCY1 genes regulation based on the use of a dual reporter screen to discover regulators. In *Saccharomyces cerevisiae*, three genes encode the PKA catalytic subunit, TPK1, TPK2, TPK3, while the regulatory subunit is encoded by only one gene, BCY1. Using a two-color GFP-RFP reporter system, Reporter-Synthetic Genetic Array, we systematically assesses the effects of gene deletions on the TPK1, TPK2, TPK3 and BCY1 promoters activity. We could define a list of positive and negative transcription regulators that were classified using Gene Ontology analysis into functionally related groups. We identified genes with roles in lipid and phosphate metabolism, cytoskeleton organization and transcription regulation. Genetic networks were constructed showing that several regulators are shared by the PKA subunits. The involvement of some of these pathways in TPKs and BCY1 transcription regulation was validated. Thus, the reporter gene assay provides a powerful means to link gene function to transcriptional control.

ST-C04.
DEEP INSIDE THE NUCLEI: DECODING THE ROLE OF ARGININE DEIMINASE DURING ENCYSTATION OF *Giardia lamblia*

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Giardia lamblia encystation is crucial for disease transmission and parasite survival outside the host. We previously demonstrated that the enzyme arginine deiminase (ADI) translocates to the nuclei late during encystation. This translocation is mainly regulated by the SUMOylation of ADI because when the enzyme has mutations in the SUMOylation site, the translocation to the nuclei is reduced and the number of cysts produced is similar to wild type trophozoites. Proteins destined for transport in the nucleus contain amino acid targeting sequences called nuclear localization signals (NLSs). ADI contains a conserved bipartite NLS and a monopartite consensus sequence. Substitutions made in the first part of the monopartite or the bipartite NLSs led to accumulation of ADI in the nuclei, indicating normal entry of ADI into the nuclei. Unexpectedly, ADI in which two hydrophobic residues (V and M) were mutated to alanine residues failed to enter the nucleus. To conclude, we show that ADI is a nucleocytoplasmatic shuttling protein. ADI entry into the nucleus depends on its SUMOylation and on the presence of particular residues on its NLS. Once in the nucleus ADI citrullinates histones which probably causes genes of the CWP family to turn off as an essential requirement to successfully complete the encystation of the parasite.

ST-C05.
AR, ER, MnSOD AND COXIV IN 17ß-ESTRADIOL AND TESTOSTERONE ANTIAPOPTOTIC EFFECTS IN C2C12 CELLS

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17ß-Estradiol (E2) and Testosterone (T) exert actions in most tissues, including skeletal muscle. In aging, this tissue can present pathologies as sarcopenia, which is associated to low hormone levels that cause a deregulation of apoptosis. We previously showed that E2 and T inhibit H_2O_2 - induced apoptosis in C2C12 cells. Here we demonstrate that E2 up-regulates the expression and activity of manganese superoxide dismutase (MnSOD) but increases the activity of cytochrome c oxidase IV (COXIV) without changes in its expression. Pharmacological and immunological assays indicate that the estrogen receptor (ER) mediates these events. In addition, the expression of MnSOD and the activity of COXIV decrease when cells are treated with H_2O_2, effects that are reversed with E2 pretreatment. Experiments with the antagonist flutamide involve the androgen receptor (AR) in the antiapoptotic action of T. Biochemical and immunological data support mitochondrial and microsomal localization of the AR in the C2C12 cells. Sucrose gradient fractionation demonstrates its presence in rafts and caveolae. Besides, the AR interacts with caveolin-1, association that is lost after T treatment, suggesting an AR translocation from membrane to inner cellular compartments. Our studies deepen the knowledge of the molecular basis of myopathies associated with deregulation of apoptosis by hormonal deficit states.

CB-P01.
Rab1b DYNAMIC AT THE COPII-COPI INTERFACE
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The first step in the secretory pathway is the transport of membranes from the Endoplasmic Reticulum (ER) to the Golgi complex. Sorting and concentration of cargo take place by the action of the COPII (coatomer protein II) complex at specialized ER domains called ER exit sites (ERES). From the ERES vesicles bud and form a compartment called VTCs (Vesicle tubular clusters) where COPII is exchanged by COPI complex. The GTPase Rab1b is required for ER-Golgi transport. We have shown that Rab1b modulates both, COPII and COPI membrane association dynamic, and that Rab1b promotes COPII function and VTCs maturation. Although it is known that Rab1b is acting in two sequential stages at the ERES, is unknown how Rab1b coordinates the COPII-COPI transition.
In this work we used confocal microscopy and performed quantitative analysis to compare COPII and GFP-Rab1b co-localization in cells with or without Brefeldin A (BFA), an inhibitor of COPI formation. Furthermore, by performing time lapse microscopy assay in living cells, we analyzed the dynamic of pmCherry-Rab1b and GFP-COPs after BFA wash out (BFA-WO). Our results indicates that COPII-Rab1b co-localization was increased in cells treated with BFA and show that dynamics changes between Rab1b and COPs while anterograde transport from the ER to Golgi is recovered. This work provides new dynamic evidence about Rab1b at the COPII-COPI interface.

CB-P02.
METHOD FOR THE ESTIMATION OF THE AMOUNT OF ACETYLATED TUBULIN
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Tubulin, the main protein constituent of microtubules, is subjected to acetylation/deacetylation at the epsilon-amino group of Lys40 of the alpha-chain. Studies on the physiological role of this postranslational modification by many laboratories produced conflictive results, in part due to the unavailability of a method to quantify the amount of acetylated tubulin (AcTubulin) in the corresponding experimental systems. We present a simple method to estimate the percentage of AcTubulin with respect to total tubulin. The method is based on the comparison of the amount of Ac Tubulin (as determined by Western blot or ELISA using a commercial antibody specific to acetylated tubulin) before and after chemical acetylation with acetic anhydride. The amount of AcTubulin is calculated as the ratio of AcTubulin before acetylation over AcTubulin after acetylation, and multiplied by 100. Validation of the method was done by applying it on preparations of known AcTubulin concentrations.

CB-P03.
BLOCKADE OF THE COOH-TERMINUS OF ALPHA-TUBULIN BY IRREVERSIBLE INCORPORATION OF L-DOPA IN CULTURED CELLS
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We previously described that L-Dopa can be incorporated into the COOH-terminus of the α-chain of tubulin by using tubulin tyrosine ligase, one of the enzymes involved in the postranslational cyclic tyrosination/detyrosination of tubulin. Now, we found that after its incorporation into tubulin, Dopa cannot be released by the other enzyme involved in the tyrosination/detyrosination cycle, that is, tubulin carboxypeptidase. Dopa-tubulin, when mixed with Tyr-tubulin in a rat brain soluble extract, assembled into microtubules as well as Tyr-tubulin. The ability to disassemble by cold or dilution of microtubules formed from Tyr- or Dopa-tubulin was also similar. Due to the unavailability of an antibody specific to Dopa-tubulin, to monitor and to measure the amount of Dopa incorporated into tubulin we used a method based on Western blot analysis of the tyrosination state of samples before and after Dopa incorporation. Dopa incorporation into tubulin was also demonstrated to occur in cultured cells in the absence of "de novo" protein synthesis. Furthermore, once incorporated into tubulin of cultured cells, dopa could not be removed by subsequent incubation in medium lacking L-dopa even in the presence of added tyrosine reinforcing the idea that dopa binds irreversibly to the COOH-terminus of α-tubulin blocking the tyrosination/detyrosination cycle.

CB-P04.
PURIFICATION OF POLYMERIZABLE, ACETYLATED AND NON-ACETYLATED TUBULINS
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Tubulin is acetylated by a-tubulin acetyl transferase on Lys40 of the α-subunit and deacetylated by HDAC6 or Sirtuin-2. Most tubulin purification methods rely on assembly-disassembly cycling of microtubules producing preparations with low (or null) amount of the acetylated isotope. We found that this is due to a deacetylating activity in brain homogenates. HDAC6 is the involved deacetylase since the activity is inhibited by Trichostatin A (TSA) and tubacin but not by nicotinamide. TSA did not influence microtubule polymerization or depolymerization *in vitro*. We took advantage of these properties of TSA to impede deacetylation of tubulin during the assembly-disassembly steps and to obtain a microtubule preparation enriched in acetylated tubulin. Inhibitory concentration of TSA in the homogenate was 3 µM and 1 µM in subsequent steps. Three-cycles-purified tubulin conserves most of the acetylated tubulin present in the starting supernatant fraction and is free of deacetylating activity. We estimated that about 64% of the tubulin molecules in these preparations are acetylated. We found no differences between both preparations with respect to several tubulin properties and associated proteins. This is the first method described so far that allows large scale purification of polymerizable tubulin containing significant amounts of the acetylated isotype.

CB-P05.
P120 CATENIN REGULATES N-CADHERIN TRAFFICKING THROUGH N-ETHYLMALEIMIDE SENSITIVE FACTOR (NSF)

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N-cadherin, a cell-cell adhesion molecule, is synthesized in endoplasmic reticulum (ER)-bound ribosomes as a precursor protein, with a prodomain that is removed in the trans-Golgi network (TGN) to generate an adhesive competent protein. Previously, we found that a deletion mutant of the N-cadherin precursor that cannot bind p120 catenin, displayed impaired trafficking and processing of the prodomain. We have confirmed the putative positive role of p120 in trafficking by shRNA p120 knockdown. To investigate further the molecular mechanisms implicated, we analyzed the complexes of the N-cadherin precursor in conditions that prevent binding or expression of p120. We found that in both conditions the amount of NSF in the complex was lower than in the control. NSF regulates vesicle fusion, an important event in intracellular trafficking. Preliminary results also showed that p120 coimmunoprecipitated with NSF. These results suggest that p120 could recruit NSF to the N-cadherin precursor complex, facilitating its trafficking. Interestingly, expression of a dominant negative NSF construct showed a greater precursor/mature N-cadherin ratio, indicating a deficiency in N-cadherin precursor trafficking to the TGN. Our results suggest a novel role of p120 in N-cadherin trafficking that could be related to its capacity to recruit NSF to the cadherin complex.

Supported by CONICET and ANPCyT.

CB-P06.
A BROAD SPECTRUM OF CONGENITAL DISORDER OF GLYCOSYLATION SUBTYPES IN ARGENTINA

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Congenital Disorders of Glycosylation (CDG) are a growing group of hereditary diseases caused by defects in N-, O-glycoproteins and glycolipids. The clinical phenotypic spectrum is highly variable, ranging from severe multisystem disorders to alteration of specific organs. Most CDG are autosomal recessive, except (EXT1/EXT2-CDG), an autosomal dominant disease characterized by the formation of multiple cartilage-capped tumors. AIM: to increase the knowledge on human glycobiology. PART A: Using Tf-IEF, HPLC, MS and LLO, we identified six patients with abnormal N-glycosylation. Three CDG-Ix patients: two compound heterozygous PMM2-CDG patients (P1 and P2) and P3 in which the exome sequencing was done. Two mutations in GALT gene were detected. In patients CDG-IIx, studies are in progress. PART B: Mutations in EXT1 and EXT2 were studied by PCR, Sanger sequencing and MLPA. We present the clinical and molecular analysis of 33 unrelated O-glycosylation patients (79% multiple osteochondroma (MO) and 21% solitary osteochondroma (SO)). The 83% presented a severe phenotype, including two patients with malignant transformation to chondrosarcoma (11%). We found the mutant allele in 69% of MO patients. Only in one SO patient a complete exon 1 deletion in EXT1 gene was observed by MLPA. Discussion: We presented an extremely wide spectrum of clinical, biochemical and molecular basis in CDG subtypes.

CB-P07.
CERAMIDE REGULATES ACROSOMAL EXOCYTOSIS IN HUMAN SPERM

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Sphingolipid metabolism involves multiple metabolic steps that constitute a complex network. Ceramide is a metabolic hub because is generated either via de novo pathway or the sphingomyelin and it occupies a central position in sphingolipid biosynthesis and catabolism. Ceramide positively regulates membrane fusion in some biological systems even though has the opposite effect in other cell types. Regulated secretion is a central issue; for instance, mammalian sperm acrosomal exocytosis (AE) is essential for egg fertilization. Since we demonstrated that sphingosine-1-phosphate, an almost immediate product of ceramide breakdown, induces AE we wondered if ceramide has any effect on exocitosis and if it is so whether it is produced by itself or through the synthesis of bioactive lipids. By using Western blot of sperm extracts, we found a ~84 kDa band corresponding to neutral ceramidase, a hydrolase that removes the fatty acyl groups from ceramides at neutral pH. It binds to sperm membranes in a calcium independent manner. The alkaline ceramidase (~31 kDa), was also present in human sperm. Neutral sphingomyelinase, which generates ceramide, is present in sperm extracts (~48 kDa). Functional assays demonstrated ceramide regulation of AE. Here, we present the first piece of evidence indicating the presence of sphingolipids metabolism enzymes in human sperm and the importance of ceramides in AE.

CB-P08.
BEYONG THE BINDING SITE: SEARCHING TARGETS OF CNBP IN VERTEBRATES

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CNBP is nucleic acid chaperone required for proper rostral head development. Various studies have identified CNBP displaying broad sequence specificity and participating in the control of translational and transcriptional processes. CNBP is highly conserved among vertebrates; however, its molecular targets are largely unknown. By using yeast inverse one hybrid assays, we have recently determined the CNBP DNA binding site consensus as a 14 bp G-enriched sequence (GA/TGGGGGA/TGGGGGG). In this work, the consensus was used to find out putative CNBP target sequences among vertebrate gene promoters (arbitrary defined as <1kbp from Transcription Start Site) using MEME/MAST software (http://meme.nbcr.net/). Further bioinformatic analyses retrieved 16 putative CNBP targets conserved among human, mouse, chicken, amphibian and fish. Analysis of these genes by Cytoscape (http://www.cytoscape.org/) revealed Gene Ontology (GO) terms related to development and general metabolism. Chromatin immunoprecipitation (ChIP-PCR) and qRT-PCR are being carried out to confirm the binding and action of CNBP on the identified genes using zebrafish as model organism.

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CB-P09.

T CELL DIFFERENTIAL MOLECULES TARGETED BY THE *TRANS*-SIALIDASE (TS) FROM *Trypanosoma cruzi*

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T. cruzi, the agent of Chagas disease, requires sialic acids to invade mammalian cells although is unable to synthesize these sugars. This gap is filled through the action of TS, an enzyme capable of transferring sialyl residues from the host's glycoconjugates to the parasite surface glycoproteins. TS is also shed and disseminated by the bloodstream altering the immune system function. An enzymatically inactive isoform (iTS) is also expressed. The differential sialylation of immune cells is a critical event in immunomodulation. Then, the sialylation pattern generated by TS seems associated with the alterations induced in the immune response. Based on this hypothesis, we analyzed by Western Blot the differential sialylation patterns by TS of different lymphocyte populations that were sialylated with azide modified sialic acid-lactose. We observed differences between activated and naive T cells, as well as between Th1 and Th2 cells sialidation pattern. We have also analysed the binding of the TS to the surface of lymphocyte cells, which result stronger for iTS than for TS. To understand this process and its possibly relevance, target molecules bound by TS were identified after affinity chromatography of T hybridoma extracts through an immobilized iTS column followed by MALDI-TOF.

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CB-P10.

***C-FOS* REGULATES THE DIFFERENTIATION OF NEURAL STEM/PROGENITOR CELLS DURING NEUROGENESIS**

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The *c-fos* proto-oncogene is expressed in many situations involving neuronal differentiation and stimulation, which suggests that the c-Fos protein plays a role in regulation of the genes involved in neuronal organization and function, such as those encoding cytoskeletal proteins, enzymes and neurotransmitters. Neurons of the mammalian CNS originate from progenitors dividing at the apical surface of the neuroepithelium. These cells show a high proliferation capacity, and an adequate control of their growth and differentiation capacity is of key importance. We proposed to determine if the absence of c-Fos has an effect during neurogenesis. For this, we compared the development cerebral cortex on day 14,5 of *c-fos*+/+ and *c-fos*-/- mice. Immunofluorescence assay showed c-Fos expression at the cortical level while different markers of terminally differentiated neurons (BIII-Tubulin, NeuN and Tbr1) indicated less number of differentiated cells in the *c-fos*-/- mice. Primary cortical cultures performed in the presence of NGF show a lower percentage of neuronal differentiation in the *c-fos*-/- mice than in the *wt* condition. Determination of the mitosis angle in apical progenitors reflected a predominant symmetric division in the knock out condition. These results suggest that the absence of *c-fos* affects the differentiation capacity of the Neural Stem Progenitors Cells during neurogenesis.

CB-P11.

DEVELOPMENT OF AN ANIMAL MODEL TO STUDY OVARIAN STEROID EFFECTS ON MAMMARY CARCINOGENESIS

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Estradiol (E) promotes cell survival in mammary cancer (MC), while in colon cancer it seems to have a protective role. We aimed to establish an animal model of MC resembling menopausal women with hormone replacement therapy which can be compared with a colon cancer model to analyze the effects of E and progesterone (P). Sprague Dawley rats of 55 days of age were treated per os with 15 mg/rat of DMBA (7, 12-dimethylbenz[α]anthracene) (day 0) and ovariectomized (OVX) or sham operated (SHAM) on day 30. From day 37, rats were treated (s.c.) twice a week with E (60 µg/kg), P (3 mg/kg), EP (at the same doses) or vehicle (VEH) for 25 weeks. At sacrifice, trunk blood and tumors were taken for hormone determinations (RIA) and histological analysis. Latency, incidence and progression of tumors were determined. E, EP and SHAM rats showed a lower latency than P and OVX. 100% of E and SHAM rats developed tumors, while the incidence for EP was 75%, for P 11% and for OVX was 0%. E increased the number of tumors per rat. Tumor growth rate was similar in all groups. E levels in E and EP rats reached physiological values. All tumors were classified as ductal carcinomas and the mitosis/apoptosis ratio tended to be higher in SHAM and EP than in E treated rats. While further molecular studies are being currently underway, we have set an appropriate model to study ovarian steroid involvement in MC to be compared to colon cancer.

CB-P12.

HORMONE RECEPTORS AND APOPTOTIC FACTORS OF REGRESSING MAMMARY TUMORS IN PRIMIPAROUS OFA HR/HR RATS

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We aimed to study the mechanisms involved in the pregnancy/lactation-induced regression of mammary tumors in primiparous rats. Female OFA hr/hr rats were treated per os with the carcinogen (15 mg/rat of DMBA (7, 12-dimethylbenz[α] anthracene) at 55 days of age) and divided into two groups: nulliparous (Nul, n=24) and primiparous (PL, n=17). Latency, incidence and progression of tumors (growth or regression) were determined. At sacrifice, tumors were removed for histological studies and for qPCR of hormone receptors: estrogens (ERa, ERß), progesterone (PR, PRB) and prolactin (PRLR), and apoptosis related genes: Bax and Bcl-2. Statistical analysis was performed by Student t test and Fisher´s exact test. Tumor incidence was similar between groups, but latency was significantly lower ($p<0.01$) in PL group. PL rats showed the highest rate of tumors regressing during the experiment ($p<0.05$) and their tumors had higher count of apoptotic bodies ($p<0.05$). The mitotic/apoptotic ratio was significantly lower in the regressing tumors ($p<0.05$). No differences in the expression of ERa and ERß between groups were found, but tumors from PL rats showed lower expression of PR, PRB and PRLR ($p<0.05$) and the Bax/Bcl-2 ratio tended to be increased. The results so far suggest that apoptosis could be one of the mechanisms involved in the higher rate of regression observed in PL rats, probably related to changes in PRL and P pathways.

CB-P13.
REGULATION OF NA,K-ATPase BY DETYROSINATED TUBULIN. IMPLICANCE IN HYPERTENSIVE SUBJECTS

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Balance between tyrosinated and detyrosinated tubulin is essential for the normal development of the cells. Previously we demonstrated that detyrosinated tubulin is increased in erythrocyte membranes of hypertensive subjects. Moreover, there is known that in erythrocytes of hypertensive subjects the Na^+,K^+-ATPase (NKA) activity is decreased in a 50 % and that the activity of this enzyme is inhibited by the formation of a complex between the NKA and tubulin. Although it is known that acetylation of tubulin is essential to form the complex has not be studied if another postraslational modifications of tubulin are involved in the above mentioned regulatory mechanism. In this work we demonstrate that detyrosinated tubulin enhanced the inhibition of acetylated tubulin on the NKA activity and changes in levels of detyrosinated tubulin in the membrane produce modifications on the NKA activity. On the other hand, we show that the increase of detyrosinated tubulin in erythrocytes of hypertensive subjects would be a consequence of a decrease in the content and the activity of the enzyme tubulin tyrosine ligase but not tubulin tyrosine carboxipeptidase. In conclusion, detyrosination/tyrosination of tubulin has an important role in the regulation of the NKA activity, and then abnormalities in its cycle would be involved in the progression of different pathologies were NKA is implicated.

CB-P14.
EFFECT OF GLUCOSE ON DISTRIBUTION AND POSTRANSLATIONAL MODIFICATIONS OF TUBULIN IN ERYTHROCYTES

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In this work distribution of different isotypes of tubulin in erythrocytes treated with high glucose concentrations was analyzed. Results shown that, in human erythrocytes, glucose induce translocation of tubulin from sedimentable fraction to the membrane. Similar results are observed in Wistar rats injected with high glucose concentrations; in this case the maximum levels of tubulin in the membrane was reached during the peak of glycemia after the treatment. When the glycemia values return to his basal values, tubulin is relocated in the sedimentable fraction. Analysis of different tubulin isotypes in the membrane showed an increase of both detyrosinated and acetylated tubulin, whereas the tyrosinated tubulin is diminishes in this fraction. These results would explain the fact that in diabetic subjects Na^+,K^+-ATPase (NKA) activity is decreased. On the other hand, analysis of tubulin content in sedimentable fraction in erythrocytes treated with nocodazol showed that tubulin is lost when cells are treated with glucose. These results suggest that microtubules are present in the sedimentable fraction and they are more dynamics in erythrocytes treated with glucose. In conclusion, glucose induces in mammals' erythrocytes a redistribution of tubulin and changes in the different isotypes, fact that might be related to the inhibition of the NKA activity.

CB-P15.
INCREASE OF HUMAN ERYTHROCYTES DEFORMABILITY BY DIACYLGLYCEROL AND PHOSPHATIDIC ACID

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During the hypertension numerous alterations of the rheological properties of the blood have been described, such as erythrocyte deformability. This parameter is determined partially by the viscoelastic properties of cell membrane, which depend on his lipid composition and the intracellular calcium concentration. Previously, we demonstrated that the membrane tubulin content affects the deformability of erythrocytes. On the other hand, we have described that plasma membrane calcium ATPase (PMCA), a P-ATPase, form a complex with tubulin, as a consequence the enzymatic activity results inhibited; this regulation is dependent on the lipidic environment where PMCA is immersed. On the base of these antecedents, in the present work we study if changes in lipidic environments are able to affect erythrocyte deformability in normotensive and hypertensive subjects. Results show that the addition of 50 µM of diacylglicerol or phosphatidic acid increases erythrocyte deformability in normotensive and hypertensive subjects. Moreover, after lipid treatment, no significant differences in the deformability of erythrocytes of normotensive and hypertensive subjects were observed. Pretreatment of erythrocytes with activators e inhibitors of PMCA and Na^+,K^+-ATPase shown that effects in erythrocyte deformability induced by lipids would be mediated by modifications in such enzymatic activities.

CB-P16.
***C. elegans* UGGT-2 IS ESSENTIAL FOR VIABILITY ALTHOUGH IT LACKS GLUCOSYLTRANSFERASE ACTIVITY**

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The UDP-Glc:glycoprotein glucosyltransferase (UGGT) is the key component of the glycoprotein folding quality control mechanism in the endoplasmic reticulum. It behaves as a sensor of glycoprotein conformation as it exclusively glucosylates glycoproteins not displaying their native conformations. Most species have only one gene coding for UGGT-like proteins while species belonging to Euteleostomi and some species of nematodes belonging to genus *Caenorhabditis* have two homologues. In humans HUGT1 but not HUGT2 displayed UGGT activity. We report that *uggt-2* is an essential gene although CeUGGT-2 lacks canonical UGGT activity. We expressed CeUGGT-1 and CeUGGT-2 in *S.pombe* cells lacking UGGT activity and only CeUGGT-1 displayed UGGT activity. To evaluate if the CeUGGT-2 C-terminal domain were active in the context of SpUGGT we developed a chimeric protein between the SpUGGT N-terminal domain and CeUGGT-2 C-terminal catalytic domain. The chimera did not display UGGT activity, we therefore concluded that CeUGGT-2 C-terminal domain is also inactive. To evaluate if uggt-2 were an essential gene we analyzed the segregation of *uggt-2(ok2510)* allele in heterozygous *uggt-2* worms (chromosome balanced with a GFP-marked translocation).More than 50% of the eggs were arrested and animals that matured to adulthood were all GFP positive, thus confirming that *uggt-2* is an essential gene.

CB-P17.
ENDOCYTIC RECYCLING OF LRP1 IN ALPHA 2-MACROGLOBULIN-STIMULATED CELLS
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The LDL receptor-related protein 1 (LRP1) is an endocytic and signaling receptor, which play an key role in the cellular migration and proliferation. Previously we demonstrated that alpha 2-Macroglubulin (α2M*) induced intracellular signaling activation via LRP1, which is characterized by PKC and MAPK activation. Our hypothesis is that the cellular function of LRP1 involves the endocytic recycling and cell surface sorting of this receptor in α2M*-stimulated cells. Hence, in this work we tried to characterize the endocytic recycling and cell membrane sorting of LRP1 in MIO-M1 and HeLa cells stimulated with α2M*. Using confocal microscopy, flow cytometry and a recombinant mini-receptor version of LRP1 (mLRP4-GFP) we demonstrated that α2M* induced the increase of LRP1 localization in Rab5 and Rab11-recycling compartments respect to cells without stimulation. Then, LRP1 immunoprecipitation techniques of biotin-labeled cell surface proteins were used to show that α2M* promoted the intracellular sorting of the constitutive LRP1 and mLRP4 to the cell membrane. This sorting was partially blocked by the negative dominant mutant form of Rab11. However, other Rab forms, probably Rab8 and Rab6, could be involved in this sorting process. Our data suggest that the LRP1 function in α2M*-stimulated cells is dependent on the endocytic recycling of this receptor.

CB-P18.
LRP1 EXPRESSION AND FUNCTION IN HUMAN BLOOD PERIPHERAL MONOCYTE (BPM) SUBPOPULATIONS
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Alpha2-Macroglobulin (α2M*) is recognized by LDL receptor-related protein 1 (LRP1), and induces the cell migration of macrophages. BPM are involved in the development of atherosclerosis. In human, at least three subpopulations of BPM may be identified (classical, intermediate and non-classical). LRP1 it has been associated with atherosclerotic processes. The LRP1 expression and function in BPM subpopulations is not well established. By flow cytometry and confocal microscopy we characterized the LRP1 expression in BPM subpopulations. By two-dimensional wound scratch assay we examined the cell migration of each BPM subpopulation isolated by cell sorter and cultured in the presence of α2M*. LRP1 was three-fold more expressed in classical than in intermediate and non-classical BPM subpopulations. In classical BPM subpopulation, LRP1 was mainly localized at perinuclear regions, increasing the cell surface expression under α2M* stimulation for 30 min. In this time, the classical BPM also formed cellular protrusions with accumulation of F-actin and LRP1, which correlated with an increased cell migration. Thus, our data demonstrate that classical BPM subpopulation are induced to migrate by the α2M*/LRP1 interaction, which may be involved with the development of atherosclerosis.

CB-P19.
RAP2B AND KEY SIGNALING PROTEINS RELATED TO AUTOPHAGY ARE RECRUITED TO *Coxiella burnetii*
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Coxiella burnetii, the etiologic agent of Q fever, is a Gram-negative obligate intracellular bacterium. It has been previously described that autophagy contributes to *Coxiella* replicative vacuole (CRV) maturation. We have recently found that a noncanonical autophagic pathway, modulated by cAMP, is involved in the regulation of *S. aureus´*s alfa-hemolysin-induced autophagy. EPAC is a cAMP-modulated exchange factor for the small GTPase RAP. In order to determine whether the EPAC/RAP pathway is involved in *Coxiella* infection, we analyzed the role of this GTPase in the process. For this purpose CHO cells overexpressing GFP-Rap2b were infected with *C. burnetii* for different periods of time. Our results indicate that Rap2b was recruited to the CRV at all times of infection. In contrast, the inactive mutant Rap2b ΔAAX was not recruited to the CRV. Interestingly, vacuole size in cells overexpressing GFP-Rap2b wt was smaller than that of vacuoles developed in non transfected control cells. In addition, we have demonstrated that adaptor molecules required for the binding of the autophagic protein LC3 were also recruited to the CRV. Our results indicate that proteins involved in key signaling pathways related to autophagy associate to the limiting membrane of the C.burnetii likely to control distinct host cell responses upon pathogen infection.

CB-P20.
RECIPROCAL ALTERATION IN THE DISTRIBUTION PATTERN OF Rab7 AND Rab24
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Autophagy is characterized by the formation of a double membrane structure called autophagosome. Autophagosomes have been reported to fuse with early and late endosomes as well as lysosomes. Thus, autophagosomes maturation is a multi-step process, which includes several fusion events with vesicles originating from the endo/lysosomal pathway. In our lab 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; however, its function is currently unknown. Rab24 was implicated in the autophagy pathway since upon autophagy induction via starvation this protein changes its distribution colocalazing with the autophagosomal marker 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 GFP-Rab7wt and RFP-Rab24T21N, we observed a drastically change in Rab7 intracellular distribution. On the other hand, the reverse cotransfection, also altered Rab24 distribution. Our results suggest that both small GTPases share interacting proteins and that they are likely involved in a common pathway.

CB-P21.
***Chlamydia trachomatis* INTERCEPTS Rab39-MEDIATED VESICULAR TRAFFICKING**
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Chlamydia trachomatis is a bacterial causative agent of major sexually-transmitted diseases and blindness in humans. During its development, this obligate intracellular bacterium resides and multiplies in a modified vacuole called *inclusion*. *C. trachomatis* subverts key eukaryotic proteins in charge of vesicular transport to prevent its degradation by phagocytosis, and simultaneously, to obtain nutrients from parasitized cells. Rab GTPases are master controllers of intracellular trafficking pathways, which cycle between a GTP-bound form (active) to a GDP-bound form (inactive). The aim of this study was to investigate if *Chlamydia* manipulates host Rab39-mediated vesicular trafficking. We observed that Rab39a associated with the chlamydial inclusion along its developing time. Rab39a colocalized with IncA and IncG, two bacterial proteins, at the chlamydial inclusion membrane. We determined that the recruitment of Rab39a is bacterial protein synthesis-dependent. Furthermore, Rab39a recruitment to the inclusions remains unaltered after treatment with drugs that interferes with the cytoskeleton, such as nocodazole, butanedionemonoxime and cytochalasin. Rab39a decorated vesicles carrying sphingolipids and labeled with lysotracker in infected cells. Furthermore, overexpression of Rab39a WT and its positive mutant Rab39a Q72L increased the chlamydial inclusion size. Therefore, these results suggest a potential role for Rab39 in chlamydial infection outcome.

CB-P22.
INTERPLAY BETWEEN RAB11, RAB14 AND FIP2 IN *Chlamydia trachomatis*-INFECTED CELLS
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The intracellular pathogen, *Chlamydia trachomatis*, replicates within a special compartment limited by membranes (called "inclusion") and takes advantage of host vesicular transport for its own benefit. Rab GTPases are key regulatory proteins of intracellular trafficking. We have demonstrated that Rab11, Rab14 and Rab11-Interacting Protein 2 (FIP2) are recruited to chlamydial inclusions and, are necessary for bacterial multiplication. Recently, it has been described that FIP2 encompasses, at its C-terminus, a Rab Binding Domain (RBD) that interacts with both, Rab11 and Rab14. The aim of this study was to assess the interplay between these three host proteins in infected cells. The degree of colocalization at the chlamydial inclusion membrane was measured by quantitative confocal microscopy. Manders´ and Pearson´s coefficients indicated almost complete colocalization between these proteins, even after Golgi disorganization or depolymerization of microtubules. Overexpression of the Rab11 GDP-bound mutant (Rab11-S25N) decreased the recruitment of FIP2, whereas the overexpression of the FIP2 mutant lacking the RBD (FIP2ΔC2ΔRBD) did not affect Rab11 association with chlamydial inclusions. On the contrary, the silencing of FIP2 diminished the binding of Rab14. These results might suggest that FIP2 coordinates the sequential recruitment of Rab11 and Rab14 to chlamydial inclusions.

CB-P23.
PLASMA MEMBRANE-BOUND SIALIDASE Neu3 IMPAIRS THE CLATHRIN-MEDIATED ENDOCYTOSIS OF TRANSFERRIN
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Gangliosides (GS) are sialylated glycolipids mainly expressed at the outer leaflet of the plasma membrane. They have been implicated in many physiological and pathological processes, e.g. in cell growth, cell adhesion and endocytosis; including its capacity to function as receptor for several toxins, viruses and antibodies. While clathrin-mediated endocytosis has been widely studied, the specific role of GS in this cellular process has not been well established. By biochemical and cell biology techniques, we found an increased internalization of the transferrin-receptor (Tf-R) complex, the archetypical cargo for internalization through clathrin-mediated endocytosis, in cell lines expressing GS with higher level of sialylation. The ectopic expression of Neu3, a GS-specific sialidase, led to a drastic decrease in Tf endocytosis, suggesting a participation of GS in this process. However, the expression of Neu3 in GS-depleted cells maintained its effect on Tf-R endocytosis. Kinetic assays carried out in Neu3-over expressing cells showed a significant reduction in the sorting of endocytosed Tf-R complex to early and recycling endosomes. Overall, the results indicate that the effect of Neu3 on the internalization of Tf is independent of its action on GS, suggesting a novel role of this sialidase on clathrin-mediated endocytosis.

CB-P24.
CALCIUM SIGNALING IN HUMAN SPERMATOZOA
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Calcium and cAMP signaling plays a pivotal role in sperm physiology, being intimately involved in the regulation of capacitation, hyperactivation and acrosome reaction. In a previous report we have proposed that the cAMP analogue 8-(p-chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphate (8-pCPT-2'-O-Me-cAMP) elicits intracellular calcium release in human sperm. Intracellular calcium stores play a central role in the regulation of cellular [Ca2+]i and in the generation of complex [Ca2+] signals such as oscillations and waves. The aim of this study was to investigate the calcium stores and calcium channels involved in the 8-pCPT-2'-O-Me-cAMP pathway in human spermatozoa. We used real time dynamic assays - with high speed and spatial resolution- and fluorescent calcium sensors. To asses this, capacitated human sperm were loaded with Fluo3-AM, then incubated in a calcium-free medium and treated with different agonist of the AR (8-pCPT-2'-O-Me-cAMP, progesterone and recombinant human ZP3). We found that all AR inducers triggered spatial distribution of Ca2+and using a kinetic assay we studied the dynamics and direction of the calcium wave. Our results indicate that 8-pCPT-2'-O-Me-cAMP, progesterone and ZP3 induce a calcium wave that starts in the posterior neck region and propagates to the acrosome region.

CB-P25.

PRENATAL XENOESTROGEN EXPOSURE AFFECTS *AMH, SOX9* AND *SF-1* mRNA EXPRESSION IN *Caiman latirostris*

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Caiman latirostris exhibits temperature-dependent sex determination (TSD). Male-to-female sex reversal after in ovo estrogen/xenoestrogen exposure was demonstrated (hormone-dependent sex determination: HSD). amh, sox9 and sf-1 genes are involved in sex determination/differentiation and steroidogenesis. Our aims were: a) to establish the expression patterns of amh, sox9 and sf-1 mRNA in the gonad-adrenal-mesonephros (GAM) complexes of neonatal TSD-male and -female, b) to compare gene expressions between TSD-females and HSD-females, c) to evaluate if in ovo exposure to xenoestrogens (E2, bisphenol A-BPA or endosulfan-END) modify amh, sox9 or sf-1 mRNA expressions in neonatal TSD-males. mRNA expressions were quantitatively compared by RT-PCR. We demonstrated a sexually dimorphic pattern of amh and sox9, with higher expression in TSD-male than in -female. sf-1 mRNA did not differ between TSD-males and -females. HSD-females exhibited a higher expression of sox9 than TSD-females. In neonatal TSD-males, increased mRNA expression of sex-determining genes was observed after in ovo exposure to END. E2 decreased the sox9 but increased the sf-1 mRNA expression. Changes induced by BPA were evident although no significant. Our results provide new insights into the potential mechanisms that lead to the gonadal histo-functional alterations observed in caimans exposed to contaminated environments.

CB-P26.

CRUZIPAIN C-TERMINAL DOMAIN SULFATES ARE INVOLVED IN THE INVASION OF *Trypanosoma cruzi* TO CARDIMYOCYTES

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Sulfation reaction plays a key role in various biological processes. Sulfotransferases catalize the transference of the sulfate group from the donor 3´phosphoadenosine 5`phosphosulfate (PAPS) to an acceptor group. Sodium chlorate is an in vitro inhibitor in the biosynthesis of PAPS. *Trypanosoma cruzi*, the causing agent of Chagas disease, contains a major cysteine proteinase, cruzipain (Cz) which bears a C-terminal domain (C-T) containing sulfated high-mannose type oligasacharides. Chlorate treated epimastigotes evidenced undersulfation of Cz, a significant decrease of sulfatides and an increase of neutral lipids as confirmed by TLC/UV-MALDITOF analysis, suggesting sulfation mechanism via PAPS. Thus, chlorate-treated epimastigotes showed different alterations by ultrastructural analysis. Interestingly, a significant decrease of the infection percentage (30%) was observed with chlorate-treated trypomastigotes using a cardiocyte cell line. After cardiac cell pre-incubation with C-T prior to and after desulfation treatment, the infection percentage decreased significantly (30%) with the desulfated form. A significant increase in trypomastigote infection (40%) after a cardiocyte pre-treatment with an anti-mannose receptor, capable to recognize sulfated molecules, suggest that the C-T domain sulfates might be relevant for the *T.cruzi* invasion through the interaction with this receptor.

CB-P27.

***IN VIVO* SPECIFICITY OF OLIGOSACCHARYLTRANSFERASE FOR TRUNCATED GLYCANS**

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The endoplasmic reticulum membrane oligosaccharytransferase complex (OST) transfers glycan $Glc_3Man_9GlcNAc_2$ from a dolichol-PP derivative upon protein *N*-glycosylation. Glycan transfer enhances glycoprotein folding efficiency. Defects in the transfer reaction due either to OST mutations or to truncated glycan structures may result in protein hypoglycosylation thus causing diseases known as congenital disorders of glycosylation type I. The OST has been shown to have strict preference for the complete glycan but no systematic studies have been performed concerning the *in vivo* transfer rate of glycans not bearing the full complement of 3 glucoses or 9 mannoses. We constructed four series of mutants of the fission yeast *Schizosaccharomyces pombe* that synthesize dolichol-PP-linked glycans bearing from 0 to 3 glucoses and 5, 6, 7 or 9 mannoses and expressed *Saccharomyces cerevisiae* carboxypeptidase Y (CPY), a protein bearing four *N*-glycosylation sites. The degree of CPY hypoglycosylation provides a good indication of OST transfer efficiency. Our results indicate that *N*-glycan mannose content does not influence glycan transfer by the OST *in vivo* and that in *S. pombe*, unlike what happens in other known organisms, three glucoses may be added to glycans in the absence of the full complement of mannoses.

CB-P28.

NMR STRUCTURE OF THE LECTIN DOMAIN OF GLUCOSIDASE II AND CONFIRMATION OF THE MODEL OF GLYCAN BINDING

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Glucosidase II (GII) removes the two innermost Glc residues from the glycan ($Glc_3Man_9GlcNAc_2$) transferred to proteins during *N*-glycosylation. It also participates in cycles involving the lectin/chaperones calnexin and calreticulin as it removes the single Glc unit added to folding intermediates and misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase, a glycoprotein folding sensor. GII is a heterodimer whose alpha subunit (GIIa) bears the active site. We have previously shown that the C-terminus Mannose 6-Phosphate Receptor Homologous (MRH) domain present in GII beta subunit (GIIß) enhances the deglucosylation activity of GII. Here we show that isolated GIIß MRH domain competes with full length GIIß for *N*-glycan binding and present its NMR structure in solution. We demonstrate that the amino acid W409, conserved in almost all GIIß MRH domains from different species, is involved in the enhancement of GII activity by MRH as mutagenesis of that residue reduces activity of heterodimeric GII *in vitro* and delays *N*-glycan deglucosylation of ER glycoproteins *in vivo* without affecting neither GIIa-GIIß interaction, nor GIIa active site. Our results show how the MRH domain of the beta subunit presents the glucose-containing arm of the *N*-glycan to the catalytic site of GII's alpha subunit.

CB-P29.
TEMPORAL DYNAMICS OF STRESS GRANULES
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Gene expression changes in response to stress; this includes the formation of stress granules (SG), foci where many transcripts are stored during stress, releasing translational machinery for the synthesis of stress response factors. SG are cytoplasmic aggregates of silenced mRNAs and a number of RNA processing factors. Since stress response is modulated by biological clocks, and a number of SG components display circadian rhythms, we hypothesized that SG could be temporarily regulated. We synchronized NIH3T3 cells and harvested them at different times. SG were induced by oxidative stress and analyzed by ICC with an anti-eIF3 antibody. We found that SG number and signal intensity present temporal changes. Area and perimeter showed only modest variations. The expression of eIF3 did not change. We found no changes in phosphorylated eIF2alpha, a protein involved in SG assembly. Then we analyzed by RT-qPCR the temporal expression of several RNA-binding proteins that are in SG and could be involved in the temporal changes observed. Interesting, *Tia1, Brf1, hnRNPQ*, and *Lark* transcripts presented temporal changes in their levels. TIA1 protein has been implicated in SG assembly; we found that its levels oscillate. LARK, which regulates the translation of the clock protein PER1 and circadian behavior, also presented temporal variations. Our results showed that SG are temporarily regulated.

CB-P30.
StarD7 SIRNA MODULATES ABCG2 EXPRESSION, CELL MIGRATION AND PROLIFERATION IN EPITHELIAL CELL LINES
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StAR-related lipid transfer domain containing 7 (StarD7) belongs to the family of START proteins ubiquitously expressed, which are implicated in lipid transport, metabolism, and signalling. Previous results indicate that StarD7 silencing decreases ABCG2 multidrug transporter level, cell migration, proliferation, and reduction in phospholipid synthesis; whereas an increase in biochemical and morphological differentiation marker expression was detected in the epithelial choriocarcinoma JEG-3 cells. The present study was undertaken to extend these data to other epithelial tumour cells to establish the StarD7 function. In order to do this, we performed StarD7 knockdown in hepatoma HepG2 and colon adenocarcinoma HT-29 cells, two cell lines in which we previously demonstrated higher StarD7 expression levels. StarD7 silencing, confirmed by qRT-PCR and western blot, led to a decrease in the xenobiotic/ lipid transporter ABCG2 at both the mRNA and protein levels. Also a concomitant reduction in bromodeoxyuridine uptake was detected. Wound healing and transwell assays revealed that HepG2 and HT-29 cell migration was significantly diminished. Altogether these findings provide evidence for a role of StarD7 in cell physiology suggesting that it has a conserved function. Current work is in progress to establish the mechanisms involved in these findings.
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CB-P31.
BREAST TUMOR MICROENVIRONMENT INDUCES C-FOS OVEREXPRESSION, ENDOCRINE-RESISTANT AND CSC ENRICHMENT
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The Epithelial mesenchimal transition (EMT) program depends on a series of intracellular signaling networks involving, among others signal-transduction proteins, ERK, MAPK, PI3K, and c-Fos. Induction of c-Fos expression in normal mouse mammary epithelial cells induces EMT and is associated with a decrease in E-cadherin expression. Previous to EMT, CD44high/CD24high cells correspond to the phenotype of the majority of cells found in breast carcinomas whereas a shift to CD44+/CD24- cells, promotes EMT and a profile associated with human breast cancer stem cells (CSC) leading to the acquisition of mesenchymal qualities and an increased ability to form mammospheres and increased metastatic potential. We found, that the microenvironment formed by macrophages induces both EMT in breast cancer cells with a stem cell–like phenotype and overexpression of c-Fos. Additionally, in cultures, this microenvironment induces proliferation, invasiveness, and migration of these breast cancer cells, even in presence of Tamoxifen. Macrophages-Breast Cancer Cells cocultured induced a sustained release of IL-6 from both cell types, leading to activation of ERK, STAT3 and c-Fos in the breast cells and also Tamoxifen resistance. Furthermore, *In vivo*, this microenvironment increases breast tumor growth when macrophages are co-injected with MCF-7 cells into immunocompromised mice and Tamoxifen resistance.

CB-P32.
MOLECULAR BASIS OF TUMOR AGGRESSIVENESS: *IN VIVO* ANALYSIS OF SIGNALLING PATHWAYS ALTERED BY PIN1
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Genetic and epigenetic alterations conspire to wire aberrant signaling circuits that modify cell behavior, propelling the acquisition of aggressive tumor phenotypes. Previously, we identified Pin1 as a critical link between oncogenic signaling and downstream mechanisms of tumor aggressiveness. Pin1 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 prolil-directed phosphorylation allows Pin1 to act as a global modulator of biological responses. 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 pathways that govern development but also cooperate with oncogenic mechanisms in tumor cells. We have isolated a sequence coding for zebrafish Pin1 and analyzed gene expression during early development. Transient overexpression of Pin1 through microinjection of 1-cell embryos showed that forebrain is altered, suggesting that cell types involved in the development of this area are particularly sensible to Pin1 deregulation.

CB-P33.
EFFECT OF LDL-Ox ON GENES RELATED TO INFLAMMATORY PROCESS

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Isolated LDL fraction from human plasmas was peroxidized *in vitro* with Cu^{++} (5 µM) for 4 h (low, L), 8 h (medium, M) and 24 h (high, H) and dialyzed overnight to wash-out the copper ions. Three types of oxidized LDL were obtained. Percentage of dead cells -evaluated by trypan dye exclusion- compared to control flasks incubated with native LDL fraction was increased in the L, M and H assays. We selected LDL-Ox (M) to evaluate the expression of genes involved in the inflammatory process (TNFα, iNOS, IL6, FAT/CD3) and 11ß hydroxysteroid dehydrogenase type 2 (11ßHSD2). RAW 264.7 cells were treated with LDL-Ox (M) for 4, 8, 12 and 24 h to a final concentration of 100 µM. The mARN level of different genes were measured by quantitative real-time PCR. Results showed an increase of TNFα, IL6 and iNOS gene expression which was more marked between 4 and 12 h. FAT/CD36 expression increased also in a range of 10-fold at 12 and 24 h versus controls indicating uptakes of fatty acids and formation of foam cells. The effect of LDL-Ox in the culture medium promotes a significant increase of 11ßHSD2 expression between 8 and 12 h. The increase of 11ßHSD2 expression indicates the oxidation of corticosterone to dehydrocorticosterone (glucocorticoid inactive form). This fact would prevent the differentiation towards an antiinflammatory macrophage profile promoted by glucocorticoids.

CB-P34.
ROLE OF GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE 2 (GPAT2) IN CELL PROLIFERATION AND SURVIVAL

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The limiting step for glycerolipid biosynthesis is the acylation of glycerol-3-phophate catalysed by glycerol-3-phosphate acyltransferase (GPAT). Although its expression seems to be restricted to very few cell types, in silico analysis showed that GPAT2 could be expressed in breast infiltrating carcinomas. For its validation, qPCR was performed on different cell lines and normal or neoplastic human mammary samples. MDA-MB 231 was the only cell line able to express GPAT2. Also, GPAT2 protein expression by IHC in an independent group of carcinomas (n=35) and normal (n=6) samples showed that GPAT2 protein is not detected in normal tissues, whereas 37% of tumours showed a positive reaction. Protein expression significantly correlated with histological grading ($p=0.02$). A stable GPAT2 KO MDA cell line, with only 5% of GPAT2 expression had 50% lower cell growing rate on MTT growing curve assays. On the other hand, a stable 10-fold GPAT2 overexpression in MDA cells was able to increase the cell growing rate by 100%. By TUNEL assay, GPAT2 overexpressing cells presented less apoptosis after 2h of 1 µM stauroporine treatment, compared to its control (37% vs 44% $p<0.01$). With an incubation of 5h, 95% of control cells showed apoptotic traits whereas only 77% of GPAT2 overexpressing cells was affected, $p<0.001$. Conclusion: the activity of GPAT2 is able to modify cell proliferation and survival.

CB-P35.
TRANSCRIPTIONAL REGULATION OF MURINE GPAT2 COMPARED TO THE HUMAN ISOFORM

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Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the first step in de novo glycerolipid synthesis. In mammals, at least four GPAT isoforms have been described, differing in their cell and tissue locations and functions. GPAT1, 3 and 4 are expressed in lipogenic organs like liver and adipose tissue. Unlike the other 3 isoforms, GPAT2, located in the outer mitochondrial membrane, is highly expressed in rat and mouse testis and certain cancer cells, tissues that do not specialize in lipid synthesis and storage. Gpat2 transcription in testis peaks at the beginning of rat spermatogenesis indicating transcriptional regulation, possibly related to hormones. We cloned the murine Gpat2 promoter region (~6.7, 3.4 and 1.3kpb upstream from the transcription initiation site) into a luciferase-reporter vector and transfected them into CHO-K1 cells. Only the 3.4 kbp construct significantly activated transcription, so we used it for further experiments. Among several hormones, cis-9-retinoic acid increased the promoter's activity, consistent with its role in testis function.
In silico data indicated epigenetic regulation for human GPAT2 gene. Human and mouse cell lines that do not express GPAT2 were treated with a DNA methylation inhibitor and GPAT2 expression was increased only in the human cell lines, evidencing DNA methylation as a possible regulatory GPAT2 expression mechanism in humans.

CB-P36.
THE T98G GLIOBLASTOMA AS A MODEL TO INVESTIGATE THE CIRCADIAN CLOCK FUNCTION IN HUMAN TUMOR CELLS

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Circadian clocks present in the brain, peripheral tissues and even in immortalized cell lines, temporally regulate a number of cellular and metabolic processes. The disruption of biological clocks may lead to diverse pathologies; however, little is known about the activity of circadian clocks in tumor cells. To this aim, we use the T98G cells derived from a human glioblastoma to investigate potential rhythmic responses under non-proliferative conditions. We looked first for a protocol to obtain quiescent cells able to be synchronized by extracellular signals: horse serum (HS for 2 h) or dexamethasone (100 nM DEX for 20 min). For this, cells were grown for 48 h in DMEM + 10% FBS to reach confluence and then, they were treated with HS or DEX. After synchronization, cells were maintained in serum free-DMEM for 24 h, collected at different times from 0 to 32 h and subjected to flow cytometry or RT-PCR for further analysis. The cytometry clearly shows that only cells synchronized by DEX were mostly arrested: 57-79% of cells were at G_0-G_1 phases at the different times examined while less than 12% of cells were mitotic. Preliminary results indicate that synchronized cells expressed the clock gene Bmal1 mRNA with higher levels during the first 4-8 h. Our results suggest that T98G cells synchronized by DEX are mostly quiescent and display circadian rhythmicity in the expression of Bmal1.

CB-P37.

ELUCIDATING ESSENTIAL PROTEINS IN THE TRANSLOCATION OF EXOPOLYSACCHARIDE I IN *Sinorhizobium meliloti*

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A Wzy-dependent like pathway, which includes the participation of ExoPTQF proteins, has been suggested to be involved in the polymerization of exopolysaccharide I (EPS I) of *S.meliloti*. However, the final steps of this process are poorly understood. Recently, by cellular fractionation and immunoblot, our group has evidenced a PCP2a family protein, ExoP, located in the inner membrane and its pivotal role in EPS I synthesis has been established. Here, we show preliminary results about the role of ExoF in this process and its potential interactions with ExoP. By bioinformatics analysis, we identified ExoF as OMA family member located in the outer membrane. Furthermore, generation of nonpolar exoF mutant resulted in a nonproducing EPS I phenotype. Fluorescence microscopy analysis of cells expressing ExoF-mCherry- and eGFP-ExoP tagged proteins, revealed that these proteins are highly dynamics during the exponential phase of growth. However, when the growth rate decreases, both proteins localize at cell poles and this moment is coincident with the beginning of EPS I export. Based on all these data, we postulate that ExoP and ExoF interact with each other to form a protein complex that allows the assemblage and translocation of EPS I. Shedding light in these mechanisms will increase our knowledge about biopolymers synthesis and export in this bacterium of agronomic and pecuary importance.

CB-P38.

CHROMATIN STRUCTURE AND ALTERNATIVE SPLICING CHOICES IN G9a DURING CELL DIFFERENTIATION

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In mammals G9a is the primary enzyme for mono- and dimethylation at Lys 9 of histone H3 (H3K9me1 and H3K9me2). There are two alternatively spliced mRNA variants of this gene, but their difference in function is still unknown. We have determined that, during neuronal and mammary cell differentiation, inclusion of the alternatively spliced exon 10 is increased. Since it is known that dynamic changes in intragenic chromatin structure could affect RNA polymerase II processivity or elongation rate, and thus modulate alternative splicing choices, we are currently studying the intragenic chromatin structure of G9a during cell differentiation. We have seen that treatment of neuronal differentiated cells with a DNA methylation inhibitor and with a histone hyper-acetylating drug revert the effect of differentiation on exon 10 inclusion, suggesting that an epigenetic component could be involved in this process. Furthermore, siRNA-mediated down regulation of G9a expression in neuronal differentiated cells changes the ratio between spliced variants, suggesting an important rol of G9a in regulating it´s own splicing. We are currently analyzing the changes in DNA methylation that could be involved in the regulation of G9a alternative splicing and trying to determine which signal transduction pathways are specific to this mechanism.

CB-P39.

NITRIC OXIDE STIMULATES MELANIN PRODUCTION DURING IMMUNE RESPONSE IN *Apis mellifera*

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The honey bee *Apis mellifera* is known to be affected by many parasites. The *A. mellifera* immune system is a central weapon against parasites. Insects have a well developed innate immunity and a characteristic humoral immune response is melanisation. Melanin is derived mostly through the action of the enzyme phenoloxidase (PO) triggered by activated host blood cells or hemocytes. Little is known on the molecular mechanisms that activate hemocytes and initiate melanogenesis in honey bees. Hemocytes adhere to nonself particles attaching them by secreting cytotoxic compounds like reactive oxygen species (ROS) and reactive nitrogen intermediates (RNIs). Nitric oxide (NO) is a multifunctional RNI generated by the enzyme NO synthase (NOS) in insects functioning as a cell signalling molecule in immune responses. We have previously reported that NO plays a role in the immune activation of *A. mellifera* hemocytes. In the present study we analyzed the role of NO in melanogenesis. The formation of melanin was studied in the presence of the NO donor sodium nitroprusside (SNP) or the NO scavenger carboxi-PTIO (CPTIO) using *A. mellifera* haemolymph. While SNP enhanced melanisation, CPTIO reduced the formation of the pigment suggesting that NO acts as a promoter of melanogenesis in *A. meliffera*. Experiments are in progress to link the NOS substrate L-Arginine with NO production and melanisation.

CB-P40.

DEHYDROLEUCODINE AFFECTS THE LEADING EDGE DYNAMIC OF HeLa CELLS

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Because metastasis is one of the major causes of mortality in cancer patients, it is important to find effective drugs to stop the cancer cell migration. Dehydroleucodine (DhL) is a natural compound extracted from an herb known as matico (Artemisia douglasiana B). Previously we found that control HeLa cells migrate at a rate of 9.1 ± 0.8 µm/h and in presence of 20 µM DhL at 4.8 ± 0.4 µm/h. The cell migration leading edge consists of dynamic protrusions of the plasma membrane like lamellipodium, filopodium and ruffles that their formation are stimulated by a family of Rho proteins, where Rac1 is the most important. In this work we studied the effect of DhL in the leading edge dynamic of HeLa cells. We performed the wound healing assay and analyzed by video-microscopy, the frequency of lamellipodium and ruffles formation. We found that control cells produce 21.8 ± 1.6 lamellipodium/h and 21.8 ± 2.5 ruffles/h, while the treated cells 6.5 ± 1.3 lamellipodium/h and 8.2 ± 0.1 ruffles/h. In addition, by fluorescence microscopy we observed a greater accumulation of Rac1 in the membrane protrusions of DhL treated cells. These results indicate that DhL diminishes the HeLa cell migration velocity because inhibits the leading edge dynamic and modifies the distribution of Rac1.

CB-P41.
DEHYDROLEUCODINE INDUCES SENESCENCE THROUGH THE p53 SIGNALING PATHWAY
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Among antitumor drugs currently studied, the sesquiterpene lactones (SLs) are of particular interest due to its cell antiproliferative activity. Dehydroleucodine (DhL) is a SL purified from *Artemisia douglasiana Besser*, a medicinal plant used in Argentina. Previous results from our laboratory showed that DhL inhibits the HeLa cells proliferation, arresting them in the G1 cell cycle phase. In this work we asked whether the antiproliferative effect of DhL is related with the induction of senescence and is involved the tumor suppressor p53. HeLa cells were treated with 0-20 µM DhL for 48 h. The cellular senescence was evaluated by the senescence-associated ß-galactosidase (SA-ß-Gal) in situ assays and the levels of p53 and p21 were assayed by Western blot. The treatment with 0 µM DhL induced 6.60 % ± 4.30 of cells positive to SA-ß-Gal and with 20 µM DhL, 53.41 % ± 1.51. In addition, the treatment with 20 µM DhL increased 27.84 % ± 3.02 and 114.01 % ± 19.6 the levels of p53 and p21 respectively. These results indicate that the DhL effect on HeLa cell arrest is due to induction of senescence through the p53-p21 signaling pathway.

CB-P42.
THE CAVEOLAR ROWS COLOCALIZE WITH MYOFILAMENTS BUNDLES IN PERITUBULAR MYOID CELLS FROM ADULT RAT
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In the mammalian testis, peritubular myoid cells (PM cells) surround seminiferous tubules (ST) and participate in their contraction. PM cells contain myofilaments (MFs) arranged in two independent layers, the outer and the inner ones with longitudinal and transverse MFs to the ST axis. Endothelin-1 induces PM cells contraction by binding to its receptors (ETRs) localized in fixed caveolae. Due to the arrangements of MFs in PM cells, we asked if the localization of caveolae and ETRs receptors follow the MFs orientation. For this purpose, ST from adult rat testes were fixed with 4% paraformaldehyde for immunofluorescence (IF) or with 5% glutaraldehyde for transmission electron microscopy (TEM). For IF, MFs were visualized with anti-alpha actin antibody (Ab), caveolae using anti-caveolin-1 Ab, and ETRs with ETAR and ETBR Abs. By confocal microscopy, caveolin-1 and ETRs appear in the outer and the inner plasma membranes of PM cells in rows following the orientation of MFs bundles in the longitudinal and transverse layers respectively. By TEM we observed a close association of caveolae in the plasma membrane with the underneath Mfs. The conspicuous colocalization among the caveolar rows, ETRs in each plasma membrane and the MFs bundles reinforces the possibility that PM cells generate contractile force in two axes.

CB-P43.
KEY AMINOACIDIC POSITIONS FOR DESMOPRESSIN (DDAVP) ANTIPROLIFERATIVE EFFECT ASSESSED BY ALA-SCANNING
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DDAVP (1-deamine-8-D-arginine vasopressin) is a synthetic analog of vasopressin with antimetastatic properties. The molecule is a well known and safe hemostatic compound that acts as a selective agonist for the vasopressin V2 receptor, expressed in endothelium and in some tumor cells, including breast and lung cancer. We have previously reported dDAVP inhibitory effect on tumor cell growth.
The aim of this work was to identify key aminoacidic positions involved in dDAVP-V2 receptor interaction responsible for desmopressin antiproliferative activity. For that purpose Ala-scanning analysis of the peptide was carried out on MDA-MB-231 breast cancer cells. Antiproliferative activity was severely impaired when positions 2-5 in dDAVP molecule were ala-substituted, highlighting the role of these amino acids in ligand-receptor interaction. In order to improve this interaction and increase its anticancer effect, substitutions at positions 4 and 5 were introduced, increasing hidrophobicity at the cyclic region of the novel peptide. This resulted in an enhanced inhibitory effect on cell proliferation and tumor growth. We conclude that the amino acids belonging to the conformational loop of the molecule are essential for interaction with V2 receptor and for the biological activity of the compound. Further studies of ligand-receptor affinity must be carried out to corroborate this hypothesis.

CB-P44.
***IN VITRO* AND *IN VIVO* STUDIES OF THE PROAPOPTOTIC PEPTIDE CIGB-300, AN INHIBITOR OF CASEIN KINASE 2**
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We have previously demonstrated that a proapoptotic cyclic peptide CIGB-300 was able to abrogate the CK2-mediated phosphorylation by direct binding to the conserved phosphoacceptor site on their substrates. Previous findings indicated that CIGB-300 inhibits tumor cell proliferation in vitro and reduces tumor growth in cancer animal models. In this work we focused on the cellular uptake of CIGB-300 in susceptible and resistant cancer cell lines (lung, prostate, lymphoma and cervix). We studied the peptide internalization under different conditions (temperature and ATP inhibitors). We also evaluated CIGB-300 intracellular trafficking pathways using caveolins and clathrins endocytic markers. Furthermore, to analyze the degradation of the peptide CIGB-300 in tumor cell lines, we measured lysosomal activity at different times of incubation. Finally we examined the antiangiogenic activity of the peptide in an *in vivo* tumor model and found that the peptide was able to prevent angiogenesis. Data show that different kinetics of peptide internalization and degradation could partly explain the behavior of susceptible and resistant cancer cell lines. In vivo the peptide was able to inhibits angiogenesis indicating that CK2 could be an important regulator of this event.

CB-P45.
INHIBITION OF P38 MAP KINASE PROMOTES TUMOR GROWTH IN A MOUSE MAMMARY CARCINOMA MODEL

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Metastasis is the major cause of mortality in cancer patients. Metastatic lesions originate from disseminated tumor cells, which often undergo a period of dormancy. Little is known about the mechanisms involved in the dormancy/proliferation transition. ERK and p38 have been suggested to play a key role in dormancy regulation: a high p38/ERK ratio results in a dormant state induction, while low p38/ERK ratio stimulates active proliferation. Previously, we have shown that the inhibition of p38 activity *in vivo* induces a shortening in the latency period for the development of F3II tumors and an increase in the multiplicity of lung metastases. The aim of this work was to evaluate the impact of p38/ERK modulation on F3II *in vivo* and *in vitro* behavior. Tumor-bearing mice were subjected to partial surgical excision, leaving a residual tumor piece. After surgery mice were treated for ten consecutive days with SB203580, a commercial chemical inhibitor of p38 kinase. Mice treated with SB203580 developed larger tumor recurrences compared to the control group. Additionally, activated ERK levels were increased after F3II incubation with SB203580. Taken together, these results suggest that p38 and ERK are important regulators in tumor dormancy in the F3II mouse mammary carcinoma model.

CB-46.
CHARACTERIZATION OF THE EXPRESSION AND SUBCELLULAR LOCALIZATION OF RABS IN *Giardia lamblia*

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G. lamblia is a protozoan parasite that inhabits the upper small intestine of many vertebrates that belongs to the earliest branch of the eukaryotic line of descent. *Giardia* possesses both regulated and constitutive secretory pathways. However, the molecular machinery involved in these processes remains poorly known and controversial. We have previously identified the complete set of SNARE and ARF proteins in *Giardia*, indicating that this protist presents a simple intramembranous system. Now, to enhance our knowledge of the *Giardia* secretory pathways and in our pursuit of the elusive Golgi apparatus of this microorganism, we performed an exhaustive analysis of the *Giardia* genome and identified 12 genes encoding RAB proteins. In higher eukaryotes, RABs are involved in regulating vesicular traffic and secretion. The expression and subcellular localization, analyzed on transfected cells under different stages of differentiation, showed the presence of particular RABs at the endoplasmic reticulum, the nuclear envelope and small and large vesicles, with some RABs localizing in a pattern that differs from all known *Giardia* sub-compartments. These results not only contribute to a better understanding of the evolution of the eukaryotic vesicular traffic pathways, but also provide light of the least set of organelles needed for a given organism to perform its cellular functions.

CB-P47.
IMMUNE PROFILES DURING *Giardia lamblia* INFECTIONS IN GERBILS

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The mechanisms involved in the immune response (IR) against *Giardia* are not known. Previous studies suggested a Th1/Th2 mixed induction. In this work, cytokines (CKs) that characterize pro-inflammatory events, Th2 and Th17 responses and immune regulation were chosen to determine the IR induced by the parasite in the gerbil model of giardiasis. Initially, levels of IL-4 and IFN-γ, which are representative CKs of a Th2 and a Th1 profiles, respectively, were determined. At 28 dpi, an increase of IL-4 in the infected compared to non-infected animals was observed. To investigate the kinetics of CKs during the infection, levels of 9 representative CKs were determined by qPCR in spleen, Peyer`s patches (PP) and mesenteric lymph nodes (MLN) at 14 and 28 dpi. The CK profiles showed differences during the course of infection. At 14 dpi, pro-inflammatory CKs increased, together with some Th2 and Treg CKs; while at 28 dpi, IL-5 was significantly up-regulated in all tissues. In PP, this was accompanied by an increase in IL-4 and a decrease of IFN-γ. These results suggest that a pro-inflammatory immune response collaborate with the control of the disease at the pick of infection, and that the IR is regulated toward a Th2 profile during the resolution of the disease. These results are the basis of future studies regarding the protective effect of our recently developed anti-*Giardia* vaccine.

CB-P48.
ANTIBODIES AGAINS VARIANT SURFACE MOLECULES STIMULATE ANTIGENIC VARIATION IN *Giardia lamblia*

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Giardia is an intestinal protozoan parasite that is considered one of the most primitive eukaryotic cells. *Giardia* colonizes the upper small intestine of humans and other vertebrates causing giardiasis. This disease is characterized by clinical manifestations ranging from asymptomatic infection to acute or chronic diarrhea. Antigenic variation in *Giardia* contributes to its worldwide prevalence and to the production of persistent and recurrent infections. The entire *Giardia* trophozoite is covered by a unique variat-specific surface protein (VSP), which constitutes the interfase between the parasite and the environment. The *Giardia* genome contains a repertoire of about 200 VSP genes, but only one VSP is expressed on the surface of each trophozoite at a particular point in time. It was always considered that antibodies against *Giardia* VSPs are cytotoxic and that VSP switching occurs even in the absence of any immune pressure. In this work we show that anti-VSP inmunoglobulins do not have a cytotoxic effect on *Giardia* but induce the rate of antigenic variation *in vitro*, suggesting that the immune system could be a major stimulus for antigenic switching *in vivo*. These results pave the way to understand the molecular links between the extracellular environnment and antigenic variation not only in *Giardia* but also in other relevant human pathogens that present similar behaviour.

CB-P49.
INFLUENCE OF EPIGENETIC FACTORS IN THE CONTROL OF ENCYSTATION AND ANTIGENIC VARIATION IN *GIARDIA*

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During eukaryotic evolution, parasites have faced the challenges of sensing and adapting to environmental and host insults to survive, colonize, and proliferate in a variety of species. *Giardia lamblia*, one of the most early-branching eukaryotes and a common cause of intestinal disease, has developed fascinating strategies to persist both outside and inside the intestine of its hosts, such as the encystation-excystation processes and surface antigenic variation. How these events are regulated remains poorly understood. This protozoan lacks several typical eukaryote features, such as canonical transcription factors, linker histone H1, complex promoter regions, relevant 5´- and 3´-UTR in mRNAs, among many other distinctive characteristics, suggesting that posttranscriptional and translational control of gene expression are key for the parasite biology. However, epigenetic factors may also play important roles in the regulation of gene expression. In this work, we describe the presence of common posttranslational histone modifications and their association with cell differentiation processes. We present evidence that the inhibition of the activity of NAD+-dependent and NAD+-independent histone deacetylases abolishes encystation and increase the rate of switching of the Variant-specific Surface Proteins (VSPs) during antigenic variation of this important human pathogen.

CB-P50.
CHARACTERIZATION OF VESICLES CONTAINING VINCULIN IN RAT RENAL PAPILLARY COLLECTING DUCT CELLS

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We have showed that bradykinin (BK) induces a dissipation of vinculin-stained focal adhesions (FA) - structures of cell attachment to the extracellular matrix- with formation of vesicles containing vinculin, PIP2 and markers of the recycling endosomal compartment. Now, we performed a biochemical characterization of these vesicles. For the analysis of membrane association properties of vinculin in these vesicles, microsomes isolated from BK treated renal collecting duct cells were incubated with increasing amounts of KCl, Na2CO3 pH 10 or Triton X-114. To verify the membrane orientation of vinculin, microsomes were subjeted to tripsin digestion with or without Triton X-100. After these treatments, the presence of vinculin was analyzed by Western blot. To study the phospholipid composition, vinculin containing vesicles isolated by immunomagnetic method were analysis by thin layer chromatography (TLC). Most of the vinculin was released with alkaline and high salt solutions, and segregated into the aqueous phase on Triton X114 solubilization and phase separation. Vinculin was sensitive to protease in the absence of detergent. The TLC revealed the presence of phosphatidylcholine and sphingomyelin. We concluded that vinculin is a peripheral vesicle-membrane associated protein, which is orientated to the citosol, and probably associated with the membranes via electrostatic interactions.

CB-P51.
BRADYKININ (BK) INDUCES AN EPITHELIAL-MESENCHYMAL TRANSITION IN NEPHROGENIC URETERIC BUD (UB) CELLS

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We studied the effect of BK on renal papillary collecting duct/UB cells of 7 days-old rats. In mammals, nephrogenesis is completed posnatally. Cultured UB cells forms large colonies with a well spread morphology and lamellipodia in the peripheral cells showing the phenotype of a migratory sheet of epithelial cells. UB cells interact with each other and with the substratum through adherens junctions and focal adhesions (FA) immunostained with vinculin. BK treatment resulted in cell scattering and FA disipation, accompanied by changes in cellular morphology from a spread to a more rounded shape. We also observed irregular cells with lamellipodia and filopodia making contacts with neighbouring cells. Pre-treatmente of cells with a selective BK-B2 receptor antagonist, Hoe 140, avoid cell scattering and FA dissipation. We interpret that in the absence of BK, UB cells form colonies and migrate collectively, and this behavior could explain the collective advance of the UB from papilla to renal cortex which take place in nephrogenesis. Taking into account that BK-B2 mRNA levels are higher in newborn kidney, we propose that in kidney development BK induces epithelial- mesenchymal transition, which causes to the cells loosen their junctional interactions and become migratory mesenchymal-like cells, which finally could undergoes mesenchymal–epithelial conversion to gives rise kidney tubules

CB-P52.
VOCHRYSIN: A NEW VANADYL COMPLEX WITH FLAVONOID ANTI-TUMORAL ACTIVITIES IN OSTEOBLAST-LIKE CELLS

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The family of polyphenolic flavonoids presents anti-viral, anti-inflammatory and anti-tumoral effects. In particular, Chrysin is an member of this family with potent anti-proliferative effects against various malignant cells. Bioavailability of the flavonoids is improved through metal coordination. On the other hand, oxovanadium(IV) compounds display interesting pharmacological activity mainly as insulin enhancers and anti-tumoral agents. In this work we report the biological effects of a complex of oxovanadium(IV) with Chrysin ([VO(chrysin)2EtOH]2 on three osteoblastic cell lines: MC3T3-E1 cells (nontransformed mouse calvaria osteblasts), UMR106 cells (rat osteosarcoma) and MG-63 cells (human osteosarcoma). Effects of the complex on cell viability and cell morphology were investigated as well as the mechanisms of action involved in the cytotoxicity. Vochrysin impaired the cell viability in the range of 25-100 µM. Moreover, the complex increased the level of Reactive Oxigen Species (ROS) 300% over basal and decreased the GSH/GSSG ratio in the same range ($p<0.001$). Vochrysin arrested the cell cycle in G2 phase after 6 h of treatment and then the cells entered into apoptosis (evaluated by SubG1 peak, Phosphatidylserine exposure and caspase-3). Altogether, these results suggest that Vochrysin is potentially a good candidate for future use in alternative anti-tumor treatments.

CB-P53.
MORPHOLOGICAL AND MOLECULAR MECHANISMS INVOLVED IN THE ACROSOME SWELLING IN HUMAN SPERM

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The acrosome reaction is an exocytic event essential for fertilization. It requires the attachment of the outer acrosomal membrane to the plasma membrane, the opening of fusion pores, and the releasing the acrosomal contents together with hybrid vesicles. In a previous report we have proposed that the swelling of the acrosome and the deformation of the outer acrosomal membrane is required for the process. By means of transmission electron microscopy, in this communication we provide evidence for this requirement: we show that all stimulators of the acrosome reaction stimulate swelling and that swelling precedes the deformation of the outer acrosomal membrane and the appearance of intraacrosomal vesicles. According to observations in other models, the contact between the outer acrosomal membrane and the plasma membrane is part of the mechanism of store operated calcium channels opening. However, by using specific inhibitors we show that swelling is downstream the opening of these channels. Our results indicate that acrosomal swelling is an essential part of acrosmal exocytosis and that it is triggered by the influx of calcium through store operated calcium channels in the plasma membrane.

CB-P54.
EPIGENETIC PROFILES OF THE BREAST CANCER CELL LINES MCF-7 AND MDA-MB-231

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Breast cancer is a heterogeneous disease in which genetic and epigenetic alterations are accumulated. One of the most studied epigenetic alterations is the aberrant DNA methylation of CpG islands. In this work, we aimed to define a 55 genes based methylation profile for 2 human breast cancer cell lines, MCF-7 and MDA-MB-231 and a human chronic myelogenous leukemia derived cell line, K-562. In order to examine aberrant promoter methylation, we have used a multigene approach called Methylation-Specific Multiplex Ligation-dependent Probe Amplification. We have determined a methylation profile of the three cell lines (107 CpG islands). Moreover, a copy number analysis was performed. Previous observations of our group in breast carcinomas had identified the methylation of WT1 gene as the most frequent epigenetic alteration in our population. In concordance with this, we observed a high methylation level of WT1 in both MCF-7 and MDA-MB-231 and absence of this alteration in K-562 cells. By Real-Time PCR we confirmed that WT1 is silenced when the promoter is methylated. The establishment of the methylation profiles of MCF-7 and MDA-MB-231 cell lines can contribute to future drug sensitivity studies. Epigenetic detections in frozen human breast tumors can be tested as possible predictive markers, by drug sensitivity assays on these cell lines.

CB-P55.
VPS4 IS NECESSARY FOR THE ACROSOME DEFORMATION DURING ACROSOMAL EXOCYTOSIS IN HUMAN SPERM

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The acrosome reaction of human spermatozoa is a complex, calcium-dependent regulated exocytosis. Fusion between the outer acrosomal membrane and the cell membrane causes the release of the acrosomal contents. Preventing the release of calcium from the acrosome, the exocytic process can be arrested at a stage where SNARE proteins are assembled in loose trans complexes. Transmission electron micrographs at this stage showed that the acrosomes were profusely swollen, with deep invaginations of the outer acrosomal membrane. These membrane deformations are part of the mechanism of vesiculation. Invagination of the acrosomal membrane is topologically equivalent to the formation of multivesicular bodies in endosomes, a process that depends on the assembly of the ESCRT complexes. We are exploring the possibility that the same mechanism is involved in acrosomal exocytosis. A dominant-negative mutant of VPS4, the ATPase responsible for the disassembly of the membrane attached ESCRT proteins inhibited acrosomal exocytosis of permeabilized human spermatozoa. Moreover, an anti-VPS4 antibody was also inhibitory. TEM images show abnormal bending of the acrosomal membrane when sperm were stimulated in the presence of the dominant negative VPS4. These observations suggest that the deformation of the acrosomal membrane necessary for acrosomal exocytosis are shaped by an ESCRT-dependent mechanism.

CB-P56.
MARCKS IS PHOSPHORYLATED DURING ACROSOMAL EXOCYTOSIS IN LIVING HUMAN SPERM

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To fertilize the egg, human sperm must secrete the acrosomal content, a process known as acrosomal exocytosis (AE). Different pathways are activated during AE and protein phosphorylation is one of the mechanisms involved in this secretory event. It is known that MARCKS sequesters PIP2 against membranes and when it is phosphorylated, the protein is released to cytosol increasing the availability of PIP2. We previously showed that MARCKS is expressed in human sperm, it participates in AE and regulates PIP2 availability and Ca2+ mobilization during AE in human sperm. In this study, we hypothesized that, to allow AE, MARCKS must be phosphorylated during AE stimulated by physiological and non-physiological stimulators. To test this we performed Western blot analysis using a specific anti-phospho-MARCKS antibody, which only recognizes the phosphorylated form of MARCKS. Human sperm were incubated with 2-ABP to prevent membrane loss and AE was stimulated by different activators in living sperm. The results showed that the phosphorylated form of MARCKS increased an 50%, 60% and 40% when AE was stimulated by calcium ionophore A23187, PMA, and progesterone, respectively, when compared to control conditions. The fact that MARCKS is phosphorylated during AE is compatible with the idea that in human sperm MARCKS might be released from membranes increasing the availability of PIP2.

CB-P57.
PERMEANT PROTEINS AS A TOOL TO STUDY CORTICAL GRANULES EXOCYTOSIS IN MOUSE OOCYTES
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One of the most important events that are produced in oocytes as a result of fertilization is cortical granule exocytosis (CGE), a secretory process that produces polispermy blockade and ensures normal embryonic development. Nevertheless, the molecular mechanism of this process remains unknown. Previous results in our laboratory show that MARCKS, a prominent substrate of PKC involved in exocytosis in different cell types, participates in CGE in mouse oocyte. In this cellular model, proteins are introduced by microinjection, which is a very valuable and useful technique, but it causes an injury to the cells which is reflected in high mortality and low yields. We decided to tune up the usefulness of permeant proteins to study exocytotic mechanisms in mouse oocyte. We used the permeant peptide corresponding to MARCKS effector domain (amino acids 154-165) conjugated to thrimethylrhodamine (TMR-ED). Oocytes were incubated with increasing concentrations of this peptide, previous remotion of zona pellucida; then cells were parthenogenetically activated with SrCl2, and cortical granules were quantified with the program Image J. TMR-DE was able to inhibit CGE and this effect was concentration-dependent. These results corroborate previous data obtained in our laboratory by protein microinjection, and show the efficiency of permeant proteins as a tool to study CGE in moouse oocytes.

CB-P58.
MOLECULAR IDENTIFICATION AND LOCALIZATION ANALYSIS OF SNAPS AND NSF DURING MOUSE OOCYTE MATURATION
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In mammals, the primary mechanism in preventing polyspermic fertilization involves cortical granules exocytosis (CGE). Several studies have suggested that CGE is a SNARE protein-mediated pathway; however, the molecular characterization of CGE is still incomplete. We hypothesized that the oocyte uses the same conserved membrane fusion machinery as neurons and human sperm, and the regulatory complex SNAP/NSF is present in mouse oocyte. We first investigated the expression of SNAP isoforms: α, ß, and γ, and NSF by RT-PCR. The results show that the α- and γ-SNAP (but not ß), and NSF are expressed in mouse oocyte. Western blot analyses indicate that these proteins are present during oocyte maturation and egg activation showing no variations between the different stages. Indirect immunofluorescence experiments revealed that α-SNAP and NSF localized mainly in the cortical region of all stages analyzed. While γ-SNAP had a similar distribution that α-SNAP, this protein also showed a cytoplasmic distribution in immature oocytes. All identified proteins: α-SNAP, γ-SNAP, and NSF are predominately observed in the cortical region, which is enriched in cortical granules at the mature oocyte, suggesting that these proteins may be involved in CGE. However, more studies are needed to elucidate if both α-SNAP and γ-SNAP are equally important in CGE or have different functions in mouse oocyte.

CB-P59.
ROLE OF α-SNAP IN THE MAINTENANCE OF N-CADHERIN-BASED ADHERENS JUNCTIONS IN MOUSE OVARIAN FOLLICLE
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Several studies suggest that N-cadherin-based adherens junctions between granulosa cells (GC) play a key role in the ovarian follicle development. α-SNAP, a protein involved in membrane fusion, has also been associated to the maintenance of cadherin-based junctions. We hypothesized that α-SNAP is a regulator of N-cadherin-based cell-cell junctions between granulosa cells and follicle development. To test this we used a spontaneous mutant mouse model for α-SNAP known as hyh (hydrocephalus with hop gait). We analyzed the expression of α-SNAP in wild type (wt) and hyh ovaries from pre-pubertal to post-pubertal stages. Western blots showed that in wt, α-SNAP increased at post-pubertal ages and after gonadotrophic stimulation; this increase correlates with the number of maturing follicles. On the other hand, hyh mice responded to hormonal stimulation, however, they showed lower levels of α-SNAP at all stages, did not display the post-pubertal increase, and the number of maturing follicles was diminished. Immunofluorescence revealed that wt GC were joined together by well-developed N-cadherin-based adherens junctions; in contrast, hyh GC showed scarce adherens junctions and a punctate cytoplasmic pattern. Several GC with piknotic nuclei were observed in the follicular antrum. Our results suggest that α-SNAP is important for the physiology of GC during follicle development in mouse.

CB-P60.
MOLECULAR AND CELLULAR RESPONSE OF HUMAN TROPHOBLAST CELLS EXPOSED TO CHLORPYRIFOS
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Chlorpyrifos (CPF) is one of the most widely used pesticides. Several reports suggest that prenatal exposure leads to cognitive impairments and increased risk of pregnancy disorders. Although placental function and differentiation is intimately related to fetal health, information on CPF´s effect on placenta is still scarce. Previously, we reported that CPF increased the expression of the ABCG2 efflux transporter and ßhCG subunit in JEG-3 choriocarcinoma cell line. Herein, we assessed primary human cytotrophoblasts (CTBs) response to CPF exposure. CTBs purified from normal term placentas were exposed to 0-100 µM CPF during 22 or 64h. Under control conditions, CTBs do not proliferate but differentiate and fuse forming syncytium-like structures. In the presence of CPF, no morphological signs of cell damage or impairment of syncytialization were detected, while mitochondrial metabolic rate, measured using MTT, was augmented. qRT-PCR revealed an early (22h) increase in ßhCG and p-glycoprotein efflux transporter and a late (64h) increase in ABCG2 expression in CPF-treated CTBs. Instead, mRNA level of the PSG3 differentiation marker and KLF6 transcription factor remained unmodified at the time points assayed. These results suggest that villous trophoblasts may activate protection mechanisms in order to preserve placental function.
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CB-P61.
METABOLITES IDENTIFIED IN AN EXTRACT OF *Baccharis articulata* INDUCE CYTOTOXIC EFFECTS ON HUMAN PBMCS

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Baccharis articulata (Lam.) Pers. is used in the treatment of different disorders. We have demonstrated that cold aqueous extract of B. articulata (Ba-CAE) induced apoptosis in human peripheral blood mononuclear cells (PBMCs). The phytochemical study of the extract showed the presence of luteolin, acacetin and chlorogenic acid. The aim of this study was determine whether these compounds are responsible for the cytotoxic and apoptotic effects exhibited by the extract. The HPLC analysis shows the presence of luteolin (L) (1.96 ± 0.27%), acacetin (A) (1.12 ± 0.14%) and chlorogenic acid (ChA) (0.29 ± 0.05%). Analysis of PBMCs viability of each compound alone determined by staining of Trypan blue dye exclusion method revealed that ChA was the most toxic compound (CC50=18µg/mL), followed by A (CC50=89µg/mL) and L (CC50=144µg/mL). Both, ChA (4µg/mL) and the combination of three compounds (L:25µg/mL+A:14µg/mL+ChA:4µg/mL), in concentrations that these are present in Ba-CAE at 1280µg/mL, showed 50.0±0.88% and 48.50±1.92% of decrease in PBMCs viability, respectively, and induced the cell death by apoptosis determined by Hoechst 33258 staining, TUNEL, and DNA fragmentation analysis. ChA not affect the Bax/Bcl-2 ratio, but neither activated the death receptor DR4. We suggested that toxic effects and death by apoptosis triggered by Ba-CAE on human PBMCs would be mainly produced by ChA.

CB-P62.
HISTAMINE ACTION IN TUMOR MICROENVIRONMENT

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Tumor microenvironment is composed of immune and endothelial cells, fibroblasts and extracellular matrix. Interactions between tumor cells and stroma are crucial for cell growth regulation and tumor metastasis. Previously we demonstrated that histamine (HA) modified epithelial to mesenchymal transition (EMT), required to transform a benign tumor into an aggressive and invasive cancer. Our aim was to evaluate HA action in the interaction between the human mammary tumor cells MDA MB231 and the normal fibroblasts CCD-SK 1059.
We obtained the conditioned media (CM) from fibroblasts treated or not with two doses of HA (0.1 and 20uM). Tumor cells cultivated with CM from control fibroblasts showed an increase in the expression of the mesenchymal marker smooth muscle alpha actin by flow cytometry, in MMP9 activity by zymography and in the migratory capacity by transwells. A decrease in the expression of the epithelial marker E cadherin was observed by western blot. The levels of phosphorylation/activation of c-Src, related to cell migration, were also increased. However these effects were reverted when MDA MB231 cells were grown with CM from 20uM HA treated fibroblasts. A diminution was also found in mRNA steady sate levels of Slug, a known inducer of EMT.
These results signal HA capacity of modifying tumor microenvironment and open a perspective for the design of therapies in the future.

CB-P63.
HISTAMINE SUPPRESSES *IN VIVO* AND *IN VITRO* TUMOR GROWTH IN HUMAN TRIPLE-NEGATIVE BREAST CANCER

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The aim of this work was to evaluate the effect of histamine on survival, tumor growth rate, metastatic capacity and expression of antigens related with the proliferative and apoptotic potential in a triple negative breast cancer experimental model. Animals bearing xenografts of the human MDA-MB-231 cell line were treated with histamine (5 mg/Kg, sc). Results indicate that developed tumors were highly undifferentiated, exhibited high levels of H4R, histamine content and proliferation marker (PCNA) while displayed low level of apoptosis. Histamine treatment significantly increased median survival (80 vs. 60 days; Log-rank Mantel-Cox Test, P=0.0025; Gehan-Breslow-Wilcoxon Test, P=0.0158) while decreased tumor growth evidenced by an augment of the tumor doubling time (13.1±1.2 vs. 7.4±0.6, P<0.01). This effect was associated with a decrease in the PCNA expression levels (83.1% vs. 63.2%, P<0.05) and an increased apoptosis. Furthermore, histamine reduced the intratumoral vascularization. A decreased tumor growth was also observed with the treatment of two H4R agonists (Clozapine, 1 mg/Kg and JNJ28610244, 10 mg/Kg) and silencing of H4R in vitro with siRNA blocked histamine-induced inhibition of proliferation.
We conclude that histamine through the H4R exhibits a crucial role in tumor progression, exhibiting a novel therapeutical potential as an adjuvant for breast cancer treatment.

CB-P64.
CPF MODULATES CELL MIGRATION AND INVASION IN HUMAN BREAST CANCER CELLS

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Chlorpyrifos (CPF) is an organophosphorus pesticide that has been described as an endocrine disruptor. We have demonstrated that environmental doses (50 nM) of CPF induce cell proliferation in estrogen dependent-breast cancer cells. Our objective was to study CPF action on cell migration and invasion in MCF-7 and MDA-MB-231 breast cancer cells. CPF at 0.05, 0.5, 5 and 50 µM were assayed. MMP-2 and MMP-9 gelatinolytic activities were evaluated by gelatin zymography. Wound-healing assay was used to evaluate cell migration. Cell invasion was studied by transwell assays using serum as chemoatractant. ß-catenin was studied by immunofluorescence microscopy. Our results showed that CPF 0.5 µM induced MDA-MB-231 (66;p<0.05) cell migration accompanied by an augment in gelatinolytic activity (20%;p<0.01). Conversely, CPF 50 µM reduced this activity (50%;p<0.001) in both cell lines. CPF 50 µM increased MCF-7 cell invasion (67%, p<0.05) with an increment in ß-catenin nuclear expression. Cell invasion was inhibited in MDA-MB-231 cells (65%;p<0.001). A selective c-SRC inhibitor, PP2, reversed CPF effects on migration and invasion processes. Our results indicate that CPF at low dose induces MDA-MB-231cell migration and MMP-2 and MMP-9 may be involved in this effect. Also, the pesticide induces MCF-7 cell invasion which may be related to nuclear ß-catenin expression in this cell line.

CB-P65.
BROMODOMAIN FACTOR 1 OF *Trypanosoma cruzi* IS LOCALIZED IN THE GLYCOSOMES, TARGETED BY AN N-TERMINAL SEQUENCE

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The bromodomain is the only known protein domain involved in the recognition of acetylated lysines. Acetylation is an ubiquitous and abundant posttranslational modification. Widespread acetylation of metabolic enzymes suggests a modification that is as important as protein phosphorylation in controlling cell function. TcBDF1 contains a bromodomain in its N-terminal half. WB analysis of *T. cruzi* lysates with antiTcBDF1, immunofluorescence microscopy of the different life cycle stages and immunoelectron microscopy of epimastigotes confirm TcBDF1´s non-nuclear localization. Colocalization assays with several markers suggest a glicosomal location.
The amino acids sequence of TcBDF1 was analyzed with the PeroxisomeDB server, which recognized in its N-terminus a peroxisome-targeting signal type 2, one of the signals that direct glycosomal proteins into the matrix. To determine if the first 27 amino acids present in TcBDF1 are responsible of its import to the glycosome, we transiently transfected epimastigotes with constructs coding the whole protein, a truncated version which lacks the first 27 amino acids or only the N-terminus targeting signal, fused to the Red Fluorescent Protein. The intracellular localization of the different fusion proteins was determined by fluorescence microscopy. Our results confirm that BDF1 possesses a PTS2, responsible of directing the protein to the glycosomes

CB-P66.
PROSAPOSIN IS DETECTED IN BULL SPERMATOZOA AND IT CHANGES ALONG EPIDIDYMAL TRANSIT

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Mammalian epididymis participates in sperm maturation through its endocytic and secretory activity. Some secreted proteins are known to be targeted to sperm membrane. Prosaposin (PSAP) is a lysosomal protein, which is found in mammalian epididymal fluid and spermatozoa. Here, we intended to study the possible presence of PSAP in the plasma membrane of epidydimal spermatozoa from bull (Aberdeen Angus). The epididymides were dissected into caput, corpus and cauda and the sperm were obtained by slicing of the tissue and centrifugation. Using specific antibodies (by IFI and flow cytometry) we detected PSAP in the sperm of bulls and the signal was stronger in the gametes of the corpus. This protein was partially released by 0.3 M ClNa, indicating a noncovalent association with the gamete. All these results were confirmed by Western blot. In addition, we have observed that the protein is mainly located in the equatorial area of the head and also in the tail of the sperm. PSAP was also detected in luminal vesicles and mostly in caput and corpus, suggesting that the vesicles could be the vehicle of PSAP for acquisition by the spermatozoa. Intriguingly, most of the changes occurs in corpus, indicating that this region could be crucial for sperm maturation. These observations could provide new insights into molecular rearrangement and protein acquisition during epididymal maturation.

CB-P67.
DNA DAMAGE-INDUCED HNRNP K SUMOYLATION REGULATES p53 TRANSCRIPTIONAL ACTIVATION

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Heterogeneous nuclear ribonucleoprotein (hnRNP) K is a nucleocytoplasmic shuttling protein that regulates mRNA metabolism and is a key player in the p53-triggered DNA damage response. hnRNP K acts as a cofactor for p53 upon DNA damage. In this context, hnRNP K and p53 levels are stabilized upon inhibition of their E3 ubiquitin ligase MDM2, and together they induce the transcription of genes involved in cell cycle arrest. In the present work, we show that hnRNP K is conjugated to SUMO in its lysine 422, within its KH3 domain. This modification is stimulated upon DNA damage and is required for the induction of p53 target genes such as p21 and 14-3-3 s. We further show that hnRNP K sumoylation is regulated by the SUMO E3 ligase Pc2: over-expression of an activity-deficient mutant Pc2 abrogates hnRNP K-triggered p53-dependent transcription. Our findings link the DNA damage-induced Pc2 activation to the p53 transcriptional co-activation through hnRNP K sumoylation.

CB-P68.
CHARACTERIZATION OF L-LEUCINE TRANSPORT IN *Saccharomyces cerevisiae* YGP1 MUTANT

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The transport of amino acids in the yeast *Saccharomyces cerevisiae* is mediated by permeases with ample or restricted specificity. In order to study the relation between L-leucine transport activity and N-glycosilation reactions we isolated mutants with a decreased transport activity for the amino acid and also with deficiency in the reactions of N-glycosylation. The mutants were isolated for their resistance to Sodium Vanadate salt, resistance for a L-leucine toxic analogous (TFL) and growth inhibition by the antibiotics hygromycine and gentamicine. We characterized by western blot assays the phenotype of the mutants through an antibody (WBP1) raised against a unit of the yeast oligosaccharyltransferase (OST1). This protein is an appropriate reporter for changes in the glycosylation pattern of proteins in the endoplasmic reticulum. Complementation of these mutants with a DNA genomic library allowed us the isolation of higromicine resistant transformants. Afterward, the transformants evidenced a transport activity increased for L-leucine uptake. The isolation of plasmids that restore the original phenotype and their ulterior sequencing permitted us to identify the YGP1 gene. Characterization of L-leucine uptake in a ygp1 knock out strain shows that the gene is resposible for the decreased transport activity and the increase in gentamincine and hygromicine sensitivity.

CB-P69.
YACON ROOTS IMPROVE INSULIN SENSITIVITY BY INHIBITING FAT STORE AND ADJUSTING ADIPOKINE PROFILE

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The metabolic syndrome is a cluster of multiple metabolic disease based on obesity and insulin resitence. The aim of the present study was to evaluate the effects of Smallanthus sonchifolius roots (Yacón), rich in fructooligosacharides (FOS), on weight loss, metabolic syndrome-related biochemical parameters, and the regulation of key genes involved in lipid metabolism of hepatic and adipose tissues in rats rendered metabolic syndrome by a high-fructose diet (HFD). Adult male Wistar rats were maintained on a rat standard chow and then animals were randomly separated into a control group (water ad libitum) and a fructose-supplemented group (fructose 10% w/v, ad libitum) After 12 weeks rats on a HFD, were randomly assigned according to the treatment: HFD or a HFD+Yacón fluor (340mgFOS/kg) twice a day. Yacon supplementation significantly lowered food intake, body weight, visceral fat-pad weights, blood and hepatic lipid, glucose and insulin levels of rats administered HFD. Feeding yacon reversed the high-fructose diet-induced downregulation of PPARalpha (peroxisome proliferator-activated receptor) and reduced the HMGCoAS (3-hydroxy-3-methylglutaryl-CoA synthase), FAS (fatty acid syntase) and GPAT (glycerol-3-phosphate acyltransferase) mRNA expression. After 2 months of treatment, rats with metabolic syndrome showed decrease in their leptin levels, as well as leptin/adiponectin ratio and HOMA-IR. These results suggest that yacon fluor improves insulin sensitivity by inhibiting fat store and adjusting adipokine profile.

CB-P70.
SORTING ROLE OF THE PERINUCLEAR REGION AND ER IN *Giardia lamblia*

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The unicellular parasite *Giardia lamblia* belongs to the Diplomonads, a group which belongs to the earliest branches in eukaryotic evolution. One of the most striking characteristics of Giardia is the presence of protein sorting even though it lacks a morphological discernible Golgi apparatus. By using bodipy FL C5-ceramide, a marker for Golgi apparatus, we showed that the membrane network comprised by the nuclear envelope and the endoplasmic reticulum (ER) possesses Golgi characteristics in both growing and encysting trophozoites. DAB photooxidation-coupled MET showed that ceramide accumulates specifically at the perinuclear region of the cell, a very dynamic zone, sensitive to brefeldin A treatment and to low temperature exposure. During encystation, there was a remarkable change in the endomembrane system, with ceramide progressively accumulating at the nuclear membrane and ER exit-sites (ERES), from which we observed that secretory granules are formed de novo. This accumulation at the ERES was found also to be sensitive to brefeldin A treatment, which causes the reabsorption of the secretion material back to the ER, although some fully formed vesicles remained, and were entirely separated from the ER. Altogether, these results suggest that the perinuclear region and ER play an important role in protein and membrane sorting in this unique eukaryotic cell.

CB-P71.
CHANGES IN MACROPHAGE LYSOSOMAL ENZYMES EXPRESSION IN RESPONSE TO *Bordetella pertussis* INFECTION

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Macrophages have the ability to recognize bacterial products and initiate an immune response to clear the microbe. Our group recently showed that the encounter of *Bordetella pertussis* with human macrophages leads to the intracellular survival of a significant number of bacteria which are able to replicate inside to macrophage. In this study we focused on the expression of lysosomal enzymes during the establishment of intracellular infection of *B. pertussis*. Using *B. pertussis*-infected macrophage model we evaluated the time course of mRNA expression at 3, 24 and 48 h post-infection. The results showed that cathepsin genes, the most important lysosomal proteases (CTSA, B, C, D, G and S), were strongly induced at 3 h post infection, but down regulated 24 h later. Similar pattern was observed in the expression of proteinase 3, azurocidin 1 and acid phosphatase 6. A significant upregulation of these proteins mRNA expression was seen at early time points but after 48h these genes were silenced showing even lower values than the uninfected control. Recently a new concept is emerging about the possibility of intracellular pathogens to manipulate gene expression program in the host cell for their own benefit. The results obtained here suggest that during infection *Bp* is able to manipulate the intracellular environment to make it less aggressive and thus allow its survival and replication.

CB-P72.
ABERRANT METHYLATION OF WT1: A POTENTIAL EPIGENETIC MARKER FOR INVASIVE DUCTAL BREAST CARCINOMAS

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The methylation of cancer related genes is a common event during tumorigenic process. The identification of oncologic methylation markers may contribute as a tool for early detection, disease follow-up and treatment response.
Previous studies of our group showed that a CpG island at position -411pb of WT1 gene, was frequently methylated in invasive ductal breast carcinomas (IDCs). In the present work, we included 96 IDCs, 30 normal breast tissues obtained from surgical margins and 25 sentinel lymph nodes, and analyzed the methylation status of WT1, and 27 more CpG islands of different cancer related genes, by MS-MLPA assay. Our results revealed that the methylation of WT1 is the most frequent alteration detected in IDCs (92.7%), and that it allows discriminating between tumor and normal breast tissue ($p<0.0001$). Besides, we could establish that the aberrant methylation is acquired since early tumor stages (Stage1 and Stage2A) and that is conserved in affected lymph nodes (24/25 sentinel lymph nodes presented methylated WT1). Real Time PCR confirmed this methylation silences WT1. Our results allow postulating WT1 as an epigenetic marker of IDC's. Besides, we postulate that this frequent feature of IDC's from our population could be an indicator to drug sensitivity, e.g.TNF-Related Apoptosis-Inducing Ligand (TRAIL), currently under study for leukemia's which do not express WT1.

CB-P73.
TRIPLE NEGATIVE BREAST TUMORS: ANALYSIS OF MICROSATELLITE INSTABILITY

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Triple-negative (TN) breast cancers are defined as tumors that lack the expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. TN tumors frequently express basal cytokeratins, EGF receptor, myoepithelial markers and rarely express E-cadherin. They are often p53 mutant and give evidence of genomic instability. The aim of this study was to evaluate instability within BAT-26 microsatellite and analyze copy number variations (CNV) and aberrant methylations in mismatch repair and tumor suppressor genes in TN tumors. For copy number and methylation analysis we used the methyl specific multiplex ligation probe amplification assay (MS-MLPA). PCR amplification of BAT-26 was performed with specific primers and resolved by capillary electrophoresis. Forty five invasive ductal mammary carcinomas were collected in this study, including 27 TN tumors. We found that TN tumors exhibit significant microsatellite instability (MSI) ($p<0.05$) when compared with non-TN tumors. This instability was not associated with the methylation nor the deletion of MMR genes, but was significantly associated with aberrant methylation of tumor suppressor genes, i.e. DLC1 gene ($p<0.05$, phi =0.488) and APC gene ($p<0.05$, phi=0.486). Here we show that TN tumors are BAT-26 unstable when compared with non-TN tumors. This instability is not associated with methylation nor deletion of MMR genes.

CB-P74.
HYPERTHERMIA IMPROVES CISPLATIN SENSITIVITY IN MISMATCH REPAIR PROFICIENT COLON CANCER CELL LINES

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Introduction. HSPB1 (HSP27) and HSPA1A (HSP72) have been implicated in resistance to antineoplasic drugs, eg cisplatin (cPt) and they have also been associated with DNA repair. Hyperthermia (H) is used in combination with chemotherapy to improve the oncology treatment. cPt induced-DNA damage is recognized by the Mismatch Repair system (MMR), ie hMLH1 and hMSH2 proteins. However, the effects of hyperthermia in cPt sensitivity have not been determined in MMR deficient/proficient tumor cell lines. Objective. To determine the effect of hyperthermia on cPt sensitivity in HCT116 and HCT116+ch2 (MMR deficient) and HCT116+ch3 (MMR proficient) cell lines. Methodology. Cells were exposed to cPt (1 hour) and H+cPt and collected at 0, 4 and 24 h after cPt. We used: RT-qPCR, alkaline comet assay, cytochemical detection of SA-ßgal activity, TUNEL and western blot. Results. cPt reduced the expression of HSPB1 in HCT116+ch3 cells ($P<0.001$). 24 h after cPt, heat shocked HCT116+ch3 cells (HS+cPt) showed increased expression of HSF1 ($P<0.001$) and hMLH1 ($P<0.001$). Hyperthermia reduced 30% cPt crosslinks ($P<0.001$) and increased senescence and apoptosis in HCT116+ch3 cells. Conclusions. A mild heat shock before cPt exposure increased senescence and apoptosis in MMR proficient cells, which may suggest that hyperthermia improves cisplatin sensitivity in human colon cancer cells.

CB-P75.
EGF PREVENTS THALLIUM-INDUCED PC12 CELLS APOPTOSIS BY MODULATING THE ACTIVATION OF p53, JNK AND p38

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In PC12 cells with or without added EGF, thallium (Tl) increases cyclins D1 and E contents, and arrests cells cycle in G0/G1. EGF also protects cells from Tl-mediated apoptosis. In this study we analysed the expression of p21 and p53, involved in cell cycle arrest. Tl(I) and Tl(III) increased nuclear p53 content which was prevented by EGF. Tl(I) and Tl(III) increased cytosolic p21 content regardless EGF presence. Even when the activation of Ras/Raf/MEK/ERK pathway by EGF is an early event, p-ERK was still increased after 24 h of Tl treatment. Tl(I) increased the pro-apoptotic MAPK p-p38, which was partially prevented by EGF. Tl(III) increased p-p38 but only at 100 µM concentration. c-jun phosphorylation by JNK1 participates in G1 to S phase transition, while its phosphorylation by JNK2 targets c-jun for degradation. Tl(I) increased p-JNK1, which was prevented by EGF. Tl(III) did not affect JNK1 phosphorylation, and low levels of p-JNK1 were detected in EGF-supplemented cells. Tl(I), but not Tl(III), increased p-JNK2, which was prevented by EGF. Finally, EGF decreased the content of c-jun in Tl(I)- and Tl(III)-treated cells nuclei. Together, results indicate that the protective effect of EGF on Tl-mediated cell apoptosis may proceed by the prevention of the activation of pro-apoptotic MAPKs.
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CB-P76.
TDP1 MEDIATES THE REMOVAL AND REPAIR OF ETOPOSIDE-STABILIZED TOP2A CLEAVABLE COMPLEXES IN HeLa CELLS

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Topoisomerase II (Top2) poisons, such as Etoposide (ETO), are widely used chemotherapeutic agents that stabilize the Top2-DNA cleavable complexes, which may result in DNA double strand breaks (DSB) formation. The aim of this work was to analyze how tyrosyl-DNA-phosphodiesterase 1 (TDP1) influences the removal of ETO-stabilized Top2a cleavable complexes and the correct DNA damage signaling and repair in HeLa cells. Stable cell lines knocked down in TDP1 (HeLa TDP1^{kd}) and a non silencing control (HeLa NS) were established by using shRNAmir sequences and further analyzed by qRT-PCR. After treatment with ETO, the removal of cleavable complexes was evaluated by the DRT (*Differential Retention of Top2*) assay by confocal microscopy. The activation of DNA damage signals, pS1981ATM and γH2AX, were analyzed by flow cytometry and the DNA repair efficiency by micronucleus (MN) formation. Our results show that Top2a cleavable complexes are processed with different kinetics in HeLa TDP1^{kd}. In addition, while γH2AX signals were induced similarly in both HeLa TDP1^{kd} and NS cells, pS1981ATM was not induced by ETO in HeLa TDP1^{kd}. Moreover, the induction of MN was significantly increased in HeLa TDP1^{kd} compared to NS cells. Thus, we conclude that TDP1 promotes the removal of ETO-stabilized Top2a cleavable complexes and participates in the repair of the induced DNA DSB in human cells.

CB-P77.
STUDY OF THE INTERNALIZATION PATHWAY INVOLVED IN INFECTIOUS BURSAL DISEASE VIRUS INFECTION

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Infectious Bursal Disease Virus (IBDV) is an important avian pathogen member of the Birnaviridae family whose genome is composed of dsRNA. With the aim of analyzing the entry pathway involved in the viral infection, we studied the possible role of endocytosis in this process. Employing Laser Scanning Confocal Microscopy and Western blotting analysis we have observed an impaired IBDV infection of susceptible cells treated with endosomal acidification inhibitors such as BafilomycinA1 and ammonium chloride. In addition, overexpression of endosomal Rab proteins (i.e. Rab5 and Rab7), wild type or mutants, allowed us to demonstrate that the viral infection trafficking occurs along early and mature endosomes. Viral infection was not blocked by Dynasore, a dynamin-dependent endosome-scission inhibitor or by the depletion of membrane cholesterol by treatment with Methyl-B-Cyclodextrin of Filipin II, which remove cholesterol from cellular membranes. Ultrastructural analysis by Cryoelectron Microscopy of infected cells, analyzed at early times post infection, revealed the presence of viral particles attached to the cell membrane and within vesicles close to the membranes. Thus, we conclude that the early capture of virus into intracellular compartments is mediated by endosomes in a dynamin and cholesterol-independent fashion.

CB-P78.
DIFFERENT STRAINS OF *Trypanosoma cruzi* PRESENT DISTINCT SUSCEPTIBILITIES TO POSACONAZOLE DERIVED DRUGS

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The use of anti-fungal azoles, which block sterol biosynthesis, against protozoan parasites has turned out to be highly successful. Inhibitors of the trypanosome sterol 14a-demethylase (CYP51) are promising candidates as anti-Chagas disease drugs. In this work we have tested VNI, a compound identified as a potent *T. cruzi* 14a-demethylase inhibitor (Lepesheva *et al.*, 2010). Our results, analyzing epimastigote and intracellular amastigote growth in *T. cruzi* Y strain, show that 500 nM and 1 µM VNI affects parasite replication reducing the values around 50 % respects to controls. Trypomastigotes are also affected since a significant reduction in the percentage of infected cells obtained after VNI treatment. Although these results show an important effect of VNI, similar experiments conducted in other *T. cruzi* strains revealed a stronger action of this compound. To analyze the origin of these differences, CYP51 gene from Y strain was cloned and sequenced. Interestingly, in contrast with CYP51s from CL Brener and Tulahuen strains, *T. cruzi* Y strain has two CYP51 genes (A and B). Chemical and structural differences in these genes could explain the higher resistance of this strain to VNI. This study will serve as the basis to design new, more potent compounds, which we will test in *T. cruzi* strains and in animal models.

PL-P01.
HEME OXYGENASE-1 AND ABSCISIC ACID AFFECT MAPK'S GENE EXPRESSION IN SOYBEAN SEEDS
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In soybean previous studies enabled the identification of MAPK3 and 6 whose activity is enhanced within the signaling pathway leading to defense reactions. In this study the effects of different compounds related to hemeoxygenase (HO-1) biosynthesis on mitogen-activated protein kinase (MAPK's) genes expression in soybean seeds were tested. To this end, 20µM hemine, 22µM ZnPPIX, 0.5mM furidine or 100µM 8-bromoguanosine 3',5'-cyclic monophosphate (8Br) were added to pre-hydrated seeds for 5 days. MAPK's genes expression was enhanced in seeds treated with hemine. This result indicates that heme catabolism could be involved in the signaling mediated by this cascade pathway. To confirm this hypothesis experiments were carried out in the presence of ZnPPIX, a potent irreversible HO-1 inhibitor. In this case, no gene induction was observed. On the other hand, 8Br, a cGMP analog, induced HO-1 gene expression but did not modulate MAPK's, indicating that this effect could not be mediated by cGMP. When the action of furidine, an abscisic acid inhibitor, was tested a diminution of HO-1 gene expression was observed. In this regard, MAPK's showed a different response, being MAPK6 the only transcript that showed a diminished respect to controls, while MAPK3 mRNA as well as MAPKK1 was enhanced. These results were confirmed by western blotting and activity determinations.

PL-P02.
NITRIC OXIDE-MEDIATED ANTIOXIDANT ENZIMES CHANGES PROTECT SOYBEAN LEAVES AGAINST UV-B RADIATION
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In previous reports we demonstrated that heme oxygenase 1 (HO) plays a protective role against oxidative stress in soybean. Here we investigated the effect of nitric oxide (NO) on oxidative stress markers and classical antioxidant enzymes activities, isoform profile and gene expression in soybean leaves subjected to a high dose UV-B radiation (30 KJ m^{-2}). Pretreatments with 0.8 mM sodium nitroprussiate (SNP), a NO donor, applied as an spray prevented chlorophyll loss, H_2O_2 and $O_2^{\cdot-}$ accumulation and ion leakage in UV-B-treated plants. The antioxidant response of plant leaves was evaluated analyzing the activities and isoform profile of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and unspecific peroxidases. UV-B treatment decreased CAT and APX specific activities 50 and 48 % respect to control values. On the other hand, we performed quantitative RT-PCR of HO, CAT, SOD and APX genes. UV-B treatment decreased significantly HO, CAT and APX gene expression levels, respect to control values. SNP pretreatments assays showed that NO reverts this response. SOD gene expression increased 2-fold respect to control. HO and CAT activities showed a positive correlation with HO-1 gene expression.
Our results indicate that NO protects against high doses UV-B radiation by regulating the response of classical antioxidant enzymes.

PL-P03.
IDENTIFICATION OF MAL DE RÍO CUARTO VIRUS (MRCV) AND WHEAT PROTEINS INTERACTING WITH MRCV P6
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MRCV causes the most devastating maize disease in Argentina. The virus infects crops such as maize, wheat, oat and sorghum, and its genome consists of 10 dsRNA segments that putatively code for 13 viral proteins. As other reovirus, MRCV replicates and assembles in cytoplasmic viral inclusion bodies termed viroplasms, composed mainly of the non-structural viral protein P9-1. Viroplasms produced by animal reovirus also contain other viral proteins and host cellular components with unknown functions. MRCV P6 is a non-structural 90kDa protein with a predicted coiled-coil motif and an N-terminal disordered region. By analyzing protein interactions of complete and deleted versions of P6 with other MRCV proteins by yeast two-hybrid (Y2H), we demonstrated that P6 is able to interact with itself and with P9-1 through its coiled-coil motif, suggesting a role as a minor viroplasm component. We also analyzed P6 interactions with plant host proteins by screening a cDNA Y2H library obtained from wheat leaves. Several possible interacting proteins were identified: a 26s proteasome non-ATPase regulatory subunit, elongation factor 1 beta', alcohol dehydrogenase, a seed imbibition protein, and a putative oxygen-evolving complex precursor, amongst other predicted proteins. Implications of these interactions and their possible role in the context of MRCV infection are discussed.

PL-P04.
EXPRESSION OF MAL DE RÍO CUARTO VIRUS (MRCV) P7-2 PROTEIN DECREASES PRE-PROCESSED mRNA ACCUMULATION
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MRCV (Fijivirus, Reoviridae) causes the most important maize disease in Argentina. The virus is transmitted by planthoppers where is able to multiply in a persistent and non-cytopathic manner. In plants its replication is limited to the phloem of grasses and causes severe symptoms. The viral genome consists of 10 dsRNA segments coding for 13 proteins. P7-2 is a non-structural protein that enhances viral symptoms when individually expressed in N. benthamiana using a PVX-based vector. Interestingly, it was shown that this protein causes a decrease in GFP fluorescence and in the accumulation of mRNAs from transiently expressed transgenes, including those coding for suppressors of RNA silencing. Special attention was directed to MRCV P7-2 given the fact that the ortholog ORF codifying for P7-2 is absent in fijivirus species unable to replicate in plants. To deepen into the molecular mechanisms underlying these effects, the mRNA levels of a GUS gene transiently co-expressed with P7-2 in N. benthamiana leaves was quantified by qRT-PCR. Since the reporter gene used carries an intron, we were able to evaluate both total and immature mRNA levels. Our results showed that the expression of P7-2 causes a decrease on the accumulation of mRNA precursos (pre-mRNAs), suggesting that MRCV P7-2 might interfere with a nuclear process such as mRNA transcription, stability or maturation.

PL-P05.
CHARACTERIZATION OF A BIFUNCTIONAL C-/O-GLYCOSYLTRANSFERASE FROM MAIZE

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Flavonoids are accumulated in the vacuoles as O-glycosylated derivatives; however, several species including cereals synthesize flavonoid C-glycosides. Maysin is the C-glycosyl flavone that predominates in silks of some maize varieties conferring resistance to lepidopteran insects. The transcription factor R2R3-MYB P1 is the main QTL responsible for its production. Recently, we demonstrated that ZmF2H1, which expression is highly regulated by P1, encodes a flavanone 2-hydroxylase, which converts flavanones to 2-hydroxy derivatives. In order to identify a C-glycosyltransferase involved in the formation of maysin, we isolated the cDNA coding for ZmUGT5 and its activity was characterized by *in vivo* and *in vitro* assays followed by identification of the products formed by LC-MS. *In vivo* activity assays in yeast co-expressing ZmF2H1 and ZmUGT5 showed the formation of flavones C-glycosides from flavanones, being 2-hydroxy flavanones generated by ZmF2H1 activity the substrate acceptors for ZmUGT5 C-glycosyltransferase activity. Surprisingly, ZmUGT5 can also O-glycosylate flavanones in bioconversion assays in *E. coli* as well as by *in vitro* assays with the purified recombinant protein. The results described show that ZmUGT5 is the first bifunctional glycosyltransferase that can form both C-glycoside and O-glycoside using natural substrates such as flavonoids.

PL-P06.
TOWARDS FUNCTIONAL CHARACTERIZATION OF THE PROLINE DEHYDROGENASE ISOFORMS IN THE PLANT DEFENCE

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ProDH is the first of two enzymes involved in the oxidation of proline into glutamic acid. In plants, the enzyme is required for normal development of the Hypersensitive Response, a defense against biotrophic pathogens. The Arabidopsis ProDH isoforms, ProDH1 and ProDH2, differ in some physiological functions. *ProDH1* and *ProDH2* genes contain *cis* elements mediating responses to salcylic acid (SA) and jasmonic acid (JA). However, their differential sensitivity to infections inducing these pathways remains unknown. To start evaluating this point we treated plants with pathogens or hormones. In wild type plants the infection with *Pseudomonas syringae* Avr-Rpm1 induced both genes, whereas SA and JA generated differential effects on their expression. In contrast, in *sid2* and *jar1* mutants impaired in the SA or JA pathways, this pathogen stimulated abnormal responses for *ProDH1* and *ProDH2* with differences among both kind of mutants. Besides, *ProDH2* was induced by the necrotrophic pathogen *Botrytis cinerea*. These results suggest that both ProDH isoforms may contribute to pathogen resistance under different infection conditions, probably by displaying non-redundant functions in plant immunity.

PL-P07.
ARABIDOPSIS PROLINE DEHYDROGENASE PARTICIPATES IN THE ACTIVATION OF PATTERN TRIGGERED IMMUNITY

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Proline Dehydrogenase (ProDH) catalyzes the limiting step of proline degradation in the mitochondria. In yeast and animal cells, ProDH hyperactivity contributes to imbalances in the production of reactive oxygen species (ROS). In plants ProDH contributes to effector triggered immunity (ETI) in response to pathogens. In Arabidopsis ProDH is required for full development of the Hypersensitive Response (HR), a localized cell-suicide reaction against pathogens that is signaled and stimulated by ROS. Arabidopsis genome encodes two ProDH genes: *PDH1* and *PDH2*. The aim of this work was to investigate if these genes are also involved in activation of defences triggered by recognition of microbial patterns (Pattern Triggered Immunity, PTI). We used bacterial flagellin (flg22) to evaluate PTI activation. We found that flg22 induced an early and transient expression of *PDH2* but not *PDH1*. Single mutants for each gene showed reduced callose deposition at the cell wall in response to the peptide. The growth curves of a weakly virulent bacterial pathogen (*Pseudomonas syringae* DC3000 DeltaCEL) indicated that *pdh2-2* as well as *pdh1-3* mutants are hyper-susceptible to this strain, which is unable to suppress PTI in wild type plants

PL-P08.
ADVANCES IN THE FUNCTIONAL CHARACTERIZATION OF ANTIMICROBIAL PEPTIDE SNAKIN-1 FROM ALFALFA

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Medicago sativa sp., commonly named alfalfa, is one of the most important forage crops in the world. We obtained several transgenic events of alfalfa over expressing a putative broad-spectrum antimicrobial alfalfa peptide snakin-1 (MsSN1). As Medicago sativa establishes a symbiotic relationship with soil microorganisms we wonder if the overexpression of this gene inhibits pathogens and beneficial microorganisms too. We evaluate the action spectrum and found that the pattern is not random suggesting a co-evolution process. We also identified some Pseudomonas fluorescens Pf-5 km mutants growing on LB supplemented with MsSN1extract. We studied those mutants in order to determine the gene which is responsible of snakin-1 sensibility in bacteria, and identified a putative target of this peptide. We also analyzed the presence of nodules in transgenic plants. Overexpression seems not to affect Sinorhizobium nodulation but increase the resistance to fungal diseases. This evidence indicates that snakin-1 is a useful gene to incorporate disease resistance within alfalfa elite germoplasm.

PL-P09.

CADMIUM TOXICITY IN TRANSGENIC TOBACCO CATALASE-DEFICIENT PLANTS

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Cadmium is a toxic pollutant that produces oxidative stress in several plant species. The aim of this work was to evaluate Cd toxicity in transgenic tobacco catalase-deficient (CAT1AS) and wild type plants (SR1). Plants of 45 d were treated for 8, 13, 25 days with 100 µM or 500 µM CdCl2. At day 25, CAT1AS plants accumulated more Cd than SR1 and showed smaller growth. At 25 d, iron and nitrates content decreased only in SR1 plants treated with 500 µM Cd. Chlorophyll content was reduced in both lines but more markedly in SR1. This was correlated with an increased expression of *Cp1*, a senescence-associated gene. Ascorbate peroxidase (APOX), catalase (CAT) and guaiacol peroxidase (GPOX) activities significantly increased with 500 µM Cd in both lines, except for CAT activity, that was undetectable in CAT1AS. At 25 d, CAT1AS plants counterbalanced their CAT deficiency by increasing the basal GPOX and APOX activities. In SR1 plants, 100 µM Cd augmented cell death, electrolyte leakage, and decreased polyamines (PAS) content at 25 d. SR1 plants treated with 500 µM Cd recovered cell viability and this could be due to the increase of the antioxidant enzymes activities, GSH and PAS content. Only CAT1AS plants increased proline content at all evaluated times. These results show that CAT1AS plants are able to activate different alternative defence mechanisms against Cd toxicity compared to SR1

PL-P10.

BIOACCUMULATION, TRANSLOCATION AND PHYSIOLOGICAL EFFECTS OF CR (III) IN EICHHORNIA CRASSIPES

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The aim of this study was to assess physiological parameters, oxidative damage and changes in antioxidant enzymes catalase (CAT) and guaiacol peroxidase (GPX) activities in response to bioacumulation of chromium in E. crassipes. Plants were treated with Cr (III) (2, 4, 6 and 8 mg/L) for 72h in a hydroponic system, taking samples every 24h. Treatments were performed in triplicate with a control group. Analyses of metal uptake indicated that most of the chromium accumulated in the roots, but was also translocated and accumulated in the stem-leaf. The highest percentage of absorption occurs the first 24h. Chlorophyll a and b as well as carotenoid content increased at low metal concentrations and short exposure times, but no change was observed in TChl/Car relationship. Both in roots and aerial parts, an increase in malondialdehyde content was observed at 72h for all treatments, and a slight increase in roots was detected at 24h. CAT specific activity showed a significant increase at 24h in aerial parts, while all treatments produced an increase in CAT activity in roots. GPX was significantly increased in both roots and aerial parts for all times and concentrations. Results suggest that chromium causes oxidative stress, evidenced by an increased lipid peroxidation and activation of antioxidative enzymes. However, no negative effects were detected on the studied physiological parameters

PL-P11.

VESICLE TRAFFICKING AND MONOMERIC GTPASES IN THE SYMBIOTIC LEGUME-RHIZOBIA INTERACTION

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Legume plants have the capacity to establish a symbiotic association with soil bacteria that fix atmospheric nitrogen. This infection process involves the mobilization of macromolecules through vesicles in the plant cell. In a previous work we have shown that a Rab GTPase of *Phaseolus vulgaris* is required for root hair elongation and the infection process initiated by rhizobia. We identified the best homolog of the Rab in *Medicago truncatula* and analyzed co-regulated genes using public microarray data. Strikingly, 40% of the genes with a high correlation index encodes for proteins involved in the different stages of budding, transport, tethering and fusion of vesicles. The gene with the highest correlation index was a monomeric GTPase of the Arf family, which belongs to a highly conserved subfamily of six proteins almost identical. Expression analysis by qRT-PCR showed that the six Arf genes have a similar expression pattern during nodulation. Pharmacological evidence indicates that activation of Arf proteins is required for nodule formation, root hair curling and noduline induction. To further understand the function of vesicle trafficking during nodulation we generated constitutively active and negative dominant forms of Arf by directed mutagenesis. We are currently using deep sequencing technology to study the superfamily of Ras GTPases during the symbiotic interaction.

PL-P12.

ISE2 DEVH-RNA HELICASE IS REQUIRED FOR GROUP II INTRON SPLICING OF CHLOROPLAST TRANSCRIPTS

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Mutations affecting processing of chloroplast RNA and translation, which are essential for organelle development, frequently cause embryo defective phenotypes. Increased size exclusion limit2 (Ise2) encodes a putative DEVH-box RNA helicase and its mutation causes embryo defective phenotype with altered plasmodesmata (PD) function and structure.
Previously, ise2 was trans-complemented with ISE2 cDNA fused to GFP coding sequence under 35S promoter (A4 lines). Microscopy studies showed ISE2-GFP colocalization with chloroplasts. A recently published proteome of chloroplast nucleoids has detected ISE2. We studied the RNA processing of plastid transcripts in albino leaves that spontaneously appeared among A4 plants and determined they were caused by decreased transgene accumulation. Northern blot assays, Western blot and Coomasie blue stained PAGE revealed altered level of RNAs and proteins from plastids. In addition, ISE2 deficiency causes inefficient splicing of three plastid genes contraining type II introns.
ISE2 is a nuclear encoded DEVH box RNA helicase that translocate into chloroplasts during early embryogenesis. Absence of ISE2 produces defects of group II introns splicing of organelle transcripts that are critical for normal chloroplast development, which might be essential for normal PD structure and functions during embryogenesis.

PL-P13.
AQUEOUS EXTRACTS FROM PLANTS WITH PHYTOTOXIC ACTIVITY
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The extensive use of synthetic herbicides directed against a limited number of targets to control weeds has accelerated the rate of appearance of resistance among them. The use of new substances with different sites of action would reduce that problem. In that sense, we propose the use of phytotoxic compounds isolated from plants. Several known vegetal species release germination and/or growth inhibitors as a strategy to colonize or invade grounds occupied by other plants. We collected samples (organs and surrounding soil) of plants that exhibited an invasive behaviour in different regions of Argentina. We also harvested plants popularly known for their medicinal use. A total of 2103 samples were processed. Aqueous extracts were obtained from each sample and their inhibitory activities tested in assays of lettuce and agronomic seeds germination. We detected that 251 extracts inhibited lettuce germination at 100%. Moreover, 4 extracts were selected because they showed the lowest IC_{50} (below 3 mg/mL) when tested for lettuce germination inhibition and produced also a post germination effect. Longer exposures of the seeds to these extracts produced an increase on inhibition of lettuce germination and growth. Particularly, the effect on germination was irreversible: after an exposition of seeds to these 4 extracts normal levels of germination were never recovered.

PL-P14.
PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF INTERACTIONS BETWEEN SHADE AVOIDANCE AND TEMPERATURE
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Light signals are essential to the growth and development of plants. Blue light is sensed by three families of photoreceptors: cryptochromes (CRY1-2), phototropins (PHOT1-2) and LOV domain (ZTL, FKF, LKP2) photoreceptors, whereas red and far red light is perceived by the phytochromes (PHYA-E).
The ability to withstand environmental temperature variation is essential for plant survival. It's well know that shade avoidance responses that are sensed by the phytochromes, induce similar phenotypes to those induced by high temperature . Based on this, we decided to characterize the temperature dependence of the phyB mutant phenotype at the hypocotyl growth level, as a model of temperature and light interactions.
To address to this aim we used physiological measures (hypocotyl length), transcriptomic (microarrays) and qRT-PCR.
The results show that at normal temperature ranges, plants are permanently sensing ambient temperature, the responses being close to linear. Further, phyB and other photoreceptors play a key role buffering this response. Consistent with this proposition, the gene expression analysis show higher levels of auxin-responsive genes only when the interaction between light and temperature signaling is evident.
In conclusion, some photoreceptors play an important role in buffering exacerbated responses to temperature.

PL-P15.
MOLECULAR CHARACTERIZATION OF A SUPPRESSOR MUTATION OF GIGANTEA IN *Arabidopsis thaliana*
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Plants flower at a certain time during the year to ensure that reproductive development occurs during the favorable season. This transition is regulated by genetic and environmental factors such as temperature and photoperiod. To isolate new genes involved in the regulation of flowering time, we performed a screening to find mutants that suppress gigantea (gi) late flowering. GI is involved in photomorphogenesis, the regulation of the circadian clock and is itself regulated by temperature; so GI is an important interaction node for light and temperature signaling. We found twenty independent mutants including gis7. The aim of this work is to make a preliminary characterization and identify the gene affected in gis7. gis7 was isolated in the Columbia accession following mutagenesis with EMS. As a crossing partner for molecular mapping we used Landsberg erecta and early flowering F2 progeny was utilized for positional cloning. Plants were grown in chambers at 23ºC in LD condition (16-h-light/8-h-dark) or SD (16-h-light/8-h-dark). Total leaf number was scored in flowering time experiments. gis7 flowers early in SD but slightly early in LD at 23ºC . gis7 mapped to a locus near FLC in chromosome 5, but preliminary evidence suggests gis7 is not an allele of FLC.

PL-P16.
CHARACTERIZATION OF *ufm1* (UBIQUITIN FOLD MODIFIER) GENE INVOLVED IN SEED DEVELOPMENT
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Seed development is a critical process in life cycle of higher plants, the spatial and temporal genes expression is a highly regulated process. A posttranslational modification of proteins is one of the main mechanisms to regulate gene expression, adding small polypeptides such as ubiquitin.
A sequence homologous to ubiquitin fold modifier 1 (*ufm1*) gene was identified in differential expression assay from inmature seeds of intra and interspecific crosses between wild species of *Solanum*. The seeds of interspecific crosses showed low viability, about 0.2%, and abnormalities at the embryo and endosperm. Mutants of *ufm1* were obtained in *Arabidopsis thaliana* but there is not information about the phenotype and function of the gene.
To analyze the *ufm1* gene effect in seed development we used an *Arabidopsis* mutant (SALK 040955). A phenotypic analysis of the vegetative parts of mutant plants showed no obvious difference compared with wild-type plants. Siliques of mutant plants, however, contained around 25% of viable seeds. These results suggest that changes in gene expression of *ufm1* affect seed development, as observed in wild potato interespecific crosses.

PL-P17.

GAIN OF CYTOSOLIC NADP-ME ACTIVITY IMPACTS ON PRIMARY METABOLISM AND STRESS TOLERANCE OF *Arabidopsis thaliana*

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NAD(P)-malic enzyme (ME) catalyzes the reversible oxidative decarboxylation of malate to pyruvate, CO2 and NAD(P)H and is present as a multigene family in *Arabidopsis thaliana*. Phenotypic and molecular characterization of plants over-expressing cytosolic NADP-ME2 was carried out. These plants exhibited lower rosette weight and root length relative to wild-type, added to an increased sensitivity to mannitol treatment on MS-plates or irrigation with PEG on soil-grown plants. Interestingly, the increased NADP-ME activity led to a decreased expression of the other constitutive ME isoforms and of another malate metabolism related enzyme, the malate dehydrogenase. In addition, this line presented higher content of organic acids and sugars relative to wild-type under typical growth conditions. However, the accumulation of these metabolites was substantially less pronounced after osmotic stress exposure. This indicates that altering malate metabolism by raising NADP-ME2 activity has profound consequences for the response of the entire primary metabolism upon normal and stress conditions. Overall, the gain of NADP-ME activity produces changes in gene expression, enzymatic activities and metabolite levels which reflect the relevance of this enzyme in the organic acid plant metabolism.

PL-P18.

COMPARISON OF MAIZE PROTEINS OF RESISTANT AND SUSCEPTIBLE GENOTYPES TO FUSARIUM VERTICILLIOIDES

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A proteomics approach was assessed to identify proteins whose level of expression was associated with maize kernel resistance against F. verticillioides infection. Proteins from a resistant and susceptible inbred have been compared using 2-D gel electrophoresis. Twenty four protein spots have been identified and sequenced. These proteins can be grouped into three categories based on their peptide sequence homology: 1) storage proteins, such as globulin 1, and late embryogenesis abundant proteins; 2) stress-related proteins, such as a superoxide dismutase, mismatch repared protein and 3) metabolism related protein such as, triosephosphate isomerase, sorbitol deshidrogenase, several heat shock proteins and a glyoxalase I protein. We establish that the resistant inbred had more constitutive expression of proteins than the susceptible inbred. Although the objective of this investigation was to identify antifungal proteins associated with Fusarium resistance, the majority of those identified were stress related proteins and storage proteins. These data suggest that kernel resistance may require not only the presence of high levels of antifungal proteins. Possession of unique or higher levels of constitutively expressed proteins may put resistant lines in an advantageous position over susceptible ones in the ability to synthesize proteins and defend against pathogens while under stress

PL-P19.

THE DEFENSE RESPONSE MEDIATED BY THE ELICITOR OF A. STRICTUM DOES NOT REQUIRE THE APOPLAST

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The production of reactive oxygen species (ROS) plays a crucial role in signal transduction and therefore their measurement and kinetics is a very useful tool in the study of defense response in plants. We have earlier reported the generation of H_2O_2 and O_2^- when strawberry (Fragaria x ananassa) plants were treated with an elicitor protein produced by the fungus Acremonium scrictum. In this study we evaluated the importance of the integrity of the apoplast to elicit the defense response. We worked with mesophyll cell suspensions ($1x10^6$ cells/mL) of 8 week strawberry plants of the cv. Pájaro. Cells were obtained by leaf homogenization, separated by differential centrifugation and resuspended in W5 buffer (pH 5.6). The intracellular and extracellular levels of H_2O_2 were measured by fluorometry using the probes H2DFFDA and Pyranine, respectively. Results showed a rapid extracellular decrease and intracellular accumulation of H_2O_2 immediately after the elicitor addition, suggesting a rapid translocation of H_2O_2 to the cytosol. After this first event, two peaks of intracellular H_2O_2 accumulation were observed, one 2 hours after induction (hai) and the other 7 hai, which coincided with the results previously observed in plants demonstrating that the integrity of the apoplast is not required in the induction of the defense mechanism mediated by the elicitor.

PL-P20.

OXIDATIVE POST TRANSLATIONAL MODIFICATIONS OF PROTEINS RELATED TOXICITY IN WHEAT SEEDLING TO CELL CYCLE ARE INVOLVED IN CADMIUM

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Abiotic stress is greatly associated with plant growth inhibition and redox cell imbalance. In the present work, we have investigated in which way oxidative posttranslational modifications (PTM) of proteins related to cell cycle may be implicated in post-germinative root growth reduction caused by cadmium, by methyl viologen (MV) and by hydrogen peroxide (H_2O_2) in wheat seedlings. Although cadmium is considered a redox inactive metal, reactive oxygen species were detected in the apex root of metal-treated seedlings. Oxidative stress hastened cells displacement from the cell division zone to elongation/differentiation zone, resulting in a shortened meristem. The number of cells in the proliferation zone was lower after MV, H_2O_2 and 10 M Cd^{2+} treatments compared to control. All treatments increased protein carbonylation. Although no modification in total Ub-conjugated proteins was detected, oxidative treatments reduced cyclin D and CDKA protein ubiquitination, concomitantly with a decrease in expression of cyclin D/CDKA/Rb/E2F-regulated genes. We postulate that ROS and oxidative PTM could be part of a general mechanism, specifically affecting G1/S transition and progression through S phase. This would rapidly block cell cycle progression and would allow the cellular defence system to be activated.

PL-P21.
INCOMPATIBLE STYLAR EXTRACT DISRUPTS F-ACTIN AND VESICULAR TRAFFICKING IN *Nicotiana* POLLEN TUBES

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The integrity of actin filaments and vesicular trafficking is essential for pollen tube polar growth. F-actin provides the track along which organella and vesicles move, carrying the material necessary to sustain the tip growth. In the self-incompatible species *Nicotiana alata* pollen tube growth is inhibited after self-pollination. Using the fluorescent markers phalloidin and FM 4-64 we analyzed the alterations of F-actin cytoskeleton and vesicular trafficking in *in vitro* cultured pollen tubes challenged with compatible and incompatible stylar extracts. Initiallty, both compatible and incompatible pollen tubes showed similar growth and an organized pattern of F-actin cables and vesicle trafficking. However, 90 min after challenging with the stylar extract, 50% of incompatible pollen tubes showed the V-shaped zone dissapeared and large accumulations of vesicles were seen in the subapical region. In parallel, the subapical actin ring and the F-actin cables along the shank became disorganized. These changes involved almost 90% of incompatible pollen tubes after three hours of culture while most of compatible pollen tubes showed organized both F-actin cytoskeleton and the endomembrane system. A comparison between pollen tube alterations in *Nicotiana* and *Papaver* self-incompatibility systems is discussed

PL-P22.
NON-FUNCTIONAL S-RNASES ARE RECRUITED TO COPE WITH PHOSPHATE STARVATION IN *Nicotiana*

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Plant T2 ribonucleases (RNases) were classified in two subfamilies, a) S-RNases, involved in pollen recognition and rejection of some self-incompatible species and b) S-like-RNases, widely distributed in the plant kingdom and involved in the response to different biotic and abiotic stresses. We have cloned a stylar cDNA from *Nicotiana alata* with structural traits of *S-RNases* but without functionality in the self-incompatibility system; therefore, it was denoted as *non-S63-RNase*. This gene was induced in plants hydroponically grown without Pi, a typical response of S-like-RNases, but not reported yet in other RNases. A 15 amino acid peptide corresponding to the hypervariable region of non-S63-RNase was used to develop a rabbit antibody. The antibody recognized a signal protein of 31 kDa in root extracts from plants grown under Pi starvation. This signal decayed significantly after preincubation of serum with the antigenic peptide providing evidence that the 31 kDa protein was the non-S63-RNase. The induction of non-S63-RNase was rapidly repressed when Pi-starved plants were replaced in a Pi-supplemented medium. In gel RNase-activity showed a band of 31 kDa which was also recognized by the anti-peptide antibody. This result suggested that the non-S63-RNase was induced in an enzymatically active form. To our knowledge this is the first report of a functional role for a non-S-RNase

PL-P23.
IDENTIFICATION AND CHARACTERIZATION OF MAIZE FRATAXIN ISOFORMS

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Mitochondria and chloroplasts perform several processes essential for cell life. In many of these, that involve for example electron transfer, gene expression regulation and environmental sensing, participates different Fe-S proteins. Moreover, both organelles contain several components that are homologous to the bacterial or yeast Fe–S cluster assembly machinery, suggesting the presence of two biosynthetic pathways.
Frataxin (FH) is a highly conserved nuclear encoded protein that has been proposed to participate as iron donor in Fe-S cluster assembly. Besides, this protein is involved in iron homeostasis, heme metabolism, ROS and REDOX control and protection against oxidative damage. However, its precise function remains unclear. This protein has been characterized in bacteria, yeast, humans and in Arabidopsis thaliana plants (AtFH). In eukaryotes, it was reported a mitochondrial localization for FH. In maize, we identified two coding sequences homologous to AtFH (ZmFH42 and ZmFH55). QPCR experiments showed that both genes are expressed mainly in young tissues. Furthermore, we cloned, purified and characterized both isoforms. Results show that at least one (ZmFH55) is a functional protein because it attenuates Fenton reaction. Finally, confocal microscopy studies on A. thaliana protoplasts transformed with GFP-ZmFH suggest mainly a mitochondrial localization for both proteins

PL-P24.
FRATAXIN DEFICIENCY AFFECT CHLOROPLAST FUNCTIONS

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Frataxin is an iron binding protein highly conserved throughout evolution. It is involved in various processes such as iron homeostasis, respiration, maturation of Fe-S proteins, heme metabolism, control of ROS and protection against oxidative damage. In yeast, mammals and plants frataxin has been described as a mitochondrial protein necessary for the normal function of this organelle. In silico studies predict mainly a mitochondrial localization for the Arabidopsis frataxin homologue (AtFH), but also a possible chloroplast localization. To determine the subcellular localization of AtFH, we constructed Arabidopsis transgenic plants carrying the AtFH transit peptide fused to GFP. Using confocal laser microscopy we determined a dual localization of AtFH in both organelles. In addition, in order to determine the roles of AtFH in the chloroplast, we analyzed several parameters related to the physiological state of the chloroplasts in AtFH deficient plants. Results showed alterations in total iron and chlorophyll content, in the photosynthetic capacity and in the activity of many Fe-S proteins. Results indicated that Frataxin is a functional protein in the chloroplast since its deficiency alters the normal physiology of this organelle.

PL-P25.
AN *IN SILICO* APPROACH TO IDENTIFY KEY GENES IN SPERMINE-INDUCED RESISTANCE IN *Arabidopsis thaliana*
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A role for spermine (Spm) in *A. thaliana* resistance to *Pseudomonas viridiflava* was previously described by our group. *A. thaliana* plants that accumulate Spm by overexpressing the *SPERMINE SYNTHASE* gene (35S::*SPMS*-9) were less susceptible to *P. viridiflava* than *spermine synthase* null mutants (*spms*-2) with reduced Spm levels, and wild type plants. Global changes in gene expression resulting from perturbation of Spm levels *in planta* was analized by transcript profiling of 35S::*SPMS*-9 and *spms*-2 plants. Analysis of gene ontology term enrichment demonstrated that many genes overexpressed in 35S::*SPMS*-9 participate in pathogen perception and defense responses. The aim of this work was to identify enrichment of cis-regulatory elements related to defense mechanisms within the subset of genes overexpressed in 35S::*SPMS*-9. Notably three known promoter motifs associated with defense responses were enriched within the gene subset. In addition, five genes (MPK3, NHL2, NHL3, AZF1 and AZF2) with a putative role in Spm-mediated resistance were found within the three subsets of genes enriched in cis-elements. Particularly, MPK3 is the *A. thaliana* orthologue of tobacco WIPK, a well-known Spm-responsive gene. The other four are probable orthologues of tobacco Spm-responsive genes. The obtained results and their implications on Spm-mediated resistance of *A. thaliana* to *P. viridiflava* are discussed.

PL-P26.
PROTOPLASTS AS A TOOL TO STUDY ALTERNATIVE SPLICING IN ARABIDOPSIS
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Arabidopsis protoplasts are cells without cell walls obtained from leaves after an enzymatic treatment. Protoplasts maintain many of the physiological properties of the plants which they come from. We have established the experimental conditions to obtain Arabidopsis mesophyl protoplasts in order to asess alternative splicing responses to light/darkness treatments as previously observed in Arabidopsis seedlings. We use the RS31 transcriptional unit as a model. In seedlings, light treatment causes an increase of the functional mRNA isoform. Protoplasts seem to show a light/darkness effect, only slightly lower than in seedlings. Furthermore, we have constructed a series of alternative splicing reporter minigenes to be introduced into the protoplasts through PEG-mediated transformation. These minigenes combine different alternative splicing events –exon cassette and intron retention or alternative 5' splice sites- with three different promoters. The idea is to evaluate if promoters affect alternative splicing in protoplasts as it was described in animal cells. Our protoplast transformation controls using a GFP reporter include detection of the expressed protein by epifluorescence microscopy and of expressed RNA by RT-PCR. Our results indicate that protoplast isolation and transformation are a useful new tool to study alternative splicing in plants.

PL-P27.
GSH, ASC AND IRON AVAILABILITY AFFECT ROOT ARCHITECTURE AND GRAVITROPIC RESPONSE IN *Arabidopsis*
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Root growth is affected by environmental factors. Among the most critical, nutrient availability can profoundly shape root architecture. The increase of the density and length of root hairs is a well studied response in plants growing under iron deficiency. However, the effect of nutrient deprivation on the primary root (PR) length, lateral roots (LR) length and density, and gravitropic response is poorly understood. It has been already established that reactive oxygen species (ROS) and auxin can modulate the PR length, LR density and length and the gravitropic response in plants. In this work, we analyzed the effect of iron availability and cell redox regulation on the root architecture in Arabidopsis plants. Glutathione (GSH) and ascorbate (Asc), two multifunctional metabolites that are important in redox balancing affect differentially the PR length and LR length and density. Under iron deficiency, Asc affects the root architecture stronger than GSH. In addition, GSH inhibits the gravitropic response in *Arabidopsis* under iron deprivation but not in iron sufficiency. A link between GSH, Asc, ROS, auxin and iron availability is discussed.
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PL-P28.
INTRA VARIETAL GENETIC VARIABILITY AMONG C.V. TORRONTÉS RIOJANO CLONES (*Vitis vinifera* L.)
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Torrontés Riojano (*Vitis vinifera L.*) is a grapevine cultivar autochthonous from South America. In Argentine it is currently used to elaborate characteristic and aromatics white wines. To begin a selection program 56 clones were collected from different sites of two wine regions of Argentine. Phenotypic variations were observed during collects. Nine clones with phenotypic variation and collected from different vineyard were selected and studied at genotypic level. Six SSR were characterized. The observed alleles per locus were the expected for Torrontés Riojano. Six AFLP and five M_AFLP markers were used to detect genetic variability. The observed variability was bigger than the awaited in relation to older varieties. In additions, all the clones showed similar genetic distance and it was not possible to group them in clusters. Our results help us to better understand the importance of clone collections in autochthonous varieties as Torrontés Riojano.

PL-P29.
MOLECULAR AND GENETIC ANALYSIS OF GRAPEVINE BERRY SIZE DETERMINATION. THE AINTEGUMENTA GENE FAMILY

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Fruit size is a highly important trait for most fruit and vegetable crops. This trait has been a main selection target from the beginning of the modern agriculture. Even though its determination is influenced by the cultural practices, several genes have been identified controlling fruit size and shape. The AINTEGUMENTA (ANT) gene is a member of the AP2/ERF family of transcription factor genes. In ovules of the ant mutant, integuments do not develop and megasporogenesis is blocked. Based on these results, it is believed that ANT regulates cell proliferation and organ growth by maintaining the meristematic competence of cells during organogenesis. On the other hand, the characterization in Arabidopsis and other plant species of seven closely related genes (ANT-like genes o AIL) also suggested their participation in specification of meristematic or division-competent states. Using the ANT and AIL known sequences we identified all the grapevine (Vitis vinifera) putative ortholog genes. After the aligned of their sequences we determined the phylogenetic relationship respect all the ANT and AIL sequences from Arabidopsis, rice and poplar. We designed qRT-PCR specific primers for all grapevine ANT/AIL genes and we analyzed their expression patterns in different tissues and stages of the plant with the aim of defining their putative role in the flower and fruit size determination

PL-P30.
BBX24 ACTS AS POSITIVE REGULATOR TO PROMOTE HYPOCOTYL ELONGATION IN SHADE AVOIDANCE RESPONSES

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Plants grown at high densities perceive through the phytochrome system a decrease in the red to far-red (R:FR) ratio of incoming light, as a warning signal of future competition, and mount morphological responses collectively known as the shade avoidance syndrome (SAS). In Arabidopsis thaliana seedlings, the hypocotyl elongation by SAS involves a rapid up-regulation of known shade marker genes activating an interacting network of various hormones that will eventually lead to cell elongation. We identified and characterized a group of B-box (BBX) proteins as new components involved in the SAS signaling pathway, which act as positive (BBX18 and BBX24) or negative (BBX19, BBX21 and BBX22) regulators. By microarray analysis, pharmacological and genetic approaches, we investigated the molecular action of BBX24 in the SAS. Global expression analysis of wild-type and bbx24 seedlings reveals that a large number of genes involved in hormonal signaling pathways are positively regulated by BBX24 in response to shade, particularly genes involved in gibberellin (GA) biosynthesis. We found that exogenous GA recovers the hypocotyl elongation of bbx24 mutant seedlings in shade conditions. In addition, the phenotype of pif4 bbx24 double mutants suggests that BBX24 is involved in the GA signaling pathway mediated by PHYTOCHROME INTERACTING FACTOR 4 (PIF4).

PL-P31.
REGULATION OF CIRCADIAN RHYTHMS BY A SM-LIKE FAMILY PROTEIN IN *Arabidopsis thaliana*

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Circadian rhythms allow organisms to time biological processes to the most appropriate phases of the day. There are many evidences that the regulation of alternative splicing contributes strongly to the adjustment of the circadian rhythms. Using a bioinformatics approach, we compared a list of genes regulated by the circadian oscillator at the mRNA level with a list of splicing factors and regulators in *Arabidopsis thaliana*. This comparison revealed that mRNA levels of 83 genes from a list of 426 splicing related genes are regulated by the circadian clock.
Leaf movement analysis in mutant plants for the majority of these genes did not affect clock function. Interestingly, a point mutation in the gene coding for LSm5, a protein of the Sm-Like family, showed a defect in clock function. We found that it has a 2.5hs longer period of circadian leaf movement than wild type plants and an early flowering phenotype in both, long and short day conditions.
To verify that the observed phenotype in the period length is due to the point mutation in the Lsm5 gene, we complemented the mutant with the Lsm5 wild type gene restoring the wild type phenotype. Here we show a "core" Sm like protein whose mRNA is clock regulated and the loss of function mutant affects the circadian clock, revealing a novel relationship between splicing and circadian rhythms.

PL-P32.
COMPARING THE ROLE OF SYMMETRICAL AND ASYMMETRICAL ARGININE METHYLTRANSFERASES IN *Arabidopsis thaliana*

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Protein arginine methyltransferases (PRMTs) are a family of enzymes that di-methylate arginine residues on protein substrates. These proteins are classified as symmetrical (sPRMTs) or asymmetrical (aPRMTs) PRMTs, depending on the position of the methyl group on the guanidino of the methylated arginine. Previous information links sPRMTs to transcriptional repression processes while aPRMTs would be associated to transcriptional activation. Here we compare the role in regulation of growth and development in A. thaliana of two of the most important members of both families: PRMT5 (sPRMT) and PRMT4 (aPRMT). Both mutants, *prmt5* and *prmt4a;prmt4b*, show alterations in the photomorphogenic process under red and blue light, but only *prmt4a;prmt4b* exhibits alterations in the chlorophyll synthesis after a red light potentiation treatment. Only *prmt5* mutants exhibit alterations in circadian rhythms and alternative splicing. Global expression analysis of the mutants revealed that these PRMTs regulate different genes, only five where co-regulated, particularly *FLC* (*Flowering Locus C*) witch explains the delayed flowering in long and short day photoperiods of both mutants. Our results show that instead of acting as antagonistic as suggested by previous information, sPRMTs and aPRMTs act independently controlling the regulation of physiological and molecular processes in *A. thaliana*.

PL-P33.
GLYCINE RICH RNA BINDING PROTEINS IN TOMATO FRUITS: A NOVEL POSITIVE REGULATION OF EXPRESSION

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In this study, the analysis of GRP1 gene family was undertaken in Micro-Tom tomato fruit. Three *LeGRP1* genes (*LeGRP1a-c*) were identified which are highly homologous to each other and to GRPs of related species. Three transcriptional products for each gene were identified, the un-spliced pre-RNA, the mature mRNA and the alternative spliced mRNA (*preLeGRP1a-c*, *mLeGRP1a-c* and *asLeGRP1a-c*). LeGRPs show the classical N-terminal end RNA recognition motif and the C-terminal end glycine-rich region, suggesting conservation of functional properties. The levels of all transcriptional forms of *LeGRP1a-c* and the total immunoreactive LeGRP1 decrease during tomato fruit development from immature green to red ripe. Particular circadian profiles of expression were detected for each transcriptional *LeGRP1* form. During ripening off the vine after mature green, the levels of all transcriptional forms *LeGRP1a-c* increased at 20ºC but not at 4ºC. The correlation analysis of the expression of all *LeGRP1a-c* transcriptional forms suggests a novel model of positive regulation of expression in tomato fruit, in which each immature form of *LeGRP1a-c* positively correlates with each other and the *mLeGRP1a-c* form correlates depending on the stage. The results obtained reveal a complex pattern of expression of GRP in tomato fruits suggesting these proteins as key regulators of important processes in fruits.

PL-P34.
ISOLATION AND PARTIAL CHARACTERIZATION OF A TRYPSIN INHIBITOR FROM *Styphnolobium japonicum* SEEDS

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With the aim of detecting protease inhibitory activity in seed extracts of *Styphnolobium japonicum* (L.) Schott, folk name "pagoda tree", a leguminous tree belonging to Fabaceae family, we process, under liquid N_2, 25 g of seeds with 100 mL of Tris- HCl buffer containing ascorbic acid as oxidative protector. The extract was precipitated with acetone and redisolved in 50 mL of extraction buffer. The resulting solution presented important trypsin inhibitory activity without detectable caseinolytic activity. The solution was lyophilized in order to obtain stable solid proteins and for maintenance purpose. The lyophilized was redisolved in extraction buffer (4 mg/mL) which contain a protein concentration of 220 µg/mL (Bradford). This solution was purified by means of affinity chromatography (trypsin agarose). The eluate was tested for protein concentration through Lowry method giving 23 µg/mL corresponding to an inhibitory activity of 98%. With the purpose of knowing electrophoretic characteristics of inhibitor, SDS-Tricina-PAGE and IEF were performed. The estimated molecular mass was 12-13 kDa and its isoelectric point nearly 5. A subsequent step will be its purification and the evaluation of potential aplication as a novel pharmacological agent.

Prospitti, A. Fellowship CICPBA Natalucci, CL Researcher CICPBA.

PL-P35.
CHARACTERIZATION OF A BACTERIAL PLANT NATRIURETIC PEPTIDE-LIKE IN PLANTS

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The bacterial citrus pathogen, *Xanthomonas axonopodis* pv. *citri*, contains a gene encoding a plant natriuretic peptide (PNP)-like protein (XacPNP) that shares significant sequence similarity and identical domain organization with PNPs. These peptides are extracellular, systemically mobile molecules that are able to elicit a number of plant responses important in homeostasis and growth. It has been shown that purified XacPNP, as well as PNPs, alters physiological responses including stomatal opening and photosynthetic efficiency. Also, it has been shown that *XacPNP* expression is induced upon infection, and lesions caused by a *XacPNP* mutant are more severe than those of wild-type strain, suggesting that XacPNP enables the pathogen to modify host homeostasis with the aim of creating favorable conditions to its own survival. To study the physiological processes in which PNPs are involved we obtained *Arabidopsis thaliana* transgenic lines that overexpress XacPNP. These lines showed higher sensitivity to saline stress compared to wild type. In order to further characterize the role of PNPs in plants and get closer to the mechanism of action of PNPs, we obtained *A. thaliana* lines that express a version of XacPNP fused to the green fluorescent protein and observed that this hormone-like peptide is associated to plant cell membranes, giving support to the proposal of this peptide as a hormone.

PL-P36.
ANALYSIS OF STRUCTURAL FEATURES OF A HRP-ASSOCIATED HARPIN FROM *Xanthomonas axonopodis* pv. *Citri*

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Phytopathogenic bacteria colonize their hosts through the secretion of effector proteins by the type III protein secretion system. This system, encoded by the hrp cluster (hypersensitive response (HR) and pathogenicity), is essential for pathogenicity in host plants and induction of HR in non-host plants. HR is a rapid response that involves cell death and slows the spread of infection. The hrp cluster of *Xanthomonas axonopodis* pv. *citri* (Xac) contains a gene that encodes a harpin protein called Hpa1. Harpins are glycine-rich heat stable proteins that can form pores in membranes and can induce HR in some plants. This sort of protein forms fibrils rich in ß sheets typical of amyloid proteins. In order to study biophysically the protein Hpa1, it was expressed in *Escherichia coli* and purified using a size exclusion column on a FPLC equipment, showing a tetrameric structure. Using the binding to Congo Red dye assay and polarized light microscopy, it was observed the formation of amyloid-like fibrils. The kinetics of amyloid-like fibrils formation in Hpa1 under different conditions using the specific dye Thioflavin T was performed, showing a fast rate of fibrils formation. In agreement to previous results about the participation of Hpa1 in plant-pathogen interaction, this fibril structure may be involved in the elicitation of HR in non-host plants as well as in virulence in host plants.

PL-P37.
METAL ACCUMULATION IN SUNFLOWER: METALLOTHIONEIN FAMILY CHARACTERIZATION

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Sunflower can accumulate significant amounts of heavy metals. Metallothioneins (MTs) –proteins deeply studied in the animal kingdom- and phytochelatins –enzymatically synthesized peptides- are among the best known eukaryotic peptidic metal chelators. Nevertheless, data on the first system (polymorphism, regulation, structure and function of these genes and corresponding proteins) in plants are scarce, so the aim of this study is the characterization of the molecular mechanisms involved in metal tolerance/accumulation in plants of interest in agriculture, such as sunflower.
In silico analysis of sunflower ESTs banks with known MT sequences or motifs has rendered 7 sequences of probable MT genes. Some isoforms are expressed at high levels in root, shoot and seeds. Four of these genes, whose sequences codify for the 4 plant MTs types described, are currently under studies. Heterologous expression in *S. cerevisiae* MT-null strains restores Cu tolerance and does not improve resistance to oxidative stress. Some isoforms enhance Cd and Zn tolerance in MT-null yeast strains. Sunflower MTs, purified from *E. coli* cells cultured in different metal rich media, render better folded polypeptides with Cd and Cu than with Zn. These results point towards a role in metal sequestration with specific metal preferences for the different sunflower MT isoforms.

PL-P38.
FUNCTIONAL REDUNDANCY OF *ARABIDOPSIS* ADC ISOFORMS IN RESPONSE TO *Botrytis cinerea* INFECTION

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Arginine decarboxylase (ADC) catalyzes the first step of polyamine (PA) biosynthesis. The model plant *Arabidopsis thaliana* harbors two ADC isoforms (*AtADC1* and *AtADC2*), which are differentially regulated by different stresses, but no information about their regulation under biotic stress is available. This study aimed to determine the role *AtADCs* in defense against infection by *Botrytis cinerea*. The effect of infection on the expression of each isoform, PA levels and lesion size were evaluated in null *adc1* and *adc2* mutants, as compared to wild-type (WT, *Col-0*) plants. Infection increased the expression of both isoforms in WT plants, as compared to non-inoculated controls. *AtADC2* expression was increased in infected *adc1* mutants, as compared to non-inoculated *adc1* plants. Reciprocally, infection enhanced *AtADC1* expression in *adc2* mutants, as compared to non-inoculated *adc2* plants. WT plants and both adc mutants exhibited increased Put levels in response to infection, as well as similar levels of tissue damage. Results demonstrate that *B. cinerea* infection induces *AtADC* expression, but none of the isoforms seems to be defense-specific, since each one was induced by infection when the other was mutated. This functional redundancy, as well as the previously reported lethal phenotype of double (*adc1-adc2*) mutants render difficult to assess the contribution of ADC to resistance.

PL-P39.
PROTEOMIC AND METABOLOMIC CHANGES INDUCED BY FROST IN ORANGE FRUIT (C. SINENSIS VAR. VALENCIA LATE)

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Exposure of citrus fruit to frost often results in the development of freeze injury during their maturation in planta. However, depending on the severity of this phenomenon, an acclimation response could be induced. The aim of this work was to characterize changes induced by frost on the proteome and metabolome of orange fruit at different points after the natural event. Following two-dimensional PAGE, almost 30 differential protein spots were detected in juice vesicles and flavedo among all comparisons made. Sixteen different proteins in 19 spots were identified. Identification of these citrus proteins by mass spectrometry and annotation according to the NCBI and Viridiplantae ESTs data bases revealed that the most affected categories were Metabolism and Energy. Ferritin levels showed an interesting behavior, being lower immediately after frost and higher at 7 days after frost respect to control. The metabolites content was assessed by GC-MS analysis. A higher concentration of sugars was evident after frost exposure, both in juice vesicles and flavedo, while the main acids were slightly or not affected. These results are consistent with a higher sink force in frost-exposed fruits respect to control, probably due to an impaired energy production, although further studies are necessary to prove this hypothesis.

PL-P40.
METABOLISM AND COMPOSITION OF BARLEY ALEURONE PHOSPHATIDIC ACID IN RESPONSE TO HORMONAL TREATMENT

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Phosphatidic acid (PA) is a lipid signalling molecule and intermediary in abscisic acid (ABA) signalling. ABA plays an important role in seed germination by inhibiting GA response in aleurone. By using aleurone 32P-Glycerophospholipids (GPL) as substrates, an active phospholipase D (PLD) was present. This PLD produced 32P-PA rapidly (min) in the presence of ABA, and transiently, indicating rapid PA removal after generation. The PA removal by phosphatase 1 and 2 isoforms was verified in aleurone membranes, the former but not the latter being specifically responsive to GA/ABA. In contrast to PA kinase, which down-regulates PA levels by GA/ABA-dependent phosphorylation to DGPP, DGPP phosphatase was unmodified. A high long-term 32P PA/PC labeling ratio after long-term (hs) incubations of aleurone layers in the absence of GA or ABA suggested that PLD targeted part of the synthesized 32P-PC. The lipid and fatty acid composition of unlabeled aleurone confirmed this. A marked accumulation of PA, highly enriched in 18:2n-6, concurred with a relatively low amount of GPL, especially low amounts of 18:2n-6-rich species of PC. The PC/PA ratio was maintained high after incubations in the presence of GA/ABA. Somewhat less GPL including 18:2-PC were formed with ABA than with GA because with ABA but not with GA part of the 18:2-PA was converted to 18:2-DGPP by ABA-responsive PA kinase.

PL-P41.
PHOSPHOLIPASE ACTIVITIES CORRELATE WITH PROLINE ACCUMULATION UNDER SALINE STRESS IN BARLEY ROOTS
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Salt stress is one of the abiotic factors that limit normal plant development and activates signaling processes. Under this condition the plants accumulate proline in the cytosol as a protective mechanism from the osmotic effect. The signal pathway for proline biosynthesis involves an increase of calcium by IP3 that originates from phospholipase C (PLC) activity while phospholipase D (PLD) activity acts as a negative regulator on this synthesis. The aim of this study was to evaluate the relationship between stress, the protective osmolyte and phospholipase activities in roots of barley germinated 4 days under salt stress (100 mM NaCl) and under osmotic stress (200 mM mannitol). Salt stress increased the levels of proline 380% while mannitol only 200%. PLC activity, measured by the formation of 3H-IP3, increased 50% under saline stress but not under osmotic stress. In contrast, PLD activity, measured by the formation of 3H-choline, increased 90% under osmotic stress but not under salinity stress. These results demonstrate a positive correlation with proline accumulation and PLC activity in barley roots under saline stress and differences in the involvement of the lipid signaling pathway.

PL-P42.
FUNCTIONAL CHARACTERIZATION AND EXPRESSION OF A FRUCTOKINASE GENE FROM A MARINE CYANOBACTERIUM
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Sucrose (Suc) is mainly synthesized by plants, unicellular algae and cyanobacteria. Two enzymes are responsible for the phosphorylation of fructose and glucose, the Suc cleavage products. Hexokinases preferentially phosphorylate glucose and fructokinase catalyze the transfer of a phosphate group from ATP to fructose to yield fructose 6-phosphate, substrate for Suc synthesis. In the genome of *Synechococcus* sp. PCC 7002, a unicellular cyanobacterial marine strain, we retrieved an open reading frame (*orf*) annotated as a member of the PfkB family. After PCR amplification of that sequence from genomic DNA, the amplicon was ligated into an expression vector and heterologously expressed in *E. coli* cells. The recombinant His-tagged protein purified by affinity chromatography, exhibited frutokinase activity with divalent-cation dependence and specificity for fructose. The *Mr* of the polypeptide determined by SDS-PAGE was in good agreement with that calculated from the predicted amino-acid sequence. RT-PCR experiments indicated that the *orf* expression parallels that of the genes for Suc biosynthesis in cells under different conditions. We conclude that the *orf* corresponds to the encoding sequence of a specific fructokinase, the first characterized from cyanobacteria, which could be providing the phosphorylated substrate for Suc biosynthesis.
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PL-P43.
MOLECULAR EVOLUTION OF PARASITIC PLANTS
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There are about 4000 parasitic plants, which originated from free-living ancestors in at least eleven independent events during the evolution of angiosperms. Little is known about the genetic changes triggered by the transition to parasitism. Parasitic plants have an intimate association with their hosts and horizontal transfer of genetic material, particularly mitochondrial, has been documented. Our goal is to gain insight into the evolution of the cytoplasmic genomes of understudied lineages of parasitic angiosperms. We collected two holoparasites and four hemiparasites that belong to unrelated angiosperm families. Genomic DNA extractions and PCR amplifications were challenging. We were able to amplify and sequence four mitochondrial genes and the chloroplastic gene rbcL. The mitochondrial genes from parasitic plants showed a high substitution rate and predicted RNA editing sites. Sequences of rbcL showed an average substitution rate (except for Cuscuta sp.) and probably encoded a functional protein. Introns were only detected in the mitochondrial gene cox1. Phylogenetic analyses of each individual gene were performed including sequences from other angiosperms obtained from international databases. Incongruences between the organismal phylogeny and the phylogenies based on individual mitochondrial genes suggested five putative cases of horizontal gene transfer.

PL-P44.
EXPRESSION AND PURIFICATION OF ANTIMICROBIAL SNAKIN-2 PEPTIDE IN BACTERIAL CELLS
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Snakin-2 (SN2) is a cysteine-rich antimicrobial peptide isolated from Solanum tuberosum tubers that was found to be active against fungal and bacterial pathogens and belongs to the snakin/GASA family. Members of this family are widely distributed among plant species and are involved in several aspects of plant development and plant responses to biotic or abiotic stress. Given their antimicrobial activity, expression in bacteria was only possible for a few members of this family and it resulted in a low production and the aggregation of the protein in inclusion bodies. To obtain purified protein for biochemical and functional studies we generated different constructs carrying SN2 coding sequence, based on the pDEST15 and pDEST17 vectors, which have a GST and a 6xHis tag, respectively. After induction of the transcription, transformed BL21-AI E. coli strain showed a reduced growth rate compared to control suggesting a toxic effect of the recombinant protein. For both constructs, tagged-SN2 expression was detected in the insoluble fraction so different buffers were tested to achieve total soluble tagged protein. Finally, employing a glutathione sepharose resin, purified GST-tagged SN2 was obtained and it is currently used to in vitro determine its activity against phytopathogens.

PL-P45.
RHIZOSPHERIC BACTERIA FOR THE MANAGEMENT OF CITRUS DISEASES
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The rhizospheric soil is the region in intimate contact with plant roots. The bacteria that inhabit the rhizosphere and have the ability to cause a positive effect on plants are called Plant Growth Promoting Rhizobacteria (PGPR). In general, biological control mechanisms and growth promotion by bacterial strains are based on microbial metabolites that adversely affect pathogens or induce systemic resistance in the plant. Our laboratory addressed a search for secondary metabolites from rhizospheric bacteria with antibiotic action on plant pathogens. Isolates were obtained from citrus rhizosphere. Among the selected microorganisms we identified strains belonging to the *Pseudomonas* and *Bacillus* bacterial genera. We studied biochemical markers associated with growth promotion i.e. siderophore production and synthesis of indole acetic acid (IAA). We also analyzed the *in-vitro* antibiotic capacity of these isolates and found that certain isolates showed activity against the plant pathogens *Xanthomonas citri* pv. *citri* and *Pseudomonas syringae*. The active compound was purified and identified as Pyochelin. We demonstrated that this siderophore has both in vitro and *in vivo*activity against *Xanthomonas spp*. Since *Xanthomonas citri* pv. *citri* is the causative agent of citrus canker, these results indicate a promising role of Pyochelin as a biocontrol agent against this disease

PL-P46.
REDOX AGENTS MODULATE CLASS I TCP TRANSCRIPTION FACTOR ACTION IN PLANTS
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TCP transcription factors are regulators of plant cell growth and proliferation that contain the TCP domain, a basic helix-loop-helix structure required for DNA binding and dimerization. These proteins can be grouped in two major classes, I and II. In this work, we identified a highly conserved Cys residue in helix I of class I TCP proteins and we show that several redox agents reversibly modulate the capacity of Cys20-containing class I proteins to interact with DNA. Mutation of Cys20 to Ser in the class I protein TCP15 abolished its redox sensitivity. Under oxidizing conditions, covalently linked dimers were formed, suggesting that inactivation is associated with the formation of intermolecular disulfide bonds. Inhibition of class I TCP protein activity was also observed *in vivo*, in yeast cells expressing TCP proteins and in plants after treatment with redox agents. Modeling studies suggest that Cys20 is probably located at the dimer interface near the DNA binding surface and explains the sensitivity of DNA binding to oxidation of Cys20. The redox properties of Cys20 and the observed effect of cellular redox agents both *in vitro* and *in vivo* suggest that class I TCP protein action is under redox control in plants.

PL-P47.
ANALYSIS OF DNA BINDING SPECIFICITIES OF PLANT CLASS I TCP PROTEINS
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TCP genes encode plant-specific transcription factors with a noncanonical bHLH domain (the TCP domain) that allows DNA binding and protein–protein interactions. Based on sequence homology, TCP proteins can be divided in two classes, I and II. SELEX experiments using rice proteins suggested that proteins from each class have distinct DNA binding specificities (GTGGGNCC versus GTGGNCCC). To gain insight into the DNA binding properties of class I TCP transcription factors, we performed SELEX experiments with different Arabidopsis class I proteins. We have observed that TCP6, TCP11, TCP15, TCP16 and TCP20 have different DNA binding preferences. In the case of TCP11, this is correlated with the presence of Thr at position 15 of the TCP domain, while Asp11 is responsible for the different DNA binding properties of TCP16. In addition, heterodimers between TCP11 and TCP15 have different binding properties than the corresponding homodimers. Interestingly, the analysis of site-specific mutants and chimeras between TCP6 and TCP20 indicated that regions located outside the basic region are responsible for their different DNA binding properties. Altogether, our results suggest that class I TCP proteins display more variability in DNA binding preferences than previously thought.

PL-P48.
A PROTEIN REQUIRED FOR HEME A SYNTHESIS DURING COX ASSEMBLY IS INVOLVED IN PLANT EMBRYOGENESIS AND SENESCENCE
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Cytochrome c oxidase (CcO) biogenesis requires more than 20 accessory proteins implicated in copper and heme a insertion, among other processes. COX10, a farnesyltransferase that catalyzes the conversion of heme b to heme o, is the limiting factor in heme a biosynthesis and is essential for its insertion in the COX1 subunit in yeast. By complementation analysis of a yeast cox10 mutant, we obtained information about the functionality of the putative Arabidopsis protein. We observed that AtCOX10 is able to restore growth on non-fermentable carbon sources when expressed in a yeast cox10 null mutant. We have also studied Arabidopsis mutant plants with a T-DNA insertion in the coding region of the AtCOX10 gene. Segregation analysis of heterozygote mutants demonstrated that it was not possible to obtain AtCOX10 knockout plants. Siliques of these plants contained 25% abnormal seeds with embryos arrested at early stages of development, suggesting that COX10 function is essential during plant embryogenesis. According to this, COX10 promoter-GUS reporter expression was observed in embryos of the same age, and normal embryogenesis was restored in Arabidopsis homozygous mutants complemented with AtCOX10 expressed under its own promoter or the constitutive 35SCaMV promoter. In addition, heterozygous mutants reached senescence before wild-type plants, suggesting a function of COX10 in this process

PL-P49.

MICRORNA390 REGULATES LATERAL ROOT GROWTH AND NODULE FORMATION IN *Medicago truncatula*

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MicroRNAs have emerged as major regulators of gene expression during development or in response to environmental stimuli. Plant roots adapt to the local environment to maximize water and nutrient acquisition by adjusting their developmental programs. Under low nitrogen availability, roots of legumes establish a symbiotic association with soil bacteria called rhizobia, which results in the formation of a new root organ specialized in nitrogen fixation, the nodule. We have identified several microRNAs that change their abundance in roots of the model legume *Medicago truncatula* at early stages of its interaction with *Sinorhizobium meliloti*. One of these miRNAs, miR390, dramatically decreased (~80%) in response to *S. meliloti*. miR390 targets the non-coding RNA TAS3 and triggers the production of tasiARFs. In turn, tasiARFs control the stability or translatability of ARF2, 3 and 4 transcripts. Consistent with the decrease on miR390 abundance, inoculation with S. meliloti led to an increase on the abundance of TAS3 precursor, a decrease in tasiARFs production and higher accumulation of ARF transcripts. Overexpression of the miR390 resulted in an increase of lateral root length, and a reduction in the number of symbiotic nodules. These results suggest that miR390 might be part of a pathway that negatively controls nodule organogenesis, but promotes lateral root growth in *M. Truncatula*.

SB-P01.
A HIGH-THROUGHPUT SCREENING FOR STRUCTURAL PROTEOMICS OF PSYCHROTOLERANT BACTERIA

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The genome of the bacterium *Biozionia argentinensis* sp. Nov. (BA), isolated in the National Antarctic Territory, was recently deciphered and is a starting point for the discovery of new proteins with biological activity at low temperatures. Structural genomics of BA will yield invaluable information about protein folding and function as well as the structural determinants responsible for the cold adaptation mechanisms. The present work describes the first high-throughput strategy for the screening of targets for structural proteomics of psychrotolerant bacteria. Using bioinformatic analysis we selected 50 genes coding for proteins of unknown function. After an initial solubility screening, 22 targets were chosen and characterized by Nuclear Magnetic Resonance (NMR) to select those proteins with suitable characteristics for three-dimensional structure determination. NMR spectroscopy and X-ray crystallography were used in parallel to increase the number of resolved structures. Here we show our preliminary experimental results.

SB-P02.
BIOCHEMICAL AND STRUCTURAL STUDIES ON HUMAN RILP-LIKE PROTEIN 1. ITS IDENTIFICATION AS HUMAN GOSPEL

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multi-functional protein, which is also involved in cell death, frequently associated with oxidative and nitrosative stress. Reversible S-nitrosylation of GAPDH facilitates its binding to the E3-ubiquitin-ligase Siah1, which possesses a nuclear localization signal that promotes the translocation of the complex to the nucleus resulting in a cytotoxic effect. In rats, it has recently been described an interactor of GAPDH which interferes with the binding between GAPDH and Siah1, preventing the apoptotic role of these proteins in the nucleus. According to this function, the authors have designated the protein GOSPEL (GAPDH's Competitor Of Siah1 Protein Enhances Life). S-nitrosylation of GOSPEL enhances GAPDH–GOSPEL binding and the neuroprotective actions of the protein.
In a database search for GOSPEL homologues in humans, we identified a protein named RLP1 (RILP-like protein 1), which is 93% identical to GOSPEL. To analyze if it shares GOSPEL properties, we have prepared the recombinant human protein and studied its capacity to be S-nitrosylated and to bind to S-nitrosylated GAPDH. We have also made structural studies of the protein, including the effect of oxidative stress on its oligomerization state. Our results would suggest that the GOSPEL-mediated neuroprotective mechanism is conserved between rodents and humans.

SB-P03.
BACKBONE DYNAMICS OF HUMAN LIVER FATTY ACID BINDING PROTEIN IN COMPLEX WITH OLEATE AND GLYCOCHOLATE

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Human liver fatty acid binding protein (HLFABP) belongs to the fatty acid binding protein family, a class of small cytosolic proteins able to translocate various lipidic molecules across the cell. It has been proposed that FABPs participate in nuclear signaling and in the regulation of normal lipid homeostasis and have recently been indicated as drug targets against the development of lipid-related disorders. The crystal structure of rat LFABP reveals a significantly larger binding cavity volume (440 $Å^3$) compared to those of other intracellular lipid binding proteins (210-330 $Å^3$), that allows it to bind two molecules of fatty acids.
The aim of this project is to determine the interaction between HLFABP and other molecules of biological relevance. To better understand the binding capacity of this protein Nuclear Magnetic Resonance (NMR) spectroscopy has been employed to study the backbone dynamics of the protein in its apo form, in complex with oleate (OA) and glycocholic acid (GCA). Our results suggest that high frequency motions (10^8-10^{12} s^{-1}) are only little affected by the addition of the two ligands. On the other side, the addition of GCA strongly affects frequency motions on a slower time scale (10^3-10^6 s^{-1}), while the addition of OA does not affect significantly these motions.

SB-P04.
A NOVEL FOOD-GRADE PARTICLE DELIVERY SYSTEM INDUCES HUMORAL RESPONSES TO CANCER-ASSOCIATED ANTIGENS

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Malignant transformation of cells is accompanied by alterations in post-translational modifications of proteins. Glycosylation pattern of mucins such as MUC1 often changes during cancer development resulting in the expression of tumour-associated carbohydrate antigens. Truncated mucin-type O-glycans are well known pan-carcinoma antigens over-expressed in several epithelial cancers. In the development of vaccines for cancer, a major obstacle is the low immunogenicity of the short MUC1 peptide. Strategies to overcome this obstacle are focused on the search of suitable adjuvants able to augment the anti-glycopeptide response. We investigated the capacity of a novel immunostimulatory particle delivery system of safe and food-grade origin, to enhance immune responses against tumour antigens. Previously, this particle system has been shown to enhance immunity to protein antigens when given mucosally to mice. Particles loaded in a non-chemical way with enzymatically glycosylated MUC1 proteins, were tested as vaccine candidates in C57bl6 mice. The formulation elicited high-level antibody responses without any additional adjuvant added and the antibodies induced were able to recognize human breast tumour cells.
These results indicate that a non-living particle system displaying tumour antigens is a proficient stimulator of immune cells and hold promise for active anticancer immunotherapy

SB-P05.
POLYPEPTIDE GALNAC-T2 INTERACTS WITH RNA POLYMERASE II AND ENHANCES TRANSCRIPTIONAL ACTIVITY

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The RNA polymerase II (Pol II) transcription machinery is responsible for the transcription of thousands of protein-encoding genes in eukaryotic cells. The structure and regulation of the Pol II machinery are critical for transcription, and participation of general transcription factors is required. In view of the binding ability of the lectin domain of polypeptide GalNAc-transferases (ppGalNAc-Ts), we studied the interaction of ppGalNAc-T2 with Pol II, the effect of acetylation of ppGalNAc-T2 on such interaction, and the role of the lectin domain of ppGalNAc-T2 in transcriptional activity. Non-acetylated ppGalNAc-T2 bound to the C-terminal domain (CTD) and glycosylated CTD of Pol II. ppGalNAc-T2 interaction with Pol II is abolished by acetylation of ppGalNAc-T2. The K521Q mutation, which mimicked acetylation of the lectin domain, had a similar effect on Pol II binding. *In vivo* assays also revealed the interaction of ppGalNAc-T2 with Pol II. Reporter gene assays reported a correlation between high ppGalNAc-T2 expression levels and enhanced transcriptional activity while K521Q mutation eliminated the transcriptional activation. These findings clearly demonstrate the important role of the lectin domain of ppGalNAc-T2 in the regulation of transcriptional activity. ppGalNAc-Ts in general may play key roles in transcriptional activity as activators that can be modulated by acetylation

SB-P06.
SETTING UP A PURIFICATION PROTOCOL FOR ACTIVE VARIANTS OF DESK, THE *Bacillus subtilis* THERMOSENSOR

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Histidine kinases (HKs) play a major role in signal transduction in prokaryotes for cellular adaptation to environmental conditions and stresses. In *Bacillus subtilis*, the HK DesK constitutes, together with DesR and Δ5_Des, the Des pathway which is responsible for cell adaptation to cold shock. The sensor region of DesK is confined to the five transmembrane segments (TMS) and is essential for sensing and transducing the cold signal to the cytoplasmic catalytic domain through still unknown conformational rearrangements.
Site-directed spin labeling (SDSL) and Electronic Paramagnetic Resonance (EPR) have proven to be useful for elucidating structures of a great variety of integral membrane proteins. These spectrometric techniques require proteins, containing a single cysteine residue, to be in a solution free of contaminating proteins implying that target proteins should be purified to homogeneity. To achieve this key issue we tested different expression systems, culture media, protease cleavage and chromatographic techniques. The molecular tools and purification steps applied in this work led us to develop a suitable protocol to purify the active variants of DesK to homogeneity. The purified variants of the thermosensor will make feasible to determine the dynamic of the lipid-induced TM helix rearrangements of DesK by means of spin-labeling EPR spectroscopy studies.

SB-P07.
LOW RESOLUTION STRUCTURE OF THE DIMERIZATION DOMAIN OF BCY1, THE REGULATORY SUBUNIT OF YEAST PKA

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The regulatory (R) subunit of protein kinase A (PKA) from mammals is dimeric in solution. The N termini from both protomers constitute the docking and dimerization (DD) domain, responsible for the quaternary structure and for the subcellular localization of the molecule. This domain consists of an X type four helix bundle fold. No structural study of this domain from other organisms is available. Here we present the first structural characterization of the N terminus of Bcy1, the R subunit of PKA from Saccharomyces cerevisiae. Using multiple sequence alignments, secondary structure prediction, and chemical crosslinking, we have shown that Bcy1 is dimeric in solution and have mapped the region of the molecule responsible for dimerization. The first 50 aminoacids of Bcy1 were cloned and overexpressed in *E. coli*. This purified fragment was shown to be dimeric both by chemical crosslinking and gel filtration, providing evidence that this is the dimerization domain of Bcy1. Circular dichroism shows that it is highly helical, thus resembling DD from mammals. Its melting temperature, followed by ellipticity at 221 nm, is concentration dependent, supporting its oligomeric nature. Denaturation is highly reversible, suggesting a great stability. SAXS experiments were also performed on this domain. These results suggest that the structure of this domain is well conserved through evolution.

SB-P08.
STUDY OF AUTOANTIBODIES AGAINST GM1 IN A RABBIT MODEL OF NEUROPATHY

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Guillain Barré Syndrome (GBS) comprises a heterogeneous group of acute motor neuropathies. Numerous publications relate the presence of anti-GM1 antibodies with the development of the disease. However little is known about the mechanisms by which these antibodies are originated. We induced an experimental neuropathy (resembling GBS) by sensitization of rabbits with bovine brain gangliosides (BBG) and keyhole limpet hemocyanin (KLH). Three groups were prepared: Group 1 immunized with BBG and KLH; Group 2 only with KLH and Group 3 only with BBG. Rabbit serum samples were obtained periodically and screened for glycolipids immunoreactivity by TLC-immunostaining. Titles were measured using ELISA (against KLH, KLH chemically oxided and GM1). Preimmune sera present low levels of anti-GM1 IgM antibodies. Rabbits immunized with BBG (Group 3) displayed small increases of those antibodies (IgM isotype). In contrast, rabbits in Group 1 showed clinical signs and their serum samples displayed IgG antibodies against GM1, indicating a critical role for KLH for antibody induction. In Group 2, we observed that most of immune response against KLH was addressed to the glycan residues. In addition low titles of anti-GM1 antibodies of IgG isotype were also observed. In conclusion, the collaboration of KLH and gangliosides is required to induce IgG-antibodies and trigger the disease.

SB-P09.

NUCLEOSIDE DIPHOSPHATE KINASE OLIGOMERIZATION: CRYSTALLOGRAPHIC AND BIOLOGICAL ANALYSES

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Nucleoside diphosphate kinases (NDPK) are enzymes involved in cell nucleotide homeostasis by the interconversion of nucleoside di- and tri-phosphates. TcNDPK1 is the canonical isoform of *Trypanosoma cruzi*, the causative agent of Chagas' Disease, and like eukaryotic NDPKs forms active hexamers. In this work we study TcNDPK1 oligomerization through molecular biology and X-ray crystallography techniques. The three dimensional structure at 3Å resolution showed that the 24 hexamers in the asymmetric unit are arranged into a helix-like oligomeric formation. The oligomerization observed in the crystalline structure was also detected in the parasite by over-expressing the NDPK gene fused to GFP, in order to reduce the intermolecular distance by the formation of weak dimers. Transgenic parasites showed a granular organization localized mainly in the anterior part of the cell that could be destabilized by changes in the intracellular salt concentration. Electron microscopy analysis indicated that these granules were filled structures without membranes. This work represents the first report of a NDPK assembled into an organized arrangement with a physiological relevance.

SB-P10.

THE DOUBLE STRANDED RNA BINDING DOMAINS FROM *Arabnidosis thaliana* DCL1

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Biogenesis of small RNAs is a complex process involving ribonuclease III like enzymes of the dicer family. In *A. thaliana* the processing of miRNA is carried out exclusively by DCL1, which produces the two cuts necessary to precisely excise mature miRNA from its precursors, pri-miRNA. Structural features which allow DCL1 to process this heterogeneous group of precursors still remain to be elucidated.

In order to understand RNA recognition by DCL1 we studied both of its double-stranded RNA binding domains (dsRBDs). The construction expressing DCL1-dsRBD2 gives a well folded protein. We obtained the structure of this domain using solution NMR. DCL1-dsRBD2 shows some significant differences when compared to canonical dsRBDs: an insertion in loop beta2-beta3 that could be involved in protein-protein interaction, and a shorter helix 1 which could give rise to higher versatility in substrate specificity. For DCL1-dsRBD1 we produced four constructs spanning the annotated domain alone and including surrounding regions. The domain is intrinsically disordered in every case. We explored different conditions and found evidence that it folds in the presence of RNA. Analysis of NMR data of the free protein shows it has no tendency to acquire secondary structure. We have calculated the structure of the protein in complex with dsRNA employing CS-Rosetta.

BT-P01.
INDUCIBLE EXPRESSION OF ROTAVIRUS VP6 PROTEIN ON THE CELL SURFACE OF *Lactococcus lactis*

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Group A rotaviruses are the major etiologic agents of acute gastroenteritis worldwide in children and young animals. Among its structural proteins, VP6 is the most immunogenic and is highly conserved within this group. In this work, the nisin-controlled expression system was used to display the VP6 protein at the cell surface of *L. lactis*. This food-grade, gram-positive and nonpathogenic lactic acid bacteria is considered a promising candidate for the development of mucosal live vaccines. The *VP6* gene was RT-PCR amplified from rotavirus EC strain RNA and was fused to a cell wall anchor fragment of *Streptococcus pyogenes* M6 protein. The construct was transformed into *L.lactis* strain NZ9000. Conditions for maximal gene expression were determined through optimizing nisin concentration and time after induction. Additionally, cytoplasmic and cell-wall protein extracts were obtained and analyzed by Western blot using polyclonal anti-rotavirus antibodies. Cultures were also analyzed by flow citometry using fluorescein-labelled monoclonal anti-VP6 antibodies. Both analysis provided evidence that VP6 was displayed on the cell surface of *L. lactis*. In conclusion, this is the first report of VP6 cell wall anchoring in *L. Lactis* and represents a potencial mucosal vaccine. Its immunogenicity and capacity to elicit a protective response will be evaluated by oral or intranasal immunization of mice.

BT-P02.
REGENERATION PROTOCOL FROM ROOTS TRANSFORMED OF PETUNIA AXILLARIS (LAM.) BRITTON, STERN & POGGENB.

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In the Floriculture Institute, within a native Solanaceae breeding program, we started to work with transformation techniques to obtain more compact Petunia plants. In the international market, potted plants have more acceptance and commercial value; therefore the development of compact national varieties is relevant for local producers. In this project we used one genotype of P. axillaris, maintained in vitro. The transformation was made with Agrobacterium rhizogenes LBA15834 over leaf laminae and petioles in MS/2 medium supplemented with 30 g/l sucrose and 7.5 g/l agar. Transformation in the hairy roots obtained was confirmed, by PCR reaction with rol A, rol B, rol C, rol D and vir C primers. For the regeneration protocol we used nine different combinations of naftalen acetic acid (NAA) and 6-bencil aminopurine (BAP) hormones. The combinations were: MS1: Control, MS2: 0.5 mg/l BAP, MS3: 2 mg/l BAP, MS4: 0.5 mg/l NAA, MS5: 2 mg/l NAA, MS6: 0.5 mg/L NAA + 0.5 mg/L BAP, MS7: 0.5 mg/l NAA + 2 mg/l BAP, MS8: 2 mg/l NAA + 0.5 mg/l BAP and MS9: 2 mg/L NAA + 2 mg/l BAP. The preliminary results showed the MS4, MS6 and MS8 as the most promising combinations. The obtained calli of these combinations were transferred to MS hormone-free medium containing 30 g/l sucrose and 7.5 g/l agar, to start plantlet regeneration. At this moment, we are working in this last regeneration step.

BT-P03.
ENGINEERING PSEUDOMONAS FOR NITROGEN FIXATION

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The beneficial rhizobacterium *Pseudomonas fluorescens* Pf-5 was genetically modified for nitrogen fixation, using the genes encoding the nitrogenase complex. This recombinant strain was able to grow in L medium without ammonium sulphate and displayed high nitrogenase activity. Furthermore, inoculation of Arabidopsis thaliana, Medicago sativa and Schenodorus arundinaceus with the recombinant strain increased plant productivity more than 100% under nitrogen-deficient conditions. Finally, we discussed the production of new recombinant inoculants.

BT-P04.
SELF-ASSEMBLED LECITHIN-BILIARY ACIDS BASED MIXED MICELLES FOR PDNA AND siRNA DELIVERY

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The physiological compatibility and the solubilizing capacity that have been recently demonstrated for phosphatidylcholine-bile salts Mixed Micelles (MM), drive to consider them as promising drug delivery systems. Our aim was to study the use of MM for cellular pDNA/siRNA delivery. Designed MM were composed of soy lecithin and Sodium Cholate in different proportions (1:1, 1:2 and 1:4) diluted in water and using pH 5 and pH 7,4 buffers. The MM were characterized in terms of their capability to bind oligonucleotides at different lipid:oligonucleotide (N/P) ratios, their cytotoxicity and delivery efficiency. Binding capability was analyzed by agarose gel EMSA. Cell viability assay was used to determined cytotoxicity. Delivery efficiency was assayed by fluorescence microscopy using the pEYFPN1 vector and AlexaFluorRed®-tagged dsRNA in MCF-7 cells.
Our results indicated that the MM 1:1 (lecithin: sodium cholate) bind pDNA at N/P ratios 500 and 1000, while the MM 1:2 and 1:4 loaded siRNA at N/P ratios 500 and 1000. Viability assays revealed that the MM prepared in pH 7,4 buffer were cytotoxic, while those prepared in pH 5 MM were not cytotoxic for N/P 500 but slightly toxic for N/P 1000. Cytotoxicity of the water MM depends on the proportion lecithin: sodium cholate.
The experimental conditions for the MM to deliver the oligonucleotides into the cells were determined by fluorescence microscopy.

BT-P05.
NEW AFFINITY CHROMATOGRAPHIC MATRIX FOR GLYCOMACROPEPTIDE PURIFICATION

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Aim: The aim of this work was the development of a novel matrix for glycomacropeptide (GMP) purification. Based on the affinity of the wheat germ agglutinin (WGA) for sialic acid and having the GMP high levels of this sugar (7-8%), the developed matrices consisted of mini-spheres of chitosan cross-linked with epichlorohydrin with immobilized WGA as the affinity ligand.
Methods: Several matrices were obtained by dripping a 2% chitosan solution in acetic acid (2 or 4%) on 2M NaOH and were cross-linked with 250 mM epichlorohydrin. For WGA adsorption a wheat germ aqueous extract was used. Preliminary GMP adsorption conditions were tested at pH 4, 5, 7 and 8.5 with pure GMP solutions. Adsorption isotherms were performed at pH 7 and 8.5. Sixteen different eluents were tested.
Results: No significant binding of GMP to the matrices was evidenced at pH 4 and 5. At pH 7 and 8.5, the GMP maximum adsorption capacities were between 137.0 and 57.1 mg/g at pH 7, and between 149.4 and 79.1 mg/g at pH 8.5. The best eluent was 1M glucosamine.
Conclusions: We have synthesized chromatographic matrices based on chitosan that after adsorption and cross-linking of WGA, showed optimal adsorption and elution for pure GMP. Therefore, the developed matrices are promising for GMP purification from cheese whey, thus increasing its added value.

BT-P06.
SELECTIVE CAPTURE OF RECOMBINANT BOVINE LACTOFERRICIN BY AFFINITY ADSORPTION WITH TRIAZINIC DYES

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Antimicrobial peptides (AMPs) are the first defense line against pathogens in many organisms. Bovine lactoferricin (Lfcin B) belongs to the AMPs family and has 25 amino acids with a net charge of +8 under physiological conditions. It is present in the N-terminal region of the bovine lactoferrin. Besides its antiviral, antifungal, antiparasitic, anticancer and antibacterial activity, it has a synergistic effect with some conventional antibiotics. In this work, Lfcin B was expressed alone or as a fusion protein with a glutathione-S-transferase (Lfcin B-GST) using Baculovirus Expression System - Sf9 insect cells. When Lfcin B-GST was expressed and accumulated in cytoplasm or secreted to the culture supernatants, yields were 1.9 ± 0.4 and 1.1 ± 0.1 mg of Lfcin B/l, respectively. These values were 10 fold greater than Lfcin B expression without GST. For its recovery and purification, five triazinic dyes were screened using Surface Plasmon Resonance technology. The Yellow HE-4R and the Red HE-3B dyes showed the highest affinities for Lfcin B with Kd values of 1.1 ± 0.3 x 10-7 M and 1.6 ± 0.4 x 10-5 M, respectively. These dyes were immobilized on Sepharose-4B matrix for further purification studies. The observed levels of Lfcin B-GST expression and its specific interaction with these low cost ligands are promising for the scaling up using lepidopteran larvae for industrial purposes.

BT-P07.
CONTROLLED ADHESION AND PROLIFERATION OF SAOS-2 CELLS BY TUNING NANOPOROUS TITANIA / SILICA COATINGS

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Herein, we demonstrate that it is possible to tune adhesion and proliferation of human osteoblastic cell line (Saos-2) by tailoring the nanopore size of a film oxide coating. The designed strategy was based on the tailored preparation of mesoporous coatings by combination of sol-gel and supramolecular templating. Non mesoporous Si films and mesoporous Si with an average pore diameter of 4 and 9nm, as well as, non mesoporous Ti films and mesoporous Ti with an average pore diameter of 6 and 10 nm were prepared by evaporation induced self-assembly.
Cellular response was dependent on both physicochemical and nano-topographical stimuli. Particularly, it was shown that in the short term (i.e.: 3-6 hours), the initial events at the surface are governed by the hydrophilic/hydrophobic properties of the surface that creates an interface to which the cell responds with different degrees of adhesion. Moreover, in the long term (days), the nanotopography and chemistry of the surface, determines how cells will attach and proliferate. In this sense, it was demonstrated that it is possible to tune cell adhesion and proliferation by controlled nanoporosity introduced during film formation. It is important to emphasize the simplicity of the procedure used to prepare these nanostructured surfaces that avoids the use of chemical functionalization.

BT-P08.
ABSOLUTE COPY NUMBER ANALYSIS OF TRANSCRIPTION FACTORS USING MULTIPLEX, TARGETED PROTEOMICS

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Many biological processes are mediated by differential gene expression, which is largely controlled by transcription factors (TFs). The biochemical and regulatory properties of TFs are in part dictated by their cellular concentration. To understand TF function, we therefore need to derive absolute TF copy number data. However, due to their low cellular abundance, such data has been difficult to obtain. Here, we present the development of a sensitive selected reaction monitoring (SRM)-based mass spectrometry assay, allowing us to simultaneously determine the absolute copy number of up to 10 proteins over a wide concentration range. We apply our approach to monitor the nuclear abundance of key TFs including the adipogenic master regulators PPARγ and RXRα over the course of terminal adipogenesis. We find that the concentration of TFs differs dramatically, fluctuating from 250 to >300,000 copies per nucleus, but that their dynamic range during differentiation is limited, varying at most five-fold. We also formulate a genome-wide TF DNA binding model to explain the significant increase in PPARγ binding sites during the final differentiation stage, despite a concurrent saturation in PPARγ copy number. This model provides unique, quantitative insights into the relative contributions of binding energetics, copy number, and chromatin state in dictating TF DNA occupancy profiles

BT-P09.
CHITIN-GRAPHENE OXIDE NANOSHEET HYBRID MATERIALS: APPLICABILITY AS BIOSORBENTS

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There is great interest in developing low-cost materials with sorption properties for several targets. This interest relays on a large amount of by products and industrial waste that can be used as biosorbents of a wide variety of compounds with high affinity and capacity. The target of these materials could be undesired pollutants, such as heavy metals or organic compounds, or specific products from different industries such as proteins or hormones.
The aim of this work is to study the development of nanostructured hybrid materials for use as biosorbents. These consist of a biopolymer, chitin, together with graphene oxide nanosheets (GON) in its composition. Chitin endows the material with an inert and stable support. The surface area of GON adds adsorption capacity to chitin together with the hybrid mechanical reinforcement. The driving purpose of this work is the development of versatile answer to the pollution of natural waters and sewage effluent applicable for industrial producers. Also, the versatility of the materials was tested in tum on the adsorption of proteins as a model for industrial purification. GON-chitin mass rations from 0 to 2 were evaluated in their material mechanical properties and adsorption behavior against model organic pollutants and proteins. Ratios of 0.5 and 1 showed have better mechanical stability and are capable to adsorbe over 95% of eosine.

BT-P10.
BISPHOSPHONATES INCORPORATION INTO SILICA NANOPARTICLES AND THEIR EXPOSURE TO BONE CELL CULTURES

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Implants based on the application of polymers colonized by cells such as osteoblasts to regenerate bone tissue are being widely studied. Some of them use nanocomposites that are the combination of polymers with filler nanoparticles (NPs). Bisphosphonates such as ibandronate are drugs used to treat certain diseases involving bone resorption and could be applied locally in bone implants increasing periprosthetic bone density leading to a stronger and successful implant fixation.
The aim of this work is to synthesize different NPs containing ibandronate: solid silica NPs obtained by the Stöber method, chemically modified silica NPs with amino groups (APTES), and hollow silica nanoparticles (HSNPs) with or without $CaCO_3$. The drug content was studied by means of capillary electrophoresis and the effect over cell cultures was evaluated using bone cells. All tested NPs were approximately 350 nm. The content of drug incorporated to silica NPs was 44.7 mg/100 mg NP for HSNP, 25.5 for HSNP with $CaCO_3$ (0.05% p/p), 11.6 for APTES modified NP and 9.16 for Stöber NPs. HSNPs which incorporated higher amounts of Ibandronate were exposed to cell cultures to evaluate their intrinsic toxicity (without drug) using the MTT assay. It was observed that hollow silica NPs were toxic for cells when NPs concentrations were higher than 2.4 mg/ml but this effect was not seen for hollow silica NP with $CaCO_3$.

BT-P11.
CLONING AND OVER-EXPRESSION OF INULINASE GENE FROM *Aspergillus kawachii* IN *Pichia pastoris*

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Inulinases comprise an important group of enzymes for fructose and fructooligosaccharides production, extensively used as sweeteners and functional food additives. *Aspergillus kawachii* produces inulinases (INU), but due to its low expression levels, its cloning and over expression are required for its industrial application. *A. kawachii* INU ORF, lacking its original signal peptide, was cloned into a *Pichia pastoris* expression vector under the regulation of the α-mating factor signal sequence. Since INU primary transcript presents an intron which *P. pastoris* is not able to remove during maturation, its in vitro deletion was developed; generating the pPICZ-a-a-INUDIDSP construction. After plasmid propagation in *E. coli* DH5α, this construction was used to transform *P. pastoris* GS115 cells. The INU gene was integrated into the yeast genomic DNA, confirmed by colony PCR. All analyzed clones induced by methanol, were able to express an inulinase after 24 hours, identified by tryptic digestion followed by MALDI TOF analysis. Nevertheless, the expressed recombinant protein was no active under standard inulinase activity assay. A new cloning strategy is under development based on bioinformatics tools, towards the analysis of intron and signal peptide sequences and/or potential post translational modifications in order to consider a more appropriate expression system, such as *S. cerevisiae*.

BT-P12.
ISOLATION OF BIOSURFACTANT-PRODUCING MICROORGANISMS AT THE PETROCHEMICAL AREA OF CAMPANA

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Biosurfactants are amphipathic molecules which have the capability to reduce the medium surface tension and can be used in food, pharmaceutics and petroleum industry. Isolation and identification of biosurfactant-producing microorganisms were carried out from hydrocarbon-contaminated soil and water of a petroleum distillery. Isolation was performed by enrichment cultures in Erlenmeyer flasks with 25 ml of mineral salt medium (MSM) containing 4,5% of a mixture of three different hydrocarbons (HC) or polluted water (AgLag) as carbon and energy source. Temperature, pH and agitation conditions were maintained at 25°C, 7.00 and 110 rpm, respectively. Thirteen morphologically distinct colonies were identified and screened for biosurfactant and bioemulsifier production. Six of these colonies were able to reduce the growth media surface tension more than 30%, all of them grown with HC. A microplate assay was carried out to confirm surface tension results using sterilized distilled water as standard. In addition, a 24 hour-emulsification test (E24) was performed. Four organic phases were tested (soy oil, fuel RON=95, diesel and kerosene) in cell-free supernatants. Three samples resulted positive to this assay (E24 > 50%). It can be concluded that six biosurfactant-producing colonies were isolated with a potential application in the industrial production of biosurfactants.

BT-P13.
PEGYLATION EFFECT ON STAP3 CYTOTOXIC ACTIVITY

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PEGylation (i.e. the covalent link of PEG strands) is a technique used to improve pharmaceutical properties of bioactive proteins and peptides, mainly improving the serum half-life, and reducing the immunogenicity and the enzymatic degradation. We have previously reported the purification and characterization of potato aspartic proteases (StAPs) with antimicrobial/ antitumor activity. Selective cytotoxic activity of StAPs suggests that, these proteins could be used to develop alternative drugs to contribute to resolve the increasing resistance of pathogenic bacteria to conventional antibiotics. In this work we have analyzed the ability of PEGylation to improve StAPs antimicrobial activity. PEGylation of StAP3 was performed at pH 6 and 8, obtaining higher performance at pH 8. Results obtained show that, PEG-conjugated StAP3 exerts cytotoxic activity towards Fusarium solani. IC50 estimated to PEG-conjugated StAP3 was 9 µg/ml, whereas this value for free StAP3 was 28 µg/ml. Like free StAP3, PEG-conjugated StAP3 is able to interact and to permeabilize spores and hyphae plasma cell membranes in a dose dependent manner. The results obtained here indicate that PEGylation increases the cytotoxic activity of StAP3.

BT-P14.
***IN VIVO* ANALYSIS OF *St*AP3 CYTOTOXIC ACTIVITY**

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The increasing resistance of pathogenic bacteria to conventional antibiotics has become a serious problem in health care, which requires alternatives to be developed. One strategy is the use of antimicrobial proteins/peptides (AMPs) which act by the disruption of the microorganisms plasma cell membrane. Previously, we have reported the *in vitro* cytotoxic selective activity of potato aspartic proteases (*St*APs) on human pathogen microorganisms and cancer cells, but not on human erythrocytes and T lymphocytes. The aim of this work was to evaluate the *in vivo* cytotoxicity of *St*AP3 using the Balb/c mice model. Mice were treated with a single dose of *St*AP3 (from 1 to 10mg protein/kg) or physiological solution (control). Results obtained show that no changes in the body weight, behavior, morphology and histology of the organs and tissues of treated mice compared with control mice, was observed at all times analyzed (1, 2, 8, 14 and 24h and 1, 2, 3, 4, 5, 6, 7, 8 and 9 weeks). Additionally, not significant changes in the serum levels of urea, creatinine and transaminases, were determined in treated mice, at all times and concentration assayed. Pharmacokinetic studies indicated that *St*AP3 is bioavailable in the serum from 2h to 14 days. These results allow us to continue with the studies *in vivo* with *St*APs as potential new drugs for infectious diseases and cancer treatment.

BT-P15.
PURIFICATION OF *Solanum elaeagnifolium* ASPARTIC PROTEASES (SEAPS) WITH ANTIMICROBIAL ACTIVITY

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Solanum elaeagnifolium (trompillo or silverleaf nightshade) is an endemic plant from the northeast of Mexico and southwest of United States. This plant in some places of Mexico has been used for decades in the manufacture of artisanal filata-type asadero cheese. The milk-clotting activity of *S. elaeagnifolium* has been attributed to aspartic proteases. The aim of this work was to purify aspartic proteases from leaves of *S. elaeagnifolium*. Purification was performed from leaves of *S. eleagnifolium* by ammonium sulfate precipitation; ion exchange chromatography and Pepstatin A affinity chromatography. SDS-PAGE analysis of proteins eluted from the affinity column revealed three bands (*Se*APs) with molecular weights of 86, 45 and 27 kDa approximately. In order to identify these proteins, bands were recovered, digested with trypsin and submitted to a MALDI-TOF spectrometric analysis. Proteins corresponding to the three bands isolated, matched with the sequences of plant aspartic proteases, specifically with potato aspartic proteases (*St*APs). Additionally, we determined that, like *St*APs, *Se*APs were able to interact and permeabilize microorganism plasma cell membranes, and exert cytotoxic activity in a dose-dependent manner. These results suggest the presence of saposin-like domain into the sequences of mature *Se*APs and therefore, new biotechnological applications for *Se*APs.

BT-P16.
SURFACE MOLECULES OF *Giardia lamblia* PROTECTS BIOACTIVE PEPTIDES FOR ORAL ADMINISTRATION

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Giardia is an intestinal pathogen that undergoes antigenic variation, a mechanism by which trophozoites continuously switch its major surface molecules. These surface antigens belong to a family of cysteine-rich Variant-specific Surface Proteins (VSPs), which are integral membrane proteins that cover the entire surface of trophozoites for protection within the upper small intestine. VSPs present unique characteristics that make them ideal candidates to transport drugs through the gastro intestinal track: they are resistant to acidic pH and proteolytic degradation and adhere to the intestinal mucosa. As a proof-of-principle, we initially used insulin as a prototype drug to be delivered by the oral route. We tested if the combination of insulin with a VSP can protect this molecule from degradation and promote its systemic biological action. We also tested bioactive peptides in their capacity to remain active after being mixed with a VSP and treated with different proteolytic enzymes or confronted to acidic pH both *in vitro* and *in vivo*. For the generation of the extracellular portion of the VSPs, we selected the Baculovirus Expression System using a proprietary purification approach. Our results showed that Giardia VSPs protect bioactive peptides when administered by the oral route. The structural characteristics of the VSPs that confer these properties are under analysis.

BT-P17.
STRUCTURAL STUDIES OF *Azotobacter* SP FA-8 PROTEIN PHAP

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Phasins are proteins that are normally associated with granules of poly(3-hydroxybutyrate) (PHB), a biodegradable polymer accumulated by many bacteria. PhaP from *Azotobacter* sp. FA-8 enhances growth and polymer production in PHB producing *E. coli*, and it also showed protective effect in a non PHB producing *E. coli* strain, resulting in increased growth and higher resistance to stress conditions. In order to further characterize this phasin and shed light on its possible mechanism of action, a structural analysis was performed. The gene *phaP* was cloned in an expression vector that introduces a six histidine tag and was purified by affinity chromatography using a nickel column. SDS-PAGE in non reductive and reductive conditions revealed that the protein forms dimers. A MALDI TOF TOF analysis permitted to verify that the bands observed correspond to PhaP in its dimeric and monomeric form and it also showed that PhaP is a homodimer, formed by monomers linked by disulphide bonds that involve the only cisteine present in the protein sequence. In order to study the secondary structure of the protein circular dichroism spectrum was performed. The analysis of the spectra revealed that PhaP is composed mainly by a hélix. These results will help us elaborate hypothesis about possible mechanisms of action of PhaP and its interaction with other proteins that can be later tested experimentally.

BT-P18.
ANTRAQUINONE PRODUCTION IN HAIRY ROOTS OF *Rubia tinctorum*

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Anthraquinones (AQs) are anthracene derivatives that are synthesized by several plant families, including Rubiaceae. These secondary metabolites exhibit interesting *in vitro* properties, such as antioxidant, anticancer, antimicrobial activities. Production of these compounds by *in vitro* culture is an attractive alternative to extraction from their natural source. In this work, we established a hairy root culture of *Rubia tinctorum* and we evaluated their performance and AQ production in 250 ml erlenmeyer flasks in two different culture media: Gamborg B5 (half-saline strength; $B5_{1/2}$) and Lloyd and McCown's Woody Plant Media (WPM). The final biomass achieved was 150 g/L (fresh weight; FW) after 42 days of culture in WPM (μ= 0.11 d^{-1}), whereas biomass accumulation in $B5_{1/2}$ was significantly slower (100 g/L; μ= 0.10 d^{-1}). Intracellular AQ accumulation was enhanced in $B5_{1/2}$ (11.88 µmol/g FW after 35 days) when compared to WPM (6.44 µmol/g FW). Similar results were observed for AQ liberation to the culture medium (84.6 and 7.5 µmol/L, respectively, after 35 days). The differences found in AQ and biomass yields can be attributed to differences in the composition of the culture media (S, P and N), not only as limiting substrates but also because they might be involved in the regulation of AQ synthesis. These results are promising for further improving AQ yields and for scaling up the process.

BT-P19.
INTEGRATED PLATFORM FOR THE PRODUCTION AND PURIFICATION OF RECOMBINANT PROTEINS IN PLANT SYSTEMS

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Plant systems have emerged as safe, effective and inexpensive platforms for the production of recombinant proteins with biotechnological application. Low protein accumulation levels and the lack of efficient purification methods are major problems to be solved. Hydrophobins (HFB) are fungal proteins that alter the hydrophobicity of their fusions partners enabling efficient purification by surfactant-based aqueous two-phase system. Furthermore, they enhanced the accumulation of their fusion proteins. Dengue virus (DV) envelope protein (E) is the major structural component and the most immunogenic protein of the virus and it is involved in the induction of a protective immunity. The aim of this work is to produce E protein as a fusion with HFB in plant systems to be used for the potential production of a vaccine and for the generation of a diagnostic reagent for rapid detection of DV. A gene encoding DV serotype 2 E protein fused with HFB was successfully cloned in a binary vector and expressed in Nicotiana Benthamiana plants and cell suspensions via Agrobacterium tumefaciens-mediated transformation. GFP fused with HFB was used as a control. The results obtained indicate that fusion proteins are able to be expressed transiently in plants and stably in cell suspensions cultures of N. benthamiana. Currently, we are evaluating the integrity and the expression levels of these proteins.

BT-P20.
STABILIZATION OF MICROORGANISMS IN NANOCOMPOSITE HYDROGELS

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Immobilization of microorganisms is a current topic in biotechnology and a promising production method for bioprocess. Entrapment techniques are the most widely used for whole cell immobilization despite of their high operational stability, easy upstream separation and feasibility of scaling up the bioprocess. However, in most cases the beads are mechanically unstable. The incorporation of nanocomposites in the matrix promotes the rigidity and stability of the biocatalysts.
In this work, we used natural and synthetic polymers (agar, agarose, alginate and polyacrilamide) to immobilize by entrapment microorganisms. Calcium alginate was the best support for microorganism stabilization and different immobilization parameters as support concentration, bead size, crosslinking solution and exposure time were optimized. The optimal immobilization conditions were 4% (w/v) alginate, 3 mm bead size, 0,2 M SrCl2 as a crosslinking solution and 2 hs of exposure time. This biocatalyst was able to catalyze 70% of ribose 1-phospate in 6 h of reaction using thymidine as started subtract. Finally, this biocatalyst showed high operational and storage stability and retained full activity for more than 120 hs of use. Additionally, we evaluated the effect of bentonite, a natural nanocomposite, to improve bioprocess parameters as swelling, cell retention, compression strength and stability.

BT-P21.
LOTUS SPP.: A PROMISING TOOL FOR RHIZOREMEDIATION IN THE HUMID PAMPA

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The Humid Pampa region is the main agricultural area in Argentina. Intensive agricultural activities practiced on these soils with agrochemical applications, affect soil microbiology and generate surface and groundwater pollution. Herbicides are one of the most used pesticides; among which glyphosate stands out. Rhizoremediation is a potential strategy to minimize its environmental impact. The aim of this work was to assess glyphosate tolerance of two Lotus genus accessions, for selecting them as candidates for rhizoremediation strategies. Tolerance assays were performed in maximum bioavailability Murashige-Skoog (MS) semisolid agar media at 5 glyphosate concentrations, corresponding to a standard agronomic dose (1Kg ha^{-1} of Ultramax Glyphosate) reaching different soil depths. The seeds were scarified, surface sterilized, and then sowed in groups of ten in MS media. The germination rate, seedlings survival, growth and general status were registered. All levels showed the same germination rate at the end of the assay, and plantlet growth was observed up to a level of 5 $mg.Kg^{-1}$. Furthermore, there was no significant difference in seedling growth between control and levels of 0,5 and 1,0 $mg.Kg^{-1}$. To our knowledge, there is no previous report of glyphosate tolerance in Lotus spp. and this is a first approach towards utilizing it in rhizoremediation strategies.

NS-P01.
SCN ASTROCYTES MODULATE THE CIRCADIAN CLOCK IN RESPONSE TO TNF-α

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The immune and the circadian systems interact in a bidirectional fashion. The master circadian oscillator located in the suprachiasmatic nuclei of the hypothalamus (SCN), responds to both peripheral and local immune stimuli. Astrocytes exert immune functions in the central nervous system, and there is a growing evidence demonstrating multiple roles of these cells in the regulation of circadian rhythms. The aim of this work was to assess the response of SCN astrocytes to the proinflammatory cytokine TNF-α. SCN astrocytes cultures from PER2::luc knock-in mice treated with TNF-α at the trough of the PER2 expression rhythm showed phase delays, with no alteration in the rhythm amplitude. Conversely, cultures treated two hours after the PER2 expression peak showed a small, albeit significant, increase in the amplitude of the rhythm, with no effect on its phase. We also analyzed if SCN astroglia could secrete factors that alter circadian physiology in response to TNF-α. Conditioned media from TNF-α challenged SCN astrocytes induced both an increase in Per1 expression in NIH-3T3 cells, as well as phase delays in behavioral circadian rhythms when applied intracerebroventricularly in mice. In conclusion, SCN astrocytes respond to TNF-α both, showing an alteration on its own molecular clock, and modulating circadian physiology both, *in vitro* and *in vivo*.

NS-P02.
CIRCADIAN RHYTHMS IN *Caenorhabditis elegans*, PUTTING THE PUZZLE TOGETHER

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Circadian rhythms are ubiquitously found in nature. They are driven by endogenous biological clocks and are synchronized to environmental cues. *C. elegans* is a model organism widely used in diverse areas of research but still not well characterized in chronobiological studies. The use of *Caenorhabditis elegans* provides fundamental information about the basis of circadian rhythmicity in eukaryotes, due to its ease of use and manipulations, as well as of the availability of genetic data and mutant strains. We have found and described several circadian outputs such as locomotor activity, oxygen consumption, feeding rate and melatonin synthesis. Because it is a top soil dwelling nematode, it is subjected to daily changes in environmental stressors. In this sense, we studied stress tolerance to abiotic and biotic stressors. We found rhythmic stress tolerance patterns for oxidative and osmotic stress, peaking at daytime and nightime, respectively. When exposed to *P. fluorescens* or *P. aeruginosa* (two soil occuring bacteria that kill *C. elegans*), we found lower tolerance during nightime. In summary, our results show that the circadian system regulates changes in behavior, metabolism, abiotic stress tolerance and host-pathogen interactions. However, how this clock orchestrates all this changes and how the outputs feedback into the system still remains to be elucidated.

NS-P03.
INVOLVEMENT OF C-SRC KINASE IN NEURONAL DIFFERENTIATION MEDIATED BY ANGIOTENSIN II AT_2 RECEPTORS

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Angiotensin II (Ang II), the active peptide of the renin–angiotensin system, elicits a variety of biological effects through specific receptors, AT_1 and AT_2. Although AT_2 receptors may be involved in neuronal differentiation, the mechanism is not well defined. Thus, we decided to examine the role of Ang II and the AT_2 specific receptor agonist, CGP42112, in differentiation of SHsy5y neuroblastoma cells. The expression of AT_1 and AT_2 receptors was determined by western blot (WB). CGP42112 induced differentiation and increased the expression of ßIII tubulin, a neuritogenesis marker as assessed by optic microscopy and WB respectively. Since nerve growth factor (NGF) induces neuronal differentiation through ERK1/2 activation, we studied the involvement of this pathway in the process. While treatment for 3 days with Ang II or CGP42112 caused a decrease in ERK1/2 phosphorylation, pre-incubation with UO126, MEK1/2 inhibitor, did not affect differentiation. Previously, we have shown that activation of AT_2 receptor induced tyrosine kinase c-Src activation/phosphorylation. Therefore, we examined its contribution to neurite outgrowth process. Interestingly, the neuritogenesis induced by CGP42112 was abolished by PP2, a c-Src inhibitor. Altogether, these results suggest that activation of c-Src, but not ERK1/2, is important for neuronal differentiation mediated by AT_2 receptors in SHsy5y cells.

NS-P04.
POTENTIAL ROLE OF BUPRENORPHINE IN MULTIPLE SCLEROSIS REMYELINATION THERAPY

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Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the Central Nervous System (CNS). Remyelination is performed by oligodendrocyte progenitors to prevent axonal loss. In MS, the insufficiency of remyelination leads to the irreversible degeneration of axons which correlates with clinical decline. Therefore, the development of a regenerative strategy to promote remyelination is crucial in MS management. Though the immunological effects of FTY720 (fingolimod), the first oral therapy approved for the treatment of MS, are well established, there is controversy about its contribution on myelin repair. In that regard, while FTY720 inhibits the infiltration of lymphocytes and prevents MS progression, our previous results established that also arrests the differentiation of oligodendrocytes, inhibiting the potential remyelination. On the other hand, we have also revealed that opioid signaling regulates myelination. Here, we show that buprenorphine, an opioid analogue used for pain and addiction treatment, increases the expression of myelin basic proteins, accelerates oligodendrocyte development *in vivo* and induces oligodendrocyte differentiation *in vitro*. These evidences suggest that buprenorphine could be considered as a complementary therapy of FTY720 to promote remyelination and to prevent neuronal irreversible damage in patients suffering from MS

NS-P05.
C-FOS REGULATES AXON BRANCHING IN NEURONS
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It was previously shown in our laboratory that in addition to its nuclear activity, c-Fos associates to membranes of the endoplasmic reticulum (ER) and activates phospholipid synthesis. So, it might be associated with the molecular mechanisms that allow the higher rate of membrane genesis required for neuronal growth and differentiation. Primary cultures of hippocampal neurons were infected using a lentiviral vector that expresses a specific sequence (shRNA) against c-Fos to block its expression and no development of axonal processes was observed after 36 hours of culture. On the other hand, by immunofluoressence, we observed the presence of c-Fos co-localizing with ER markers in the neuronal soma and mainly forming structures in the branching sites of the neuronal processes. Finally, FRET technique has given positive results for the interaction between c-Fos and CTPß2. This is an integral enzyme of the membranes of the ER in neuronal cultures that plays an important role during the formation of axon branches. We also observed co-immunoprecipitation of both proteins. These results support our hypothesis of c-Fos mediated activation of phospholipids synthesis during neuronal differentiation.

NS-P06.
ROLE OF PROTEIN KINASE D1 IN GROUP I METABOTROPIC GLUTAMATE RECEPTORS TRAFFICKING
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Most of neuronal dendritic and axonal membrane protein sorting, a key process for establishment and maintenance of neuron polarity, occurs at the Trans-Golgi Network (TGN).
Metabotropic glutamate receptors belong to G protein-coupled receptor (GPCR) family of seven-transmembrane domain; they contribute to synaptic plasticity, hence in learning and memory processes. Their transport towards their final localization, dendrites or axons, is still a complex and not well-characterized process. Protein Kinase D1 (PKD1) is a major component in membrane trafficking events; in neurons, PKD1 participates in dendritic membrane proteins sorting, such as transferrin receptor (TfR). These previous observations lead us to hypothesize that PKD1 regulates Group I metabotropic glutamate receptors (mGluR) sorting, since they are dendritic membrane proteins. Using 14 DIV hippocampal neurons, we evaluate the effect in the mGluR1 and mGluR5 intracellular trafficking from the TGN to proper dendritic domain by using: i) depletion of the PKD1 by short harpin RNA, ii) a dominant negative mutant (PKD1 kinase dead), and iii) chemical inhibitors.
Taking together, the results showed that the two receptors are missorted and inserted in both dendritic and axonal membranes. Moreover, we proved that the signaling pathway whereby PKD1 is activated in neurons was through Phospholipase C (PLC) and Protein kinase C (PKC)

NS-P07.
PROTEIN KINASE D1-DEPENDENT TRAFFICKING AND SORTING OF NEUROTROPHIN RECEPTOR TrkB
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After determining the participation of several proteins, such as PKD1, in an intracellular trafficking regulatory pathway, we have decided to study their role in neuronal polarity establishment, specifically through the analysis of Tropomyosin-related kinase receptors (TrkA and TrkB) trafficking and sorting. We have observed that in the absence of its specific ligand Brain Derived Neurotrophic Factor (BDNF), TrkB is actively localized in the somatodendritic and axonal compartment of cultured rat hippocampal neurons. In the absence of an active PKD1, a remarkable change in the TrkB distribution was detected, with preferential localization in the primary dendrite and soma. In addition, there was a significant reduction in the length of dendrite branches, but no effect in their number was noticed. When non permeabilized neurons were analyzed, a reduction in the membrane expression of this receptor was observed when a small interference RNA for this kinase was expressed. In neurons transfected with TrkB and then treated with specific PKC and PLC inhibitors, both proteins involved in the PKD1-regulated pathway, we have also observed a remarkable reduction in dendritic length.
These results confirm that PKD1 play a key role in the regulation of vesicles carrying TrkB, and that the presence of this receptor at the neuronal surface would be essential for dendrite length development.

NS-P08.
A NEURAL NETWORK MODEL FOR THE SUPRACHIASMATIC NUCLEUS IN MAMALS
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Living organisms exhibit rhythmic variations in diverse biological functions. Among these there are the so-called “circadian rhythms”: variations with a period close to 24 hours. Such rhythms, which are ‘entrainable’ to external changes of similar periodicity (e.g., light/dark cycles), are also endogenous and can persist even in constant conditions. In the case of mammals, the circadian rhythms are coordinated by the suprachiasmatic nucleus (SCN), a brain structure composed of approximately 20000 neurons. Each of these neurons contain within themselves a (genetic) clock machinery and it is their synchronized expression that allows the SCN to behave as a central robust clock. This structure is often studied experimentally *in vitro*, by cutting slices of tissue and using bioluminescence techniques in order to measure genetic expression. However, discerning the structure of this neural network turns out to be unattainable experimentally. Employing a clock-neuron model (Bernard *et al*., 2007) we simulate the dynamics of a two-dimensional network with different architectures and compare some emerging properties. In particular, the embedded scale free network (Rozenfeld *et al*., 2008) is introduced, which presents simultaneously high degree of synchronization and the formation of spatio-temporal patterns.

NS-P09.
AN *IN VITRO* MODEL TO STUDY INHIBITION OF AXON REGENERATION MEDIATED BY ANTI-GLYCAN ANTIBODIES

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Axon regeneration is a response of injured nerve cells that is critical for the restoration of structure and function after PNS or CNS injuries; this response is key to recover from neurological disorders like acute immune neuropathy called Guillain Barré Syndrome (GBS). Some studies associate the presence of anti- ganglioside antibodies (anti-Gg abs) with poor recovery in GBS. Patients with incomplete recovery have impaired nerve repair. It was recently demonstrated in a passive transfer animal model that anti-Gg abs can halt axon regeneration. Defining the signaling pathways that prevent regeneration of injured axons can provide key insights to allow development of therapeutic approaches to enhance axon growth. For this, we developed an in vitro model of axon regeneration using dorsal root ganglion (DRG) explants with peripheral nerve. We observed axon inhibition of DRG neurons by treating cultures with an anti-Gg mAb (GD1a/GT1b) associated with the presence of end-bulb like structures characteristic of dystrophic growth cones. Also, cultures were infected with VSV-G-pseudotyped lentivirus vector carrying the GFP sequence taking advantage of its greater trofism for neurons to keep track of regenerating axons on nerves. This approach gave us a useful tool to characterize anti-Gg Ab-induced dystrophic growth cones and will help to clarify the pathogenic role of anti-Gg abs in GBS.

NS-P10.
MYELIN-ASSOCIATED GLYCOPROTEIN RESCUES MOTONEURONS FROM APOPTOSIS VIA RHOA SIGNALING PATHWAY

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Myelin-associated glycoprotein (MAG) is a lectin present in the periaxonal layer of myelin that engages several axonal receptors, including Nogo-R (NgRs), which have a modulatory role on programmed cell death (PCD) of motoneurons (MNs) dependent on the activation of the neurotrophin receptor $P75^{NTR}$. The small GTPase RhoA regulates diverse cellular processes such as apoptosis, through one of the effector proteins, Rho-Kinase (ROCK). The aim of this study was to analyze a possible modulatory role of MAG on PCD of MNs and elucidate the signaling pathways associated with this effect. A time course study showed that early after birth Mag-null mice have a reduction in MNs count. Also Mag-null mice exhibit increased susceptibility in an in vivo model of PCD induced by a sciatic nerve crush. Interestingly pre-treatment with a soluble form of MAG (MAG-Fc) prevented MN apoptosis in this model. Studies using an in vitro model of $P75^{NTR}$-dependent PCD on spinal cord organotypic cultures and a MN cell line confirmed the modulate role of MAG. We further report the in vivo role of RhoA signaling pathway in the protective effect of MAG against MN death. Treatment with Y27632 to inhibit ROCK was sufficient to reverse the protective rol of MAG-Fc. These findings indicate that RhoA signaling pathway plays a critical rol in the protective effect of MAG against PCD of MNs during development.

NS-P11.
BARS REGULATES MEMBRANE TRAFFICKING IN DEVELOPING NEURONS

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The ability of cells to produce highly compartmentalized membrane domains and hence to polarize is crucial for complex biological activities, such as the organization of the nervous system. Disruption of the endoplasmic reticulum (ER)-Golgi secretory pathway in developing neurons alters axon-dendritic formation. Therefore, detailed knowledge of the mechanisms underlying exiting from the Golgi is crucial for understanding neuronal polarity. In this study we have analyzed the role of Brefeldin A-Ribosylated Substrate (BARS) in the regulation of morphological polarization, the formation of Golgi outposts (GO) and the exit of membrane proteins from the TGN. The results obtained show that RNAi suppression of BARS inhibits axonal/dendritic elongation and branching, as well as the extension of GO into dendrites. In addition, using a plasma membrane (PM) protein (e.g. transferrin receptor [TfR] fused to GFP) engineered with reversible/removable aggregation domains we observed that suppression or expression of dominant negative BARS delay the exit of TfR from the Golgi apparatus. Taken together, these data provide the first set of evidence suggesting a role for BARS in neuronal polarization by regulating membrane trafficking and organelle positioning.
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NS-P12.
N-NITROSOMELATONIN ENHANCES PHOTIC ENTRAINMENT OF THE SUPRACHIASMATIC CIRCADIAN CLOCK

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Virtually all physiological processes in mammals are synchronized to the daily light:dark (LD) cycle by a circadian clock located in the hypothalamic suprachiasmatic nucleus (SCN). Photic stimulation of the retina releases glutamate through the retino-hypothalamic tract; signal transduction of light-induced circadian phase advances is mediated through a neuronal nitric oxide synthase-guanilyl cyclase pathway. We have employed a novel nitric oxide (NO)-donor, N-nitrosomelatonin (NOMel), to enhance photic synchronization of locomotor activity rhythms in hamsters. The intraperitoneal administration of this drug before a sub-saturating light pulse (LP) at circadian time 18 generated a two-fold increase of circadian phase-advances, and also accelerated resynchronization to a 6-hour phase-advance of the LD cycle. However, NOMel had no effect on light-induced circadian phase delays or on resynchronization to a 6-hour delay of the LD cycle. The LP-enhancing effects were correlated with an increased SCN-expression of cFOS. Moreover, *in vivo* NO release by NOMel was verified by measuring nitrate&nitrite levels in SCN homogenates. Here we demonstrate the chronobiotic properties of NOMel, emphasizing the importance of NO-mediated neurotransmission for circadian phase advances.

NS-P13.
CHARACTERIZATION OF SEROTONIN AS A POTENTIAL NEUROTRANSMITTER IN ECHINOCOCCUS SPP

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Objetives: 1) To search for genes related to serotonin (5-HT) metabolism and function in the cestode parasites of sanitary importance *E. multilocularis* (Em) and E. granulosus (Eg). 2) To analyse the serotoninergic nervous system ontology in Eg. 3) To analyse the effect of 5-HT in Eg protoscoleces motility.
Methodology and Results: By bioinformatic analyses, we found that the 5-HT pathway is almost complete in Echinococcus spp. genomes with some interesting divergences. The serotoninergic immunoreactivity showed a well developed serotoninergic system in protoscoleces and adult forms and a tendence towards atrophy during the development to cystic stage. The role of 5-HT as a neurotransmitter was evaluated by measuring the motility of protoscoleces in presence of 5-HT alone or in combination with 5-HT transporter and 5-HT receptor antagonists, using the worm tracker (Simonetta and Golombeck, 2007). We found that 5-HT has a specific and dose-dependent effect in the motility of protoscoleces.
Conclusions: The 5-HT pathway could be functional in Eg and Em. Eg shows a high plasticity of development of the serotoninergic system. The effects of 5-HT on protoscolex motility suggests a role as a neurotransmitter and indicates that the nervous system could be considered as a target of drugs. Molecular studies will reveal the exact role of 5-HT in these parasites.

LI-P01.
EFFECT OF ESSENTIAL FATTY ACIDS ON CELL SURVIVAL IN AN *IN-VITRO* MODEL OF BREAST CANCER

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Cancer is a multicausal disease affecting key biological events. Different studies showed that 35% of cancers are related to diet. Controling dietary factors, as essential fatty acids (EFAs), would play an important role in cancer prevention. EFAs, as Eicosapentaenoic acid (EPA n-3) and docosapentaenoic acid (DHA n-3) are highly susceptible to oxidation producing oxidants that reduce tumor cell survival, respect to arachidonic acid (AA n-6). We proposed to determine the effect of EFAs on a breast cancer cell survival. MCF-7 cell line was incubated with increased concentrations of AA-EPA-DHA. EtOH was used as control. The effect was assessed from 24 to 72 hours post-treatment. Cell viability was determined by MTT assay, cellular production of oxidants by xylenol orange assay (XO) and apoptosis by Annexin-V FITC. EPA and DHA significantly inhibited cell viability in a dose and time manner compares to cells treated with AA ($p = 0.05$). Cellular oxidant production and the percentage of apoptotic cells was significantly higher in cells treated with DHA for 72 hours ($p = 0.05$). Treatment with DHA and EPA inhibited tumor cell survival in mammary adenocarcinoma by increased release of oxidants, compared to those treated with AA which would correlate with decreased cell viability and increased degree of apoptosis.

LI-P02.
ANALYSIS OF THE ROLE OF ARACHIDONIC ACID SIGNALING ON TUMOR CELL GROWTH

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Arachidonic acid (AA) signaling plays a role in tumor growth and maintenance. It has been suggested that the regulatory effects of this polyunsaturated fatty acid (PUFA) may be mediated by cyclooxigenases (COXs) and lipoxygenases (LOXs) metabolites, key regulators of intracellular signaling networks. Here, we investigate the effects of AA on LOXs and COXs activities and its impact on cancer cell biology. Breast and pancreatic cancer cells were incubated with increased AA concentrations (5-100 ug/ml). Membrane profiles were measured by GLC. PUFA enzymatic oxidation was assessed by FOX method. COXs (12HHT) and LOXs (12-15-5-HETE) activities were measured by HPLC. Cell viability was determined by the resazurin assay, apoptosis by caspase 3/7 assay. AA treatment induces changes in membrane fatty acid profiles, increasing the n-6 AA and decreasing the n-3 and n-9 fatty acids levels. These changes modify oxygenase activity; COXs metabolite (12HHT) was lower than LOXs (12-15-5-HETE) production in cancer cells. Moreover, AA increases hydroperoxides, according to FOX assay ($p<0.05$). AA decreased cell viability and induced apoptosis in a dose dependent manner as shown by resazurin assay and the increase in activated caspases 3/7 ($p<0.05$). Together these results support the role of AA signaling in the regulation of cancer cell survival and define this axis as a potential therapeutic option.

LI-P03.
ISOLATION AND CHARACTERIZATION OF AN ACYL-LIPID DESATURASE MUTANT OF *Bacillus cereus* ATCC1457

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B. cereus is an opportunistic pathogen that can induce food poisoning and has the ability to grow at temperatures between 4ºC and 50°C. These bacteria adapt membrane fluidity during low-temperature growth by different mechanisms such as increasing the proportion of unsaturated fatty acids (UFAs) that are essential for the maintenance of membrane structure and function. In a previous work we described that *B. cereus* ATCC14579 has two acyl lipid desaturases, BC0400 and BC2983 that are involved in the synthesis of UFAs with double bonds in Δ10 and Δ5 positions, respectively. When cells of *B. cereus* growing at 37ºC are shifted to 20ºC only Δ5-UFAs are increased, indicating that adaptation of the membrane fluidity at lower temperatures might depend on BC2983 activity. In this work we report the construction and characterization of a *B. cereus* mutant in Δ5-Des, LSC2983, by a simple and efficient method that uses a temperature-sensitive vector that carries a selectable marker. The impact of this mutation on FA composition analyzed by GC-MS showed that strain LSC2983 did not synthesize Δ5-UFAs. This mutant grows as wild type strain in minimal medium at low temperature showing that Δ5-UFAs synthesis is not essential for *B. cereus* growth indicating that an additional mechanism is also involved in cold adaptation.

LI-P04.
ROLE OF PHOSPHATIDYLCHOLINE DURING NEURONAL DIFFERENTIATION

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Neuronal differentiation is a process that requires membrane biosynthesis. Phosphatidylcholine (PC), the most abundant phospholipid in membranes of eukaryotic cells, is mainly synthesized through the Kennedy pathway. During retinoic acid (RA) induced differentiation of neuroblastoma cells, PC synthesis is induced by increased expression of CTP:phosphocholine cytydilyltransferase alpha and choline kinase. We demonstrated that cell lines that overexpressed these enzymes have increased levels of PC and they undergo neuronal differentiation in the absence of RA.
Interestingly, we found that the addition of PC liposomes promotes neuronal differentiation by activating ERK signalling cascade, mimicking the RA effects. In addition, we demonstrated that PC-induced differentiation is dependent of phospholipase A2 activity suggesting a role of lysophosphatidylcholine (LPC). In fact, PC derivative metabolites such as LPC, choline and phosphatidic acid (but not lysophosphatidic acid) induce neuronal differentiation. These results allow us to propose that PC, and derivative metabolites, are not only important structural membrane building blocks, but could also stimulate signalling pathways driving neuronal differentiation, which means a significant progress in the identification of specific signals regulating neuritogenesis.

LI-P05.
A NEW IMPLICATION OF C-FOS IN PHOSPHOLIPID SYNTHESIS: ACTIVATION OF PAP1 ACTIVITY
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We have previously shown that c-Fos activates phospholipid synthesis through a mechanism independent of its genomic AP-1 activity. To accomplish an overall activation of the synthesis, only particular enzymatic activities are positively affected. In this work, we showed that recombinant c-Fos increases in vitro Phosphatidate Phosphohidrolase (PAP1) activity from cellular homogenates. PAP1 catalyzes Phosphatidic Acid (PA) conversion to Diacylglicerol, both of which have essential functions in signaling cascades and lipid biosynthetic pathways. The regulation of PAP1 activity may govern the pathways by which phospholipids are synthesized and control the cellular contents of important signaling lipids. To precisely characterize the phenomenon, we used purified Lipin1 (responsible of most PAP1 activity in mammals) obtained recombinant from E. Coli. Lipin1 activity was measured with or without c-Fos in PA/Triton X-100 mixed micelles and the kinetic parameters determined. Finally, we established through FRET-FLIM microscopy there was a direct physical association between c-Fos and Lipin1 in cells. Results support our general hypothesis: c-Fos directly interacts with the phospholipid synthesizing enzymes whose metabolic steps it activates, and highlight the role of c-Fos in events requiring large amounts of phospholipid synthesis.

LI-P06.
IRON IONS EFFECT ON LIPID PEROXIDATION IN MODEL MEMBRANES AND ON MITOCHONDRIAL ACTIVITY OF CHO CELLS
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We have attempted to gain understanding of the mechanisms responsible for lipid peroxidation (LP) in a simple model system, made by dispersing retinal lipids in the form of liposomes (Ls). Although LP in model membranes may be very different from that in biological membranes, the results obtained in the former may be useful to get information about processes in the more complex biological environment. In spite of the relative simplicity of LP of Ls, these reactions are still relatively complex because they depend on many factors such as liposome type, reaction initiator and reaction medium. Here we describe: a) a method to prepare small Ls with natural phospholipids and b) the results obtained by the analytical techniques (DLS, TBARS production, determination of conjugated dienes production and analysis of fatty acid profile by CG-MS) used to follow LP initiated by iron ions in these Ls. Results show that Fe+2 ions were more efficient initiators than Fe+3. The effect of iron ions was also investigated in CHO-K1 cell cultures. As tested by MTT assay, a significant reduction ($p < 0,001$) in mitochondrial activity to 80% of the control value was observed for 1 mM Fe+2 decreasing to 61% in the case of 4 mM. A significant reduction ($p < 0,001$) in mitochondrial activity to 86% of the control value was observed for 1 mM Fe+3 decreasing to 63% in the case of 4 mM.

LI-P07.
PURIFICATION AND CHARACTERIZATION OF LIPOVITELLIN FROM EGGS OF THE WOLF SPIDER SCHIZOCOSA MALITIOSA
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Vitellins are essential in ovigerous species for embryogenesis and viability of progeny, but in spiders, only have been described the egg lipovitellins in the species Polybetes pythagoricus. Thus, there is virtually no information in arachnids. The aim of this work was to purify and characterize lipovitellins from Schizocosa malitiosa spider. Lipoproteins were isolated by ultracentrifugation and their lipids, proteins and fatty acids were analyzed by a combination of chromatographic techniques and electrophoresis. Two lipovitellins of high and very high density, 42 and 11.1% total lipids, respectively were isolated, the HDL named LV1 with density of 1.13 g/ml, and the VHDL named LV2, density of 1.24 g/ml. Sphingomyelin was the major component in both of them, representing 48.3 and 76% of total lipids, respectively. Major fatty acids were 18:2>18:1>16:0>18:0 in LV1, and 16:0>18:1>18:2>18:0 in LV2. Native LV1 has a MW of 559 kDa, and 3 apolipoproteins of 116, 87 and 42 kDa. Native LV2 is more heterogeneous with 5 particles of MW 492, 405, 293, 213 and 48 kDa. The 5 have the same apolipoprotein composition with 4 subunits of 135, 126, 109 and 70 kDa, though in different ratios. The description and characterization of LV1 and LV2, the first egg lipoproteins described in the family Lycosidae, lead to get an insight into molecular and biochemical aspects of such a large group as spiders.

LI-P08.
CHANGES IN ENVIRONMENTAL OSMOLALITY REGULATE LIPID METABOLISM IN RENAL EPITHELIAL CELLS
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Cells in the renal papilla, due to their function in concentrating urine, are surrounded by high osmolalities. To survive in these adverse conditions, they have protective mechanisms. We showed that renal papilla is the kidney zone that has the highest synthesis and phospholipid (PL) synthesis and turnover, and that this helps to preserve the membrane structure and thus cell viability. As PL synthesis requires an adequate supply of fatty acids, here we evaluate the levels of triacylglycerides (TG) and their relationship with PLs in the different kidney zones and in MDCK cells submitted to osmolalities ranging from 298 to 579 mOsm/kg H2O. Lipids were separated by TLC and quantified. Also we studied the expression of several enzymes involved in the synthesis of PL and TG by RT-PCR. We observed that renal cortex has the highest content of PL but the lowest of TG and PL synthesis. In contrast, renal papilla has the lowest content of PL but the highest of TG and PL synthesis. In MDCK, hyperosmolality significantly increases PL and TG content in a time and concentration dependent manner. Hyperosmolality also increases the expression of PL and TG enzymes. TGs are stored in lipid droplets, so we evaluated their size and number by Oil-Red-O staining in MDCK, observing that they both increase in hyperosmolality. These data show that changes in environmental osmolality regulate PL and TG metabolism in renal

LI-P09.
NATURAL ISOPRENOIDS ENHANCE CHOLESTEROL-LOWERING EFFECT OF STATINS IN HUMAN LIVER CELLS

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Cholesterol (C) excess in blood produces coronary diseases which are the main cause of world death. Statins (ST), as simvastatin (SV), are competitive inhibitors of HMG-CoA reductase widely used as hypocholesterolemic drugs, which occasionally cause diseases such as hepatic dysfunction and weakness. Natural isoprenoids (NI) as linalool (LN) and cineole (CN) are hydrocarbons derived from the mevalonate pathway in plants capable of decreasing cholesterol synthesis (CS) by different mechanisms than statins. The possibility to diminish effective doses of ST by dietary supplement with NI arises as an interesting therapeutic alternative. We studied the action of the combination of LN, CN and SV on CS and C content (CC) in HepG2 cells. Cells were treated with LN, CN and SV, alone or combined in pairs, at concentrations which did not inhibit cell proliferation. CC and ^{14}C-acetate incorporation into C were determined. Our results showed a decrease in CS and CC when cells were treated with LN, CN, SV and their combinations. R index was calculated to determine if the combinations decrease additively (R=1) or synergistically (R>1) the CS and CC. We found values of R>1 in all combinations for CS while the combination LN-SV had an additive effect on the lowering of CC. Our results suggest a possible benefit and future uses of ST and NI combined in the treatment of hypercholesterolemic diseases.

LI-P10.
GERANIOL AS A NUTRIGENOMIC HYPOLIPIDEMIC AGENT

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Hyperlipidemia can trigger various metabolic disorders such as obesity, diabetes and cardiovascular diseases. Nowadays, several natural compounds with antihyperlipidemic properties have been in the spotlight in the field of medical sciences. To evaluate the *in vivo* effect of geraniol (GOH) -a terpenoid derived from herbal plants- on lipid metabolism, nude mice were fed with 25; 50 or 75 mmolGOH/kg diet for 21 days. Three hours before sacrifice, animals were injected with 25 µCi of ^{14}C-acetate. Total plasma total cholesterol and triglyceride levels of mice fed with 50-75 mmolGOH/Kg diet were significantly reduced (20 and 30-90%, respectively). GOH decreased the incorporation of ^{14}C-acetate into fatty acids (34-75%), total (25-72%) and nonsaponifiable lipids (53-62%) in the liver of treated mice, and reduced 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) activity (25-42%). Preliminary results revealed that GOH regulated the expression of HMGCR and LDL receptor (LDLR) at transcriptional and/or posttranscriptional levels; only higher doses showed to upregulate them. Posttranscriptional inhibition of HMGCR was suggested by the decrease of enzyme activity and protein levels observed in mice fed with all doses of GOH. These results showed that GOH may enhance LDLR gene expression with LDL clearance capacity, and decrease lipid biosynthesis. This study suggests that GOH can be a potential pharmaceutical agent for the prevention and treatment of hyperlipidemia.

LI-P11.
DIFFERENTIAL MODULATION OF THE MEVALONATE PATHWAY BY DIVERSE *Lippia alba* CHEMOTYPES

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Lippia alba (Mill) N.E.Br. is a plant with several chemotypes (CHMs) commonly used in traditional medicine. The biological activity of these plants are attributed to their essential oils (EOs). These EOs present a diverse and high concentration of monoterpenes characteristic of each CHM. Many monoterpenes exhibit antitumor activity that has been attributed to multiple pharmacological effects on the mevalonate pathway (MP). We have reported that EOs of tagetenone (T), carvone (Ca), piperitone (P) and citral (C) CHMs inhibit cell proliferation (CP) in HepG2 and A549 cells. The aim of this work was to study the effects of these EOs on the MP in these cell lines. Cells were treated with increasing non inhibitory CP doses of EOs. Incorporation of ^{14}C-acetate into intermediates and final products of the MP was determined by autoradiography of nonsaponifiable lipids separated by TLC. Exogenous cholesterol (CHO) uptake and outflow were evaluated. CHO synthesis was inhibited in a dose-dependent manner with all EOs. Synthesis of other products decrease proportionaly with P, whereas with Ca, C, and T an increase in ubiquinone and other intermediates was observed. This increase was more markedly in A549 cells. These findings suggest that different synergic effects of monoterpenes present in the EOs modulate the MP at different levels, some of them at a later step of the ramification point.

LI-P12.
MONOTERPENES AFFECT COMPOSITION AND ORGANIZATION OF NEUTRAL LIPIDS AND ASSOCIATED PROTEINS IN Hep G2

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Lipid droplets (LDs) are dynamic organelles implicated in cellular lipids homeostasis. Stored lipids from LDs are used for synthesis of hepatic VLDL, bile salts and membranes. Geraniol (G) is a monoterpene present in essential oils of aromatic plants. We have demonstrated that treatment of Hep G2 cells with G 50 µM decreased cholesterogenesis, and that concentrations above 300 µM are required for inhibiting protein prenylation and phosphatidylcholine synthesis. These findings let us hypothesize that geraniol could have an effect on the composition and organization of cellular lipids. Cells were treated with G 200 and 400 µM. LDs were analyzed by microscopy and separated into six bands by sucrose gradient ultracentrifugation of sonicated cells. Total lipids (L) -from the major upper band (b-A) and whole cell- were extracted and cholesterol (Cho), cholesteryl esters (CE), triacylglycerides (TAG) and phospholipids (PL) were determined. Constitutive proteins of b-A were separated and quantified by SDS-PAGE/western blot. We found that in treated cells: 1) TAG, Cho and CE content decreased while PL and L increased; 2) LDs number increased with preserved size; 3) b-A volume increased with conserved L/Protein ratio, decreased TAG, CE, Cho and PL and increased perilipin, indicating that other lipids are stored in these LDs. Geraniol produced alterations in cell lipid composition and Lds organization.

LI-P13.
FATTY ACID SYNTHESIS IN *Bradyrhizobium* TAL1000 PEANUT-NODULATING: TEMPERATURE EFFECT
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High temperature is known to cause bacterial protein aggregation and denaturation and to cope with this stress factor a cellular response occurs, such as changes in the ratio of unsaturated to saturated (U/S) fatty acids (FA).
The aim of this work was to clarify the effect of high temperature on mechanism of monounsaturated fatty acid (MUFA) synthesis in *Bradyrhizobium* TAL1000.
B. TAL1000 was grown in B- medium wih or without radioactive substrates for 16 h at 28°C and shifted to 37°C for 2 h. Cells were harvested, total lipids were extracted (Bligh and Dyer, 1959) and FA methyl esters were prepared using BF_3 in methanol. Non labelled FA were analyzed by GC and labelled FA were separated according to unsaturation degree by $AgNO_3$ TLC. The radioactivity of each fraction was then quantified.
We demonstrate that 18:1 FA decreased a 45.35%, whereas 18:0 FA increased 41%. Both 19:0 cyclopropane and 20:3 FA increased by heat shock. These changes affected the degree of unsaturation, changing the U/S ratio from 2.77 to 1.27. Also heat shock caused a decrease of 32%, 15% or 30.5% in radioactive recuperation in the MUFA fraction when 18:0 or 16:0 or sodium acetate was the substrate, respectively. The FA desaturarion in *B*. TAL1000 is down-regulated by temperature to maintain membrane fluidity and a two hour exposure to 37°C is required for a change in the quantity of MUFA to occur.

LI-P14.
THE MOUSE LIVER DISPLAYS CIRCADIAN RHYTHMS IN LIPID CONTENT AND EXPRESSION OF SYNTHESIZING ENZYMES
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Biological clocks are present in different tissues distributed all through the body. In the liver, circadian clocks regulate diverse metabolic processes under a daily basis. In this respect, the temporal control of phospholipids synthesis and particularly of phosphatidylcholine (PC) in the mammalian liver is little known. In the present work, we investigated the circadian variation in the composition of membrane phospholipids (PC, PE, PI). Focused on PC, the most abundant phospholipid, we examined the temporal expression of its synthesizing enzymes choline kinase (CK), cytidylyltransferase (CCT) and phospatidylethanolamine methyl transferase (PEMT). For this, C57BL/6J mice synchronized to a 12:12 h light dark (LD) cycle or kept in constant dark (DD) were euthanized at different times and livers collected for further assays. mRNA levels were determined by RT-qPCR using the clock gene Bmal1 as a positive marker of circadian rhythmicity. We found significant changes in the content of different phospholipids (PI, PE, etc) and in the ratio of PC/PE in both LD and DD. Also, a significant circadian oscillation was found in mRNA levels of CKa in LD and DD and of PEMT in DD, and a slight LD variation in CKa protein and activity. These results strongly suggest that there is a tight clock driven-control on the phospholipid metabolism and expression of synthesizing enzymes in the liver of mice.

LI-P15.
FIRST INSIGHT INTO THE MAJOR EGG PROTEIN FROM THE APPLE SNAIL *Pomacea insularum* PERIVITELLIN FLUID
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Pomacea insularum, is a south american freshwater snail that is becoming a plague in Europe rice fields causing important economic loses and ecologic risk. Egg proteins (perivitellins) are important components of the perivitellin fluid that play several function during embryo development. Here we report the first data on the characterization of the major *P. insularum* perivitellin. Ultracentrifugation of egg cytosol in NaBr gradient showed three major protein peaks, the largest corresponds to a carotenoprotein complex hereafter named *Pomacea insularum* PV1 (PiPV1). This complex is responsible of the bright reddish color that characterizes the conspicuous egg masses. It is a lipoprotein with a hydration densitity of 1.25 g/mL that falls into the VHDL range. It is a 292 KDa oligomer composed of four subunits with masses between 25 and 35 KDa, been the oligomer held together by non-covalent forces. Here we report the biochemical features of PiPV1 and compare the particle with *P. canaliculata* and *P. scalaris* carotenoproteins, the only ones so far studied in molluscs. These proteins play important roles providing protection against factors such as sun radiation, egg desiccation, as well pathogens or predators. Further structural and functional analyses will expand our knowledge on embryo defensive proteins, giving an insight into the adaptive radiation of these invasive freshwater snails.

LI-P16.
TEMPERATURE REGULATED FATTY ACID DESATURASES IN *Mycobacterium smegmatis*
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Despite their relevance in mycobacterial physiology, knowledge of biosynthetic pathways and regulatory mechanisms controlling desaturase expression are extremely scarce. Only one gene, *desA3*, was annotated as stearoyl-desaturase in *Mycobacterium tuberculosis*. *M. smegmatis* adapts to temperature changes by adjusting mycolic acids and fatty acids content. Our bio-informatic analysis in mycobacteria revealed several genes with homology to *desA3*. Interestingly, *M. smegmatis* has four of such genes. To address their possible role in temperature adjustment, we cloned the promoter region of *MSMEG*_1886 and *MSMEG*_1211 upstream of the *lacZ* gene and analyzed the effect of temperature and UFAs on their transcriptional activity. ß-galactosidase activity showed no changes at different temperatures when *MSMEG*_1886 was analyzed, but a decrease was seen for *MSMEG*_1211 with higher temperature. UFAs of C16-C18 repressed the expression of both genes. We generated a knock-out mutant in *MSMEG*_1886 and evaluated their growth rate at 25, 37 and 42ºC. The mutant grew in the absence of oleic acid (albeit at slow rate) at 37 and 42°C, but was unable to grow at 25°C. Fatty acid analysis revealed that C18:1 was severely reduced and C16:1 was absent. These results show that *MSMEG*_1886 and *MSMEG*_1211 are C16-C18 fatty acid desaturases regulated by end product, with *MSMEG*_1211 being regulated by temperature.

LI-P17.
RETINOIC ACID MODIFIES LIPID METABOLISM IN ISOLATED ADULT AND AGED RAT CEREBELLUM NUCLEI
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Nuclear lipid metabolism gives rise to several lipid second messengers that seem to be involved in the regulation of nuclear structure and gene expression. The purpose of the present research was therefore to study the metabolic pathways involved in the metabolism of phosphatidic acid (PA) and its regulation in isolated nuclei from the central nervous system. This study was also conducted in isolated nuclei from aged animals allowing us to analyze the effects of neurodegeneration processes on PA metabolism as a result of ageing. Adult (4 mo) and aged (28 mo) rat cerebellums were homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Using radiolabelled substrates we demonstrated lipid phosphate phosphatases (LPPs), diacylglycerol lipase (DAGL), monoacylglycerol lipase (MAGL), lysophosphatidate phosphohydrolase (LPAPase) and phospholipase (LPAase) as well as PA-phospholipase type A (PLA) activities. We further studied their regulation by the nuclear agonist retinoic acid (RA), which was observed to decrease DAGL and MAGL activities. Significant aged-related changes in the above-mentioned enzymatic activities as well as in its regulation by RA were observed. Taken together, our results demonstrate a RA-regulated PA metabolism in rat cerebellum nuclei which could be involved in neurodegeneration processes.

LI-P18.
AGED-RELATED CHANGES IN 2-AG METABOLISM ENZYMES EXPRESSION AND ACTIVITY IN RAT CEREBRAL CORTEX
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Endocannabinoid 2-arachidonoylglycerol (2-AG) is synthesized by the enzymes diacylglycerol lipase (DAGL) and lysophosphatidate phosphohydrolase (LPAase). Its hydrolysis is carried out by monoacylglycerol lipase (MAGL) although other enzymes, such as fatty acid amide hydrolase (FAAH) and serine hydrolase ABHD may also be involved. The aim of this study was to analyze 2-AG synthesis and hydrolysis during physiological aging. Cerebral cortex membrane and soluble fractions; and synaptosomes from adult (3 mo) and aged (28 mo) rats were isolated by differential centrifugation and the synaptosomes were purified in ficoll gradients. LPAase, DAGL, and MAGL activities were assayed using radiolabeled substrates, and their products were quantified from aqueous or lipid phase, previously separated by TLC. The expression of DAGL, MAGL and FAAH was analyzed by Western Blot. Both DAGL α and ß were expressed in membranes while the second was only expressed in synaptosomes. The expression and activitiy of DAGL changed during aging. 2-AG hydrolysis showed no changes in the membrane fraction during aging in coincidence with the absence of changes in MAGL expression. 2-AG hydrolysis was observed to be higher in synaptosomes whereas MAGL expression decreased during aging. Our results show that the expression and activity of the enzymes involved in 2-AG metabolism are differently modulated by aging.

LI-P19.
METAL-INDUCED OXIDATIVE STRESS ACTIVATES DIFFERENT LIPID SIGNALING PATHWAYS IN DOPAMINERGIC NEURONS
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The characterization of the mechanisms mediating the effects of metal-induced oxidative stress on neuronal dysfunction and death is central in the understanding of the pathology of several neurodegenerative disorders such as Parkinson's disease. In this work, we characterized the cellular responses that operate in dopaminergic neurons (N27 cells) exposed to an overload of transition metals such as iron (Fe, 1 mM), copper (Cu, 10 and 50 µM) or their combination for 24 hs. Under these experimental conditions, reactive oxygen species measured by fluorescence microscopy, and lipid peroxidation levels increased as a function of metal concentration. Maximum levels of lipid peroxides were observed in the presence of Fe + Cu. Cell viability, determined by MTT reduction, strongly decreased in the presence of Cu and with the combination of both metals. Under these experimental conditions, an increase in the levels of Akt phosphorylation in Ser-473 was observed. Bcl-2 expression showed the same profile that Akt phosphorylation. In addition, the expression and the activation of the secretory and cytosolic isoforms of phospholipase A2 (PLA2) were differentially affected by metal overload. Our results demonstrate that phospholipid deacylation processes catalyzed by PLA2s and PI3K activation are involved in the response of dopaminergic neurons to metal-induced oxidative stress.

LI-P20.
SPHINGOMYELIN SYNTHASE1 ACTIVITY IS IMPLICATED IN MDCK CELLS EPITHELIAL-MESENCHYMAL TRANSITION
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We have demonstrated that sphingomyelin (SM) biosynthesis is essential for hypertonicity-induced MDCK cell differentiation. Under inhibition of SM synthesis, MDCK cells instead of differentiate switch to mesenchymal phenotype, thus performing an epithelial to mesenchymal transition (EMT). We aim to study the sphingolipid metabolic pathway as well as the sphingomyelin synthase isoform 1 (SMS1) involvement in such process. MDCK cells were subjected to hypertonicity and concomitantly treated or not (control) with 15 µM D609 (SMS inhibitor) or siRNA-SMS1. Sphingolipid metabolism was determined by using radioactive precursors in the presence or absence of cicloserine (CS) or Fumonisin B1 (FB1). By using D609 as well as siRNA SMS1 the characteristic polarized phenotype of the cells was lost and it was not retrieved by a concomitant treatment with CS or FB1; suggesting no intermediates accumulation participation. Acquirement of mesenchymal phenotype was accompanied by alterations in amount and localization of the epithelial markers (E-Cadh, Cad16 and ZO-1) and mesenchymal marker Vimentin. These results demonstrate implication of SM synthesis in the EMT. Is important to note that EMT has been implicated in the development of cancer and renal fibrosis, consequently SMS1 activity emerges as a possible target molecule for the study of such important human pathologies.

LI-P21.
CHARACTERIZATION OF A PUTATIVE OCTANOYL TRANSFERASE FOR LIPOATE BIOSYNTHESIS IN TRYPANOSOMATIDS

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Lipoic acid (LA) is a cofactor of 2-oxoacid dehydrogenases (2OADH) and glycine cleavage (GCC) complexes. Owing to this enzymes are essential for cellular viability, it biosynthesis metabolism represents a potential chemotherapeutic target against parasites like *Trypanosoma cruzi* and *T. brucei*. A bioinformatic analysis allowed us to found orthologous genes for the enzymes lipoate synthase, octanoyl transferase and lipoate protein ligase, involved in LA de novo synthesis and protein lipoilation. In order to functionally characterize the Tb11.01.1160 gene product, putative *T. brucei* octanoyl transferase, we conducted phenotype reversion assays in mutant yeast strains unable to synthesize LA and incapable to grow in medium lacking a fermentable carbon source (YPG). We observed the functional complementation of the yeast octanoyl transferase activity (Lip2) but not of lipoate synthase (Lip5) or amidotransferase (Lip3) activities. This could be an initial evidence for Tb11.01.1160 as a gene coding for octanoyl transferase in *T. brucei* (TbLip2). On the other hand, *Saccharomyces cerevisiae* could lipoilate E2 subunits of 2OADH only through the amidotransferase activity of Lip3, using lipoil-H protein (subunit of GCC) as substrate. The fact that lip3 mutant was not complemented, suggest that TbLip2 transfer octanoate specifically to H protein, in a similar way as Lip2 in yeasts.

LI-P22.
STEROL BIOSYNTHETIC GENES DO NOT REFLECT STEROL COMPOSITION OF *Capsaspora owczarzaki* GROWN *IN VITRO*

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In a phylogenetic analysis of Rieske-type sterol C7-desaturases (Neverland/DAF-36 family of proteins), highly conserved in animals, and described by our group in the protists Tetrahymena and Paramecium, we found two orthologues in the genome database of *Capsaspora owczarzaki*, a unicellular symbiont of the snail Biomphalaria glabrata. Capsaspora occupies a pivotal phylogenetic position between choanoflagellate protists (the closest relatives of Metazoa), and other opisthokonts (such as Fungi), turning into a good model for studying the evolution of sterol metabolism. An in silico analysis on the *C. owczarzaki* genome database revealed a complete conservation of the pathway for de novo synthesis of ergosterol. However, the GC/MS analysis of the sterol profile of an axenic culture grown in a medium supplemented with fetal calf serum (supplying cholesterol), suggest the cells are able to convert the incorporated cholesterol into ergosterol through a series of desaturations at positions C7(8) and C22(23) of the sterol moiety as well as the introduction of a methyl group at carbon 24 in the lateral chain. These results lead us to hypothesize that the metabolic pathway for de novo ergosterol synthesis may be blocked by the presence of cholesterol in the media, and that cholesterol is converted into ergosterol as a requirement to maintain membrane homeostasis.

LI-P23.
OXIDATIVE STRESS STATUS IN CATTLE: RELATIONSHIP WITH PASTURE QUALITY AND NUTRITIONAL SUPPLEMENTATION

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Both mineral composition of the soils and dietary supplementation are key aspects in cattle's nutritional status. This work aimed to determine if quality differences between two pasture fields might be accountable for problems in bovine breeding efficiency. We compared the basic hematologic parameters, plasma cholesterol (CHOL) and triacylglycerides (TAG) contents, and the oxidative status from two groups of cows and their respective calves from the Chascomus area (Buenos Aires, Argentina) that were bred over a 6-month period with or without nutritional supplementation. Cows had decreased CHOL, TAG and lipid peroxidation products upon minerals and vitamins nutritional supplementation, regardless the quality of the pasture. Feedlotting calves had lower CHOL, TAG, and lipid peroxidation values, and higher total antioxidants content in plasma respect to the pre-weaning period. Grass-fed calves had higher osmotic fragility than feedlot calves, being the remaining parameters similar to those from feedlot calves. Minerals and vitamins supplementation caused an overall beneficial effect on the parameters measured. In summary, results suggest that feedlotting does not introduce a significant improvement of the oxidative parameters in calves, while mineral and vitamin supplementation decreases cattle oxidative status and overcomes the nutritional quality of the pasture.

LI-P24.
L-FABP FACILITATES NUCLEAR LIPID DROPLET OLEIC ACID MOBILIZATION AND ESTERIFICATION

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Cellular nuclei (N) are an evolutionary development of the eukaryotic cell that enables a critical compartmentation of the processes of replication, transcription, premRNA splicing, and ribosome assembly among other cellular functions. We have determined that N lipids are located in nuclear membranes and Nuclear Lipid Droplets (nLD) (mainly composed of TAG, CE and C). nLD could be involved in N lipid homeostasis and serve as a buffering system that provides or incorporates lipids of signaling paths and transcription factors.
Taking into account previous results, exogenous 18:1n-9 is mainly esterified to nLD TAG and CE, the aim of the present work was to determine the role of L-FABP in 18:1n-9 incorporation, mobilization and release. Firstly N were labeled ([14C]N) *in vitro* with [1-14C]18:1n-9 free or L-FABP bound, ATP and CoA; then [14C]N were incubated without 18:1n-9, plus ATP, CoA at increasing concentrations of delipidized L-FABP. Under these conditions, exogenous 18:1n-9 was incorporated to N as FFA and esterified to TAG>CE>GPL; esterification increased when 18:1n-9 was L-FABP bound and with incubation time. Nuclear and nLD 18:1n-9 was mobilized from TAG, CE, PE and FFA pools and its release to cytosol increased with L-FABP concentration.
In conclusion, L-FABP facilitates 18:1n-9 esterification to nLD and its mobilization within the nucleus as well as its release to cytosol.

MI-P01.

POTENTIAL PROTECTION ROLE AGAINST SOYBEAN PROTEASE INHIBITORS OF *Nezara viridula* GUT BACTERIA

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Southern green stinkbug (*Nezara viridula*) is one of the most relevant pests of soybean crops, which feeds on the seeds in spite of their strong defenses against herbivory. We propose that the microbial community in the gut of stinkbugs may have a role in helping digestion and tolerance of chemical defenses of soybeans, such as cystein protease inhibitors (CysPI). The aim of this study was to determine the effects of gut bacteria activity on CysPI. Stinkbugs were collected from soy fields, dissected and guts were removed in sterility. Cell counts and isolations, and 16S ribosomal sequencing were performed. Soybean flour media was fermented with four selected gut bacteria, with 10^4 UFC/mg gut count, for 24h - 37°C. Extract total protein was also performed. All strains assayed diminished inhibitory activity of soybean flour CysPIs. Inhibitory activity of CysPI´s decreased 80% when soy flour was fermented with a strain identified as *Enterococcus faecalis*, 40% with *Enterobacter* sp, 30% with a *Pantoea* sp and less tan 10% with *Klebsiella* sp. None of these strains showed cystein protease activity, nor protease activity on skim milk agar plates. Fermented products had pH around 5, as insect´s gut normal pH. Our results suggest that gut bacteria have a potential role of deactivating protease inhibitors when insects feed on soybean pods.

MI-P02.

DEVELOPMENT AND VALIDATION OF A GLYCOCONJUGATE MAGNETICS BEADS-BASED ASSAY FOR HUMAN BRUCELLOSIS

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The O-polysaccharide (OPS) of *Brucella spp.* is a homopolymer of N-formylperosamine identical to the OPS from *Yersinia enterocolitica* O:9. We took advantage of this similarity to explore the application of a recombinant *Y. enterocolitica* O:9 OPS-protein conjugate (OPS-AcrA) as a novel antigen for diagnosis of human brucellosis. In this work, we have developed, optimized and validated an indirect immunoassay using OPS-AcrA coupled to magnetic beads. OPS-AcrA was produced in *Y. enterocolitica* O:9 cells co-expressing the OTase PglB of *Campylobacter jejuni* and the protein acceptor AcrA. Introduction of PglB in *Y. enterocolitica* resulted in the transfer of the OPS from its lipid carrier to AcrA. The assay was validated using a panel of characterized serum samples obtained from healthy individuals and patients of different clinical groups. To determine the cutoff values a receiver-operating characteristic analysis was performed. The cutoff value that concurrently optimizes sensitivity and specificity was 13.20% which resulted in a diagnostic sensitivity and specificity of 100% and 98.81%, respectively; a cutoff value of 16.15% resulted in a test sensitivity of 93.48% and a test specificity of 100%. Due to the low cost in terms of production of the antigen, reduced incubation time, and simplicity we propose the glycoconjugate-beads assay as a Point-Of-Care test for diagnosis of brucellosis.

MI-P03.

PROTEIN INTERACTIONS INVOLVED IN CYCLIC ß-1,2-GLUCANS METABOLISM: IMPORTANCE OF A COILED-COIL DOMAIN

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Cyclic ß-1,2-glucans (CßG) are periplasmic homopolysaccharides that play an important role in several symbiotic and pathogenic relationships. *Brucella abortus* CßG synthase (Cgs) is an integral inner membrane (IIM) protein which catalyzes the four reactions (initiation, elongation, phosphorolysis, and cyclization) required for the synthesis of CßG. Once synthesized in the cytoplasm, CßG are transported to the periplasm by the CßG transporter (Cgt) and succinylated by the CßG modifier enzyme (Cgm). Cgt and Cgm, as well as Cgs, are IIM proteins. In this work, we used a bacterial two-hybrid system and confirmatory techniques to study the interaction network among these three IIM proteins. Our results indicate that Cgs interacts with Cgt and Cgm, and that Cgt interacts with Cgm. We have also observed that each one of these proteins form homotypic complexes (homodimers). Furthermore, analyses carried out with Cgs truncated mutants, Cgs in-frame pentapeptide insertion mutants and a Cgs deletion mutant revealed that Cgs-dimerization as well as Cgs interactions with Cgt and Cgm are mediated by a coiled-coil motif located in the NH-terminal domain of the protein. We propose that Cgs, Cgt and Cgm form an inner membrane protein complex necessary to coordinate the synthesis, transport to periplasm and succinylation of CßG.

MI-P04.

FUNCTIONAL CHARACTERIZATION OF TWO SUBSTRATES OF THE *Brucella abortus* TYPE-IV SECRETION SYSTEM

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The delivery of effector proteins into the host cell is the central function of the VirB protein complex, the Type IV Secretion System of *B. abortus*. Effector proteins target, modulate and subvert diverse cellular processes allowing the pathogen to evade lysosome fusion and to create an organelle permissive for intracellular replication called the *Brucella*-containing vacuole (BCV). To identify VirB substrates, we performed a combined in silico-in vivo screening and found 3 proteins that are translocated in a VirB-dependent manner: BPE043, a hypothetical protein with prediction of 4 apolipoprotein domains; BPE005, a putative cyclic nucleotide-binding protein and BPE275, a member of the rhomboid family. To unveil the function of these proteins, we generated *B. abortus* clean deletion mutants of those genes and analyzed their phenotype. Our results revealed that two mutants displayed different but remarkable defects in virulence. *B. Abortus* Δ*bpe005* showed a significant increase in LAMP-1 acquisition and was affected in the biogenesis of the replicative BCV, thus, its survival and intracellular replication was impaired. On the other hand, *B. abortus* Δ*bpe275* displayed reduced adhesion to host cell but the intracellular replication stages were not affected in this mutant. These results remark the importance of VirB and effector proteins for the *Brucella*-host cell interaction.

MI-P05.
LOVHK IS RELATED TO A GENERAL STRESS RESPONSE SYSTEM IN *Brucella abortus*
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Bacteria can detect and respond to environmental changes through the two component signalling systems (TCS), which consists of a sensor histidine kinase (HK) and its cognate response regulator (RR). In previous experiments we have characterized an HK protein in *Brucella abortus* 2308: LOVHK. When the N-terminal LOV domain is exposed to blue light it promotes the autophosphorylation of the HK domain, which initiates a signal transduction pathway that ends with an increment in *Brucella* virulence. Using two-hybrid assays and phosphotransfer experiments we have identified two possible RR as interacting partners for LOVHK: one RR only has a REC domain and the other RR also has an additional domain. Phosphotransfer assays suggest that the first RR could be functioning as a phosphate sink for LOVHK, while the second RR could be responsible of a specific cellular response. The second RR has a 72% homology to an anti-anti sigma factor which is involved in the General Stress Response (GSR) characteristic of alfaproteobacteria. Using qRT-PCR we confirmed that the GSR system is active in *B. abortus*, and that it is downregulated in a LOVHK mutant. Light activation of the GSR system is still under evaluation. In conclusion, our results suggest that LOVHK activates a stress response system that could modify *Brucella* virulence.

MI-P06.
CHARACTERIZATION OF TCCEST, A NOVEL *Trypanosoma cruzi* PROTEIN ASSOCIATED TO THE FLAGELLAR POCKET
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The flagellar pocket is a small invagination of the plasma membrane of *Trypanosoma cruzi* where the flagellum exits the cellular body and is the only site for endo- and exocytosis. Here we characterize a novel *T. cruzi* protein, termed TcCesT, with homology to bacterial CesT chaperone involved in secretion through the type III system. Also, TcCesT bears a eukaryotic Rad-23 ubiquitin-like domain (UBL) domain, likely involved in receptor mediated endocytosis and nucleotide excision repair (NER). TcCesT is conserved in TriTryps and localizes to the flagellar pocket, as shown by immunocytochemistry and immunoelectron microscopy analysis in wild type and TcCesT overexpressing parasites. Expression data showed that TcCesT is restricted to replicative *T. cruzi* forms, namely epimastigotes and amastigotes. To further characterize TcCesT, colocalization analyses were performed. TcCesT partially colocalized with both ConA-rhodamine in endocytosis assays and with ubiquitin, suggesting a possible interaction through its UBL domain. A functional NLS, probably involved in regulated nuclear translocation of TcCesT associated to its NER domain, was identified. Finally, TcCesT transfected parasites showed impaired survival under starvation conditions. Taken together, our results suggest that TcCesT may be involved in *T. cruzi* endo- and exocytosis processes probably required for parasite growth.

MI-P07.
ROLE OF MISMATCH REPAIR SYSTEM IN THE GENETIC RECOMBINATION OF *Pseudomonas aeruginosa*
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The DNA Mismatch Repair (MMR) is a physiologic mechanism to prevent post-replicative mutations and/or recombination between partially homologous sequences. To analyze the recombination process in *Pseudomonas aeruginosa*, we previously constructed a LacZ-based system to measure recombination *in vivo* between identical (homologous) or partially divergent (homeologous) DNA sequences. Here, we show that the DNA divergence reduced the recombination rate by 38-fold in a wild type strain. Inactivation of either *mutS* or *mutL* gene significantly increased the homeologous recombination respect to a wild type strain (~28-fold for *mutS* and ~370-fold for *mutL*). These mutants also affected the recombination rate between identical sequences, although in a lesser extent (~4-fold for *mutS* and ~15-fold for *mutL*). In addition, we determined that mutant strains expressing MutSR842E (a full-length dimeric version of MutS) or MutS$^{\beta}$ (a MutS version unable to interact with ß-clamp) were as proficient as the wild type strain to inhibit the homeologous recombination. These results indicate that both homologous and homeologous recombination are affected by the absence of MMR factors whereas the oligomerization state of MutS as well as its interaction with ß-clamp do not have any effect. We are currently analyzing the proficiency of MutS and MutL to inhibit the recombination catalyzed by RecA in a vitro system.

MI-P08.
OXIDATIVE STRESS PREVENTS COLONY FORMATION IN AN *Escherichia coli* IMPAIRED IN CATECHOLS SYNTHESIS
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Under iron-limited conditions, *Escherichia coli* produces the catecholate siderophore enterobactin. We proposed that enterobactin has a new role as an intracellular protector against oxidative stress. We also showed that an *E. coli* impaired in enterobactin synthesis (*entE*) is unable to develop colonies on solid M9 medium. Iron supplementation did not recover colony formation, indicating that this phenotype is not due to iron deficiency. It is known that the viable but non-culturable phenotype is associated to oxidative stress. As we propose that catechols may act as radical scavengers, the absence of colonies in an *entE* mutant context could be related with an increment in the oxidative stress. Comparing with the wild type strain, the *entE* mutant showed greater sensitivity to hydrogen peroxide, paraquat and antibiotics which promote reactive oxygen species. The double mutant *sodA entE* displayed a pronounced colony impairment phenotype and were more sensitive to the agents tested. Finally, we observed that a normal growth was achieved when ascorbic acid was added to the medium or when the mentioned strains were grown in anaerobiosis. Results suggest that the incapacity to form colonies could be due to hostile conditions caused by oxidative stress and support our hypothesis implying cathecols siderophores in a oxidative stress protection function.

MI-P09.
MALTOSE UTILIZATION IN *Enterococcus faecalis* INVOLVES A NOVEL MALTOSE-6-P PHOSPHATASE

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In *Enterococcus faecalis* the genes involved in maltose metabolism are organized in a divergent fashion. The *malPBMR* operon encodes putative maltose phosphate phosphorylase (*malP*), ß-phosphoglucomutase (*malB*), aldose-1-epimerase (*malM*) and transcriptional repressor (*malR*) whereas the *malT-mapP* operon codes for a PTS enzyme II (*malT*) and a putative maltose phosphatase (*mapP*). In order to confirm the role of MalP and MapP enzymes in the pathway, the corresponding genes were cloned, expressed and the recombinant proteins purified. MalP enzymatic activity was only detected when maltose was used as a substrate, indicating that it is in fact a maltose phosphorylase. Since PTS sugars are phosphorylated during transport, our hypothesis was that MapP could be responsible of intracellular maltose-6-P dephosphorylation, providing the substrate for MalP. Effectively, MapP in vitro phosphatase activity was confirmed by a spectrophotometric assay and by mass spectroscopy. Furthermore, in order to demonstrate that MapP activity in vivo, we constructed a *B. subtilis* mutant, devoid of the 6-P-a-glucosidase that hydrolyses maltose-6-P (MalA). MapP expression allowed this strain to grow on maltose-containing minimal medium, supporting the *in vitro* observations. The results presented in this work help to clarify the unusual maltose metabolism present in *E. faecalis*.

MI-P10.
EVOLUTION OF METHICILLIN-RESISTANT *S. aureus* CLONES IN THE COMMUNITY AND HOSPITAL SETTING, ARGENTINA

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MRSA is a successful healthcare (HA) and community (CA) associated pathogen showing high virulence. We aimed to investigate the molecular epidemiology of MRSA infections in both community and hospital setting in Argentina. Consecutive S.aureus clinical isolates were collected during Nov-2009 from 66 hospitals in 20 Argentina provinces and Buenos Aires city. Healthcare-onset (HO) infection was defined when MRSA was obtained after 48 hours of hospitalization. A MRSA was considered an HA strain if it was resistant to at least 2 non-beta-lactam antibiotics. MRSA were studied by local and global molecular typing. Out of 591 total *S. aureus* isolates, 375 (63%) were from community-onset (CO) infections and 322 (54%) were MRSA (37% CA and 17% HA). MRSA proportion differed significantly between CO (58%) and HO (49%) infections. The molecular typing determined that most harbored SCCmecIV (74%) (IVc, 35%, IVa, 31% and minor variants) followed by SCCmecI (20%). CO-infections were associated mainly to PFGE type N-ST30-SCCmecIVc-t019-PVL+ (46%) and I-ST5-SCCmecIVa-t311-PVL+ (37%) clones. In contrast, HO-infections were associated mainly to PFGE type A-ST5-SCCmecI-t149 (46%) and I-ST5-SCCmecIVa-t311-PVL+ (20%) clones. The epidemiology of MRSA is changing in Argentina, with higher rates in the CO- than in the HO-infections, mainly associated with the spread of two CA-MRSA clones harboring SCCmecIV.

MI-P11.
STUDY OF THE AUTOPHAGY PATHWAY INVOLVEMENT IN THE JUNIN VIRUS INFECTION

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Autophagy is a process whereby double membrane vesicles (autophagosomes) form around a portion of the cytoplasm and fuse with lysosomes where enzymatic degradation occurs. This process was described as a protection against viral invasion; however, viruses have been able to subvert this pathway in their own benefit. We analyzed the role of autophagy during Junin virus (JUNV) infection. We have observed that cells overexpressing EGFP-LC3 (a well-known autophagy marker), infected with JUNV showed an increased number of LC3 dots in the cells similar to starved- or BafilomycinA1 treated-cells which leads to autophagosome formation or accumulation, respectively. We analyzed the conversion of LC3-I (cytosolic) to LC3-II (associated to autophagosomes) by Western blot observing that the level of LC3-II in JUNV-infected cells was similar to that observed in starved-cells. Moreover, cells pre-treated with rapamycin, a pharmacological autophagy inductor, enhanced virus yield with respect to the control situation. In addition, we assayed the replication capacity of JUNV in Atg5 knock-out cells (a key molecular component of the autophagic pathway), but no differences were found. These results allowed us to conclude that JUNV infection leads to an autophagic response in the infected cells; however, a functional autophagy pathway does not seem to be required for efficient virus replication.

MI-P12.
COMPARISON OF LIPID A MOIETIES OBTAINED FROM *Bordetella bronchiseptica* LPS CORE MUTANTS

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Bordetella bronchiseptica (*Bb*) can infect a variety of mammals including humans. Defective mutants of the lipopolysaccharide (LPS) structure are used to understand their role in bacteria–host interaction. On a *Bb* 9.73 background, three mutants were generated: *Bb*LP39, defective in the expression of *waaC* gene which codes for a heptosyltransferase of the core region; *Bb*3394, defective in a gene involved in core substitution with a GalNAc and *Bb*3398, defective in the glucose transfer to the first heptose of the core. In this work the Lipid A moieties were released from the LPS mutants, analyzed by mass spectrometry and compared with the wild type strain. It was interesting to note that while the wild type strain presents a hexa-acylated diglucosamine backbone substituted with a phosphoglucosamine unit, lipid A from *Bb*LP39 presented a pyrophosphate group. On the other hand, Lipid A obtained from *Bb*3394 mutant showed a hexa-acylated backbone carrying two phosphoglucosamine units. However, *Bb*3398 mutant showed a hexa-acylated backbone modified with phosphoethanolamine and pyrophosphorylethanolamine groups. How inactivation of genes involved in the core biosynthesis affects Lipid A structures is not clear. However these modifications are known to alter LPS toxicity as well as vary the charge of Lipid A involved in protection of the bacteria.

MI-P13.
PLANT GROWTH PROMOTION AND SIDEROPHORE PRODUCTION BY A *Lotus tenuis* ENDOPHYTIC *P. fluorescens* STRAIN

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Phosphate (P)-solubilizing bacteria constitute an important group of microorganisms able to improve plant growth and development. Growth promotion through increased P uptake is mediated by bacterial secretion of organic acids and is often combined with the production of other metabolites, such as siderophores, which play a role in biological control of soilborne phytopathogens. Siderophores are low-molecular-weight, Fe-chelating ligands synthesized by microorganisms that help a particular microorganism to compete effectively against other organisms for available Fe, restricting their growth. Furthermore, siderophores are beneficial to plants by solubilizing iron otherwise unavailable for the plant.
In this work, the isolate *P. fluorescens* M25 promoted *L. tenuis* growth both *in vitro* and in microcosm experiments. On the other hand, this isolate was resistant to a large group of antibiotics and produced siderophores under Fe-limiting conditions. Interestingly, these siderophores are similar to those produced by the *Lotus* pathogen *P. syringae* DC3000. Our results suggest that *P. fluorescens* M25 could promote plant growth under P- and Fe-limiting conditions such as restrictive alkaline soils that can be colonized by *L. tenuis*. Adittionally, this strain could antagonize *L. tenuis* pathogens by restricting their growth through the production of siderophores and other biocontrol metabolites.

MI-P14.
IDENTIFICATION OF SUMO TARGETS IN *Trypanosoma brucei* BY PROTEOMIC ANALYSIS

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SUMOylation is a conserved post translational modification that involves the covalent attachment of a small ubiquitin-like protein called SUMO to a variety of proteins participating in diverse cellular processes including transcriptional control, nuclear transport, DNA repair and signal transduction. The functional consequences of SUMO attachment depend on each particular substrate but are based on the alteration of the target interaction surface, leading mainly to changes in its activity or subcellular localization.
Previous studies performed by our group and others have shown the importance of SUMO in the biology of trypanosomatids. SUMO resulted essential for *T. brucei* procyclic (PC) and bloodstream (BS) forms and affect a number of biological processes. as can be inferred from a global proteomic map of SUMOylated proteins in *T. Cruzi*.
The aim of this work is to improve the proteomic identification of SUMO targets in *T. brucei* by performing SUMO chromosomal tagging. This strategy enables SUMO expression at physiological levels, avoids the competition with the endogenous form, while providing tags for tandem affinity purification of SUMO and SUMO conjugates. We have successfully purified SUMOylated proteins from PC and BS hemizygote clones, and obtained their proteomic profile. We are currently validating these results in vivo for the top 20 targets.

MI-P15.
FIRST NITRIC OXIDE SYNTHASE (NOS) IDENTIFIED IN CYANOBACTERIA: THE NOS FROM *Synechococcus* PCC 7335

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Nitric oxide (NO) is a free radical involved in several physiological processes in all living organisms. NO is synthesized by nitric oxide synthases (NOS) from the substrate L-arginine (L-Arg). In most plants and cyanobacteria, canonical NOS enzymes are lacking, and NO is synthesized by other enzymatic and non-enzymatic pathways. Recently, our lab characterized the first NOS from the green marine algae *Ostreococcus tauri* (OtNOS), a member of the plant kingdom (Foresi et al, 2010). Later, through bioinformatic analysis we found a sequence coding for putative NOS in the cyanobacteria *Synechococcus* PCC 7335 (SyNOS). SyNOS has high similarity with OtNOS. SyNOS contains the two oxygenase and reductase domains as mammalian NOSs do and an extra globin domain in the N-terminal. The NO production in *Synechococcus* PCC 7335 was studied using the NO specific fluorescent probe DAF-2FM DA. The addition of the L-Arg induces the production of NO in a dose dependent manner. The exposure of a *Synechococcus* PCC 7335 to UV-B radiation causes an induction of 2-fold of NO release indicating a putative function of NOS-derived NO in responses to UV-B. The full length of SyNOS was cloned and expressed in E. coli. The characterization of the biochemical and structural properties of SyNOS is in progress.
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MI-P16.
THE S-LAYER GLYCOPROTEIN FROM *Haloferax volcanii* PRESENTS NOVEL N-LINKED OLIGOSACCHARIDES

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The S-layer glycoprotein from *H. volcanii* has been used as a model for determining the steps involved in haloarchaeal protein N-glycosylation. This protein displays a known pentasaccharide at positions N13 and N83. The analysis of the glycans present in this glycoprotein is of increasing interest as post-translational modifications are known to be involved in the proper folding of proteins specially needed to survive in adverse physical environmental conditions. In this work, membrane fractions of *H. volcanii* H26 were fractionated by SDS-PAGE. The gel band corresponding to the S-layer glycoprotein was excised and digested with peptide:N-glycosidase F. The released oligosaccharides were separated and analyzed by HPAEC-PAD and by MALDI-TOF MS. Mass spectrum performed in the negative ion mode, presented a main ion attributed to a NAcGlc-NAcGlc(Hex)-SQ-Hex structure bearing different number of methyl substituents. However, when the analysis was performed in the positive ion mode, a main peak attributed to NAcGlc-NAcGlc(Hex)-$(SQ\text{-}Hex)_6$ was detected. MALDI-LID-MS/MS analysis of the main ions confirmed the assigned structures. Interestingly, MS/MS analysis in the negative ion mode was essential to determine the different structures. As far as we know, these high molecular weight oligosaccharides have not been reported in any haloarchaeal glycoprotein so far.

MI-P17.

HEME A BIOSYNTHESIS IN *Trypanosoma cruzi*, AN ESSENTIAL COFACTOR FOR CYTOCHROME *C* OXIDASE COMPLEX

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Trypanosoma cruzi, the etiological agent of the Chagas disease, presents nutritional requirements for heme. This parasite must acquire heme from different hosts and distribute it to the different hemeproteins. We are interested in elucidating the heme traffic to the mitochondrion and its conversion into heme A, the cofactor only seen in cytochrome c oxidase complex (C*c*O). It is assumed that the C*c*O is essential all along the life cycle of the parasite. In our lab, we identified the enzymes involved in heme A biosynthesis in *T. cruzi*: TcCox10 (heme O synthase) and TcCox15 (heme A synthase), and characterized their function in the yeast S. cerevisiae. The mRNA level of these genes were analyzed by qRT-PCR and they were detected along the different parasite life stages. We obtained specific antibodies against TcCox10 and TcCox15 and the presence of this proteins was observed in different life stages of the parasite by western blot analysis and their mitochondrial localization was analyzed by indirect immunofluorescence detection. We obtained nonfunctional proteins by site direct mutagenesis, overexpressed them in *T. cruzi* epimastigote and a dominant negative effect was observed. These preliminary results indicate the relevance of this pathway, at least for the epimastigote stage, suggesting the heme A biosynthesis might be essential for *T. cruzi* parasite.

MI-P18.

A MURAMIDASE IN *Brucella abortus* IS INVOLVED IN THE EARLY STAGES OF INTRACELLULAR REPLICATION

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Secretion of proteins in Gram-negative bacteria is a high-energy consuming process as it requires the translocation across two membranes and the peptidoglycan. To achieve this, bacteria have evolved complex secretion systems that cross these barriers and specific peptidoglycanases that degrade the peptidoglycan to allow the proper assembly of the secretion machinery. Analysis of the genome of *Brucella abortus* allowed us to identify a gene that we named *sagA* (Secretion Activator Gene A) coding for a putative lysozyme-like protein. We demonstrated that this protein has peptidoglycanase activity, that a strain with a clean deletion of the gene displayed a defect in the early stages of the intracellular replication in cells and that this is dependent on the lytic activity. While neither the attachment nor the invasion of the strain was affected we showed that the mutant had a defect in excluding the lysosomal marker LAMP-1 but not in acquiring the reticulum endoplasmic marker calnexin, indicating that the gene participates in the early steps of the intracellular trafficking but not in the establishment of the replicative niche. Examination of the genome of *B. abortus* resulted in the identification of a gene highly similar to *sagA*, designated *sagB*, also required during the early stages of intracellular replication. Our results suggest that these genes do not have redundant functions.

MI-P19.

MICROBIAL MEMBRANE AND SURFACE INTERACTION: A PHYSICOCHEMICAL APPROACH ANALYSIS

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Biofilms are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix. They can cause significant problems in many areas, such as medical and in industrial envairoments.

Primary adhesion constitutes the first step in biofilm formation. This initial and reversible stage is mediated by complex physicochemical interactions, including hydrophobicity and surface charges.

The aim of this study is to determine the cell surface characteristics of a *Pseudomonas spp*. strain isolated from an industrial contamination and grown in three different growth media. We propose a set of physicochemical criteria regarding three parameters: hydrophobic/hydrophilic character, Lewis acid-base interactions, and charge properties. Investigations of these three aspects were performed by using the microbial adhesion to solvents method and the DRIFT method.

The correlations between this parameters and bacteria primary adhesion to stainless steel and aluminum surfaces will be presented.

MI-P20.

***Staphylococcus aureus* ABC TRANSPORTER IS REGULATED BY SAOUHSC01313-01314 TCS UPON TEMPERATURE CHANGE**

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Staphylococcus aureus, a G+ bacterium, is an opportunistic pathogen capable of causing severe diseases. *S. aureus* methicilin-resistant (MRSA) strains are a major cause of nosocomial and community-acquired infections worldwide. A sudden change in temperature may be a signal for the pathogen of being inside the host, inducing the expression of virulence-related genes. Since two component systems (TCSs) allow bacteria to sense environmental parameters, we studied two contiguous genes located in *S. aureus* chromosome NCTC 8325, SAOUHSC01313-01314 coding for a putative histidine kinase (HKsa) and response regulator (RRsa), respectively. These proteins showed homology with *B. subtilis* DesKR TCS that regulates *des* transcription at low temperature. Linked upstream of this TCS are located two genes, SAOUHSC01311-01312, encoding a putative ABC transporter that carries in its promoter a regulatory box identical to that of *des*. In this work, we investigated whether signal sensed by HKsa mediates activation of ABC transporter. We performed ß-gal assays of *B. subtilis des*KR mutants carrying a transcriptional fusion of SAOUHSC01311 promoter to *lac*Z. We found that P*sa*01311-*lac*Z is activated when HKsa-RRsa are expressed under a conditional promoter, indicating that the *S. aureus* TCS, without additional factors, regulates ABC transporter expression.

MI-P21.
INTRACELLULAR SURVIVAL OF *Streptococcus pneumoniae* DEPENDS ON THE TWO-COMPONENT SYSTEM COMDE
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It has been described that the uptake of pneumococcus involved clathrin coated-vesicles, and bacteria proceed into vacuoles decorated by Rab5 and Rab7, the classical route to the lysosome. However, there is no data about the existence of a specific pneumococcal containing-vacuole and the intracellular life of this pathogen.
Using an in vitro infection model, we observed a decrease in the number of intracellular bacteria in the first hours, although it remains alive in macrophages and pneumocytes for at least 5 hours. By immunofluorescence microscopy, long bacterial chains were observed intracellularly. In macrophages, inhibition of lysosomal acidification by chloroquine increased bacterial clearance, suggesting that bacterial survival mechanism depends on lysosomal pH. On the other hand, we focused our study in bacterial mechanisms that would allow pneumococcus to survive inside cells, for example, signal transduction systems. We carried out survival assays using two-component systems mutants, and we found that the comE mutant evaded bacterial clearance and replicates inside the pneumocytes. These results suggested that pneumococcus was able to survive within macrophages and pneumocytes for several hours, and that its survival mechanism depends on lysosomal acidification. In addition, we showed that ComE is involved in controlling survival/death balance of pneumococcus inside cells.

MI-P22.
INHIBITION OF POLY(ADP-RIBOSE) POLYMERASE INTERFERES WITH *Trypanosoma cruzi* INFECTION AND PROLIFERATION
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Poly(ADP-ribosylation) is a covalent modification of proteins catalyzed by poly(ADP-ribose) polymerases (PARPs). Poly (ADP-ribose) (PAR) metabolism plays a role in a wide range of biological processes such as DNA damage repair, chromatin modification, transcription and cell death. In humans, 17 different genes have been identified that encode members of the PARP superfamily, in contrast, there is only one PARP present in *T. cruzi* (TcPARP). In *T. cruzi*, PARP enzyme appears to play a role in DNA repair mechanisms and may also be involved in controlling the different phases of cell growth. Here we describe the identification of potent inhibitors for *T. cruzi* PARP with a fluorescence-based activity assay. The inhibitors were also tested on *T. cruzi* epimastigotes, showing that they reduced PAR formation in vivo. The best inhibitor, Olaparib, reduced growth rate by 50% at 25 nanomolar. PARP inhibition also decreases drastically the amount of intracellular amastigotes (the replicative form of the parasite). Knocking down human PARP-1 decreases both the amount of amastigotes and trypomastigotes in cell culture, indicating that the effect would be mainly due to inhibition of human PARP-1. The result suggests that the inhibition of PARP could be a potential way to interfere with *T. cruzi* infection.

MI-P23.
MOLECULAR CHARACTERIZATION OF VIRULENCE FACTORS IN *Brucella abortus*
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Virulence of *Brucella* depends on the capacity to survive within the host cells. To achieve this the bacterium has evolved strategies to escape recognition by the immune system. Although much has been done on the mechanisms that allow the bacterium to survive within the host cell, few virulence factors have been identified to date. The aim of this work was to study and characterize a new virulence factor involved in the intracellular survival. Using a bioinformatic approach we searched the *Brucella* genome for genomic regions with potential horizontally transmitted characteristics with the premise that they might contain novel virulence factors. With this approach we identified several gene candidates that were flag-tagged to determine protein localization during infection. One of them, Bab1_1492, is secreted from the bacteria and, most probably, localizes to the *Brucella* containing vacuole. A deletion mutant in this gene showed a significant defect in the intracellular replication rate during the first hours of infection in professional and non-professional phagocytic cells. To further characterize this gene we evaluated the internalization process and the intracellular trafficking and showed that although the mutant strain has greater internalization, it has an increased degradation rate, consistent with the altered intracellular replication kinetic.

MI-P24.
EFFECTS OF NITRIC OXIDE (NO) ON T CELL RESPONSES IN CHRONIC CHAGAS DISEASE MICE
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We have previously shown an inhibitory effect of NO on the degranulation capacity of CD8+ T cells in a murine chronic infection with *Trypanosoma cruzi*. Herein, the action of NO on the *T. cruzi*-specific apoptotic and secretory capacity of cytokines by T cells in C3H/HeN mice infected with *T. cruzi*Sylvio-X10/4 was evaluated. In addition, the degranulation capacity of CD8+ T cells in relation to NO levels was investigated. Splenocytes and cardiac infiltrates were co-cultured with autologous monocytes in the presence of an NO donor (SNAP), NO inhibitor (L-NAME) or controls with media. The frequencies of CD8+CD107+ and CD8+Anexin+ T cells were measured by flow cytometry, while the levels of IFN-γ and IL-2 were determined by a capture ELISA assay. An inverse significant correlation between NO levels and the frequencies of CD8+CD107+ T cells specific for *T. cruzi* was found (slope=-3.99; p=0.02). *T. cruzi*-infected splenocytes produced high levels of IFN-γ, that was inhibited by SNAP and reverted by L-NAME addition. Apoptosis of CD8+ T cells was low and was not modified by SNAP or L-NAME. IFN-γ and IL-2 were secreted by cardiac infiltrates from chronically infected mice. The addition of SNAP induced a decrease in the levels of both cytokines, which was reverted by L-NAME. These findings support that NO might inhibit *T. cruzi* specific Th1 responses in chronically infected mice

MI-P25.

A GACS DEFICIENCY AFFECTS BACTERIOCIN PRODUCTION IN *Pseudomonas putida* MGR37

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The plant growth-promoting rhizobacterium, *Pseudomonas putida* MGR37 secretes bacteriocins which kill a wide range of bacteria related to the *Pseudomonas* genus. The aim of this work was the identification of genetic determinants involved in the production of such compounds. In this sense, a collection of *P. putida* MGR37 mutants was generated by Tn5-B21 mutagenesis and screened by the inability to produce antimicrobial compounds. An isogenic Tn5-B21 mutant that had lost the capacity to inhibit the growth of *P. fluorescens* CTR212 was selected and further characterized. This mutant, designated 646 carries a single transposon insertion into an ORF homologous to a *gacS* gene from several members of *Pseudomonas* genus. This gene encodes for a histidin kinase sensor of the two-component system GacA/GacS. In addition, the mutant 646 showed a cell phenotype of non-attached aggregates and a diminished in the biofilm formation. These results suggest that a mutation in the *gacS* gene affects a variety of phenotypes which are important for the bacterial competitiveness within the environmental. Complementation assays are being carried out to demonstrate the implication of the two-component system GacS/GacA in the production of antibacterial compounds such as bacteriocins.

MI-P26.

CHARACTERIZATION OF *Pseudomonas fluorescens* SF39A MUTANTS AFFECTED IN BACTERIOCIN PRODUCTION

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The objective of the present study was to identify genes involved in the biosynthesis or regulation of a bacteriocin produced by native strain *Pseudomonas fluorescens* Sf39a.
Random transposon mutagenesis of strain SF39a was carried out through the use of mini-Tn*5*Km1. Km-resistant clones were selected and screened for bacteriocin production. To identify the gene interrupted by the insertion of the transposon, flanking DNA region was cloned and sequenced in the mutants.
Three transconjugants affected in the bacteriocin production were found: mutants 451 and 495 showed a decreased bacteriocin production while mutant 375 presented an enhanced bacteriocin production.
The opposite phenotypes observed in clone 451 and 375 resulted from inactivation of the *ptsP* gene and *cbrA* gene, respectively. The former encodes a phosphoenolpyruvate phosphotransgerase and the latter encodes a sensor/histidine kinase of the two-component system CbrA/B. Both genes, *ptsP* and *cbrA*, play an important role in various virulence-related processes, such as mobility, biofilm formation and siderophore production. While, in the mutant 495 the transposon was inserted into a gene encoding a putative exported protein.
These results provide new information about a possible regulation and export mechanism in the bacteriocins produced by plant-associated *Pseudomonas*.

MI-P27.

ROLE OF *Trypanosoma cruzi* POLYPIRIMIDINE TRACT BINDING PROTEIN IN DICISTRONIC pre-mRNA PROCESSING

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Trypanosome gene expression clusters are co-transcribed by RNAPII to yield polycistronic pre-mRNAs. During mRNA maturation, polycistrons are processed into monocistrons by two coupled reactions, *trans*-splicing at the 5' and polyadenylation at the 3' end. We have previously observed that alternative *trans*-splicing can produce dicistronic pre-RNA molecules or transcripts with long 3'untranslated regions. These pre-RNAs should be processed by a post-transcriptional *trans*-splicing event to produce mature mRNAs. Our hypothesis is that *trans*-splicing is a regulated process serving as another mechanism for gene expression regulation. We focus in the identification of pre-RNA molecules and the analysis of *trans*-splicing regulation factors. In this context, we have previously described a dicistronic unit containing coding sequences for two U-rich RNA binding proteins. Now, we found that dicistronic RNAs from parasites transfected with fusion-tagged recombinant constructs are not translated into protein products. Furthermore, DRBD4, a trypanosome homologue of the PTB splicing regulator, is involved in the dicistronic processing. Finally, sequence analysis of a cDNA library obtained from co-immunoprecipitated DRBD4-associated RNAs allowed for the identification of 24 target transcripts, including 15 new putative dicistronic units, most of them coding for hypothetical and RNA-binding proteins.

MI-P28.

Azospirillum brasilense flmAB: ITS ROLE IN GLYCOSYLATION, MOTILITY AND ATTACHMENT TO MAIZE ROOTS

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Azospirillum brasilense is a diazotrophic motile microorganism found in the rhizosphere of cereals. The polar flagellum was shown to play a role in the adhesion of cells to plant roots. Previous studies have confirmed that the polar flagellin is glycosylated in *A. brasilense*, however, the genes required for such glycosylation as well as the role of this modification, have not been described yet. The aim of present study was to identify the genes of *A. brasilense* responsible for the glycosylation of the flagellin. *In silico* analysis revealed *flmA* and *flmB* genes as putative candidates. Mutations in either *flmA* (dehydratase) or *flmB* (aminotransferase) resulted in a severely impaired swimming phenotype. TEM analysis revealed the presence, in both mutants, of a truncated polar flagellum. Moreover, the attachment of these mutant strains to maize roots was significantly lower than the wild-type strain in competition assays, suggesting the participation of the polar flagellum in the establishment of the plant-bacteria interaction. Purified flagellins from the wild-type, mutants and complemented strains were separated by SDS-PAGE and then stained for the detection of sugars. Contrary to the wild-type strain, in both mutant strains no glycosylation signals were detected suggesting that the products of genes *flmA* and *flmB* are required for glycosylation of the flagellin.

MI-P29.
INSIGHTS INTO THE LIPOIC ACID METABOLISM IN *Staphylococcus aureus*
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Lipoic acid (LA) is a cofactor of several key enzymes involved in oxidative and single carbon metabolism. In the model gram-negative bacterium *Escherichia coli*, LipB transfers octanoate to target proteins and these octanoylated domains are converted into lipoylated derivatives by lipoyl synthase (LipA). Exogenous free LA can also be scavenged by the lipoyl ligase. However, in the gram-positive *Bacillus subtilis* two additional proteins are required: LipL and GcvH. Analysis of gram-positive pathogens genomes revealed that homologues to these genes can be found in most of them. The aim of this work was to study lipoylation mechanisms in *S. aureus*. The function of LA genes was inferred from genetic and physiological experiments. Although *S. aureus* encodes two lipoyl ligases homologs only one was able to functionally replace *B. subtilis* LplJ. The expression of *S. aureus* octanoyltransferase (LipM) restored growth of the respective *B. subtilis* mutant. Besides, *S. aureus* lipM mutant was unable to grow in minimal medium and this deficiency was rescued by the addition of the products of LA-dependent enzymes, suggesting that LA biosynthesis pathway might be conserved among gram-positive pathogenic bacteria. The knowledge about LA metabolism in pathogens is of great relevance because it might be an excellent target for the development of new antimicrobials.

MI-P30.
INTERRUPTION OF THE MUTS-ß CLAMP INTERACTION PROMOTES MUTATIONS COMPATIBLE WITH THE POLIV ACTIVITY
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The DNA mismatch repair (MMR) system corrects mismatched base pairs resulting mainly from DNA polymerase III replication errors. Interaction of the replication processivity factor ß clamp with the mismatch repair protein MutS is involved in early steps of MMR in eukaryotes and *Bacillus* sp. In a previous report, we demonstrated that the MutS-ß clamp interaction does not play a role in the MMR of *P. aeruginosa* by characterizing a strain harboring a chromosomal *mutS* allele (*mutS*^{ß}) which encodes a MutS mutant that does not interact with ß clamp. To establish the functional consequences of this interaction, we analyzed the *nfxB* mutation spectrum from the *mutS*^{ß} and parental strains. The *mutS*^{ß} strain, at difference of the parental strain, exhibited some mutations associated to the low fidelity DNA polymerase IV (PolIV) activity. Then, we determined the effect of enhance PolIV expression levels on the *nfxB* mutation spectrum. PolIV-associated mutations were increased in the *mutS*^{ß} strain whereas the *nfxB* mutation spectrum did not change in the parental strain. We propose that MutS could be limiting the action of PolIV by its interaction with ß clamp.

MI-P31.
DETECTION OF *parA* AND *parB* GENES IN MYCOBACTERIOPHAGES
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We have isolated and characterized 18 mycobacteriophages from environmental samples. They did not differ from most of the previosuly described ones in the predominant physical features (virion size in the 100-400 nm, genome size in the 50-70 kbp, morphological features of the Siphoviridae family). Mycobacteriophage genomes were sequenced using a GS-FLX 454 pyrosequencer (INDEAR service, Argentina). Completed sequences were analyzed by the dotplot generator Gepard, annotated with the genome editor DNAMaster and the alignment and phylogenetic studies were performed by ClustalW and MEGA5.
Twelve mycobacteriophage genomes were solved in one contig; their genome comparison by dot plot allowed for their classification in four groups, mainly matching groups reported in databases and a novel singleton which did not fall in this classification.
The genome of "First", a new phage (53028 bp in length) isolated during this work and capable of infecting *Mycobacterium tuberculosis,* contains 93 ORFs and a single tRNA. In spite of behaving as a temperate phage, we were unable to identify an integrase by bioinformatic analysis. However, we detected the presence of *parA/ parB* genes in this mycobacteriophage and in other four ones capable of infecting *M. tuberculosis.* Thus, an inheritance mechanism, less frequently described in other phages, seems to be highly relevant for mycobacteria phages.

MI-P32.
ANALYSIS OF GENOMIC SEQUENCE OF TCH RESISTANT MUTANT FROM *Mycobacterium tuberculosis*
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2-thiophen carboxylic acid hydrazide (TCH), used to differentiate between clinical isolates of *Mycobacterium tuberculosis* and *Mycobacterium bovis*, exhibits high activity at the low microgram level only against these two pathogens. However, there is no information on its possible mechanisms of action. Our preliminary results showed that TCH does not inhibit mycolic acid synthesis, altthough it causes cell lysis and requires KatG, the catalase-peroxidase activator of the anti-tubercular drug Isoniazid. Aliquots of the reference strain *M. tuberculosis* H37Rv cultures were plated with different concentrations of TCH. In this way, we obtained 8 TCH resistant mutants and determinated their genomic sequence by Whole Genome Sequencing. Our bioinformatics analysis revealed a mutation in four of those mutants, located in the promoter region of *ahp*C (alkylhydroperoxidereductase). This mutation was previously described in the literature only as associated with mutations in *kat*G. However, our mutants presented a wild-type sequence of this gene. The other four mutants displayed mutations in non essential genes (i.e. a putative malate synthase, a sensor of a two component system) or intergenic regions (upstream of the Rv3717 gene, involved in peptidoglycan recycling).We are currently constructing a merodiploid *kat*G strain to isolate TCH mutants in order to identify the molecular target for this drug.

MI-P33.
MORE THAN THE EYE MEETS: FAST-GROWING MYCOBACTERIA SUSCEPTIBLE TO THIACETAZONE AND ISOXYL

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Thiacetazone (TAC) and Isoxyl (ISO) are anti-tubercular drugs that do not inhibit fast growing mycobacteria, such as *Mycobacterium smegmatis* (MSMEG). Our findings on TAC and ISO hypersensitive (Hs) MSMEG mutants led us to study each phenotype. The HadABC complex (b-keto acyl ACP dehydratase) and the mycolic acid methyl transferases (MAMTs) of mycolic acid synthesis are involved in TAC resistance in *M. tuberculosis* (Mtb). However, their over-expression failed to restore growth of the strain TAC Hs UNR18 at high temperature. No nucleotide alteration was detected in these genes in MSMEG. Interestingly TAC-treated MSMEG and UNR18 at non permissive temperature lacked α_2-mycolic acids. On the other hand, the Mtb stearoyl desaturase DesA3 has been postulated as a target for ISO, however our results showed no increase in resistance by its over-expression. Deletion of the *MSMEG_*1886 Δ*desA3* homolog- caused growth alterations and lack of palmitoleic acid, suggesting the existence of other(s) fatty acyl desaturase(s). Bio-informatic analysis showed the presence of several of such desaturases in mycobacterial genomes that might mediate natural resistance to ISO. Supporting this hypothesis, MSMEG Δ*desA3* was four-fold more susceptible to ISO than the parental strain. Taken together we demonstrated that TAC and ISO have targets in MSMEG.

MI-P34.
CYTOCHROME P450 REDUCTASES OF *Trypanosoma cruzi*: SUBCELLULAR LOCALIZATION AND FUNCTIONAL ANALYSIS

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Cytochrome P450 (CYPs) reductases are an extensively described superfamily of enzymes involved in the synthesis of endogenous compounds such as steroids, as well as in the detoxification of foreign compounds, including therapeutic drugs. A single Cytochrome P450 reductase (CPR) is known to interact with the CYPs in most organisms. We have identified and characterized a gene family consisting of three Cytochrome P450 reductases (CPRs) in *T. cruzi*, named TcCPR-A, TcCPR-B and TcCPR-C over expression. Although recombinant TcCPR-A, -B and -C were able to complement CYP activities in an *in vitro* reconstituted system, only TcCPR-B and -C overexpression in the parasite increased ergosterol membrane content, while TcCPR-A resulted lethal. We present here the results of complementation in yeast: expression of TcCPR-B and -C in the CPR knock-out *Saccharomyces cerevisiae* strain WR can restore yeast normal growth, while expression of TcCPR-A cannot. This suggests a different role for TcCPR-A, in agreement with our previous observations. Since TcCPR-B and TcCPR-C presented different localization in previous immunofluorescence studies, their putative transmembrane domains were fused to GFP. Chimeric proteins presented similar localizations to the wild type, in the reservosome and ER respectively. This would indicate that the N-terminal transmembrane domain fragments cloned contain sequences that commit protein localization.

MI-P35.
EFFECT OF STIGMASTANE DERIVATIVES ON HERPES SIMPLEX TYPE 1 REPLICATION AND APOPTOSIS IN NEURONS

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The stigmastane derivatives (22S,23S)-22,23-dihydroxystigmast-4-en-3-one (1) and (22S,23S)-3ß-bromo-5a,22,23-trihydroxystigmastan- 6-one(2) prevent Herpes Simplex Virus type 1 (HSV-1) multiplication *in vitro* and reduce the incidence of herpetic stromal keratitis in a murine model of corneal infection. Considering that at least 90% of the population is infected with HSV-1 and the primary site of virus latency are sensory neurons, we decided to investigate the antiviral effect of both compounds in neuronal cells and also its involvement on cell apoptosis. Three different cellular lines (PC-12, Neuro-2a and SH-SY5Y) were infected with strains KOS and KOS Cgal+ of HSV-1 and then treated with compounds 1 and 2 under no cytotoxic concentrations. The results obtained by plaque assay and stain in situ for ß-gal activity showed that the compounds inhibit up to 99% the replication of both strains of HSV-1 in all cellular lines tested. On the other hand, the study of apoptosis by flow cytometry produced encouraging results. Cell treatments with both compounds under cytotoxic concentration as well as HSV-1 infection induced neuronal apoptosis. Despite of this, the addition of compounds 1 and 2 to the infected cells diminished cell death by apoptosis. We report here that compound 1 and 2 not only have antiherpetic properties but also exert an anti-apoptotic effect in neurons infected with HSV-1.

MI-P36.
POLYAMINE CATABOLISM IN THE PLANT PATHOGEN *Pseudomonas syringae*

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In bacteria, many mechanisms regulate polyamine (PA) intracellular levels. The research conducted on this subject has often been limited to biosynthesis and uptake rather than catabolism. This work aimed to explore the PA catabolic pathways existing in the plant pathogen *Pseudomonas syringae*. Putrescine and spermidine were the most abundant PAs in this strain. In addition, bacteria were able to grow in culture media amended with these compounds as nitrogen sources, proving the existence of catabolic routes for them. In this trend, the analysis of the bacterial genome identified four genes belonging to two different degradation pathways in which PAs are oxidatively deaminated. qPCR performed on two of these genes demonstrated the existence of a regulatory mechanism that reduces their expression when putrescine is added to the culture medium. In turn, the PA analogue 1,12-dodecanediamine, which inhibits PA oxidases from plants and mice, blocked bacterial multiplication at 10 µM, suggesting that these enzymes play a major role in the growth of the microorganism. In addition, the inhibitor was unable to prevent the infection of *Arabidopsis* plants, even though it should be kept in mind that this outcome may be due to a balance between the inhibition of bacterial growth and the attenuation of host defense mediated by plant PA oxidases previously reported by our group.

MI-P37.
PYROSEQUENCING ANALYSIS OF RHIZOSPHERIC AND LEAF-ENDOPHYTIC BACTERIAL COMMUNITIES OF TOMATO PLANTS

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Plant-associated bacteria are of key importance for sustainable agriculture. Bacterial communities associated to tomato plants, and particularly those that colonize leaves as endophytes, are poorly known. Pyrosequencing of the V4 region of the 16S rRNA gene was performed on rhizospheric and leaf-endophytic DNA samples. Tomato plants were grown in a greenhouse in soil collected from an organic tomato plantation and were used to obtain rhizospheric and endophytic DNA. A total of 19,403 and 16,562 sequences were obtained from rhizospheric and endophytic samples, respectively. Based on their similarity, sequences were clustered using UClust, thus obtaining 3,453 total OTUs (Operational Taxonomic Units). Rarefaction curves revealed significant differences in biodiversity between both communities. OTUs abundance was lower in the endophytic than the rhizospheric community. Leaf-endophytic samples saturated at about 80 OTUs while soil samples increased over 1,700 OTUs. Endophytic communities were represented mainly by unknown bacteria, while among classified bacteria, γ-Proteobacteria was the main group. Almost 45% of the rhizospheric communities was represented by Verrumicrobia and Acidobacteria. These results demonstrate that a relatively small group of rhizospheric bacteria is able to colonize plant leaves as endophytes, the majority of which are unknown.

MI-P38.
SCREENING OF ESSENTIAL GENES FOR COLD GROWTH IN *Pseudomonas extremaustralis*

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P. extremaustralis is an Antarctic bacterium capable to grow and survive at low temperatures with high stress resistance in association with the accumulation of large amounts of polyhydroxybutyrate (PHB). A mini Tn5 library was constructed to detect essential genes for growth under cold conditions. The resulting collection was screened in Luria-Broth agar plates supplemented with 0.25% sodium octanoate and were incubated at 10°C during 7 days; controls were performed at 28°C. Five mutants were detected and two of them, C42 and C60, were identified using a PCR based strategy.
The sequence analysis indicated that the transposon insertion site for C42 was located in the pyrroloquinoline quinone biosynthesis cluster (pqq), specifically in the *pqqB* gene and in the *wabH* gene coding for a glycosyltransferase for C60. At 28°C in liquid cultures both clones presented similar final OD_{600nm} compared with the wild type strain and showed PHB accumulation. However, the C42 clone showed a higher growth rate during exponential phase, while the C60 exhibited a long lag phase and high adherence. In contrast, none of the mutants grew at 10°C while the wild type strain reached an OD_{600nm} value of approximately 1.The phenotype was not altered by changes in the carbon source. The results describe for the first time the involvement of these two genes in cold survival and adaptability.

MI-P39.
EFFECT OF PHOSPHATE ON DIFFERENT VIRULENCE TRAITS OF UROPATHOGENIC *Escherichia coli* ISOLATED FROM PROSTATITIS

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Uropathogenic *E. coli* (UPEC) strains, the most frequent cause of urinary tract infections, has several virulence factors to colonize host tissues. Biofilm formation allows cells to persist a long time in the genitourinary tract and interfere with bacterial eradication. We have shown that laboratory *E. coli* cells grown in media containing phosphate (Pi) concentrations =35 mM were defective in biofilm formation. The aim of this work was to determine a possible Pi-dependent effect on biofilm formation, production of attachment factors and antibiotic resistance in *E. coli* prostatitis isolates. A total of 58 strains collected from patients were analyzed for *in vitro* biofilm formation by the O'Toole and Kolter method technique and confocal microscopy. Curli and cellulose production was evaluated with Congo Red and Calcofluor assays, respectively. 65.5% of the isolates were considered positive for *in vitro* biofilm formation, but different behaviors were observed in respect to the media phosphate concentration. MIC for nalidixic acid and ciprofloxacin was determined in planktonic and static cultures changing Pi concentrations. When antibiotics were added after 24 h over a preformed biofilm, no sensitivity was observed. This work is a first approach that shows how environmental Pi concentrations could modulates different phenotypes related with UPEC virulence.

MI-P40.
INORGANIC PHOSPHATE MODULATES BIOFILM FORMATION AND STRESS RESISTANCE IN *S. thyphimurium*

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In several organisms, inorganic polyphosphate (polyP) formation was shown to be critical for attributes such as motility, quorum sensing, biofilm formation, resistance to stress, and stationary-phase survival. We have previously reported that, stationary *E. coli* cells grown in media containing at least 37 mM phosphate (Pi) maintain high polyP levels and high viability. They also exhibit resistance to H_2O_2 and low capacity to form biofilm. Here, the effect of different Pi concentrations on cell viability, polyP level, oxidative stress resistance and biofilm formation capacity (BFC) was evaluated througout the growth curve of *S. thyphimurium*. Viability, oxidative stress resistance and polyP levels were high in stationary phase cells grown in high Pi concentration media (=25 mM). Biofilm formation in polystyrene, glass, polypropylene and stainless steel were evaluated in static condition at 30ºC with O'Toole and Kolter method. A preference for glass or polypropylene surfaces was observed. At 24 h, BFC was dependent on the Pi concentration of the planktonic preinocula and of the incubation media. BFC was maximal at 20 mM Pi. According to our results, we conclude that Pi concentration is an important signal for biofilm formation and stationary phase fitness in *S. thyphimurim* cells.

MI-P41.
MANNITOL 2-DEHYDROGENASE ACTIVITY AT DIFFERENT GROWTH PHASES IN *Lactobacillus reuteri* CRL1101

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Mannitol is a sugar alcohol used in food, chemical, and pharmaceutical industries. Microbial mannitol production is being investigated as an alternative approach to the current industrial chemical production. Lactic acid bacteria, particularly *Lactobacillus*, are efficient mannitol producers in presence of fructose through mannitol 2-dehydrogenase (MDH) enzyme activity. In this work, the highest MDH activity (3.94 U/mg protein) was observed in *Lactobacillus reuteri* CRL1101 log phase cells (8 h) grown under agitated conditions in MRS broth (glucose 2%, w/v) supplemented in lag phase with fructose (5%, w/v). Although in these conditions, mannitol production (16.4 g/l) was higher than those in static culture (10.4 g/l), the highest mannitol production (40.3 g/l) was obtained in the static culture after 72 h. The CRL1101 *mdh* gene (99% identity with *mdh* of *L. reuteri* ATCC 53608 and *L. reuteri*; GenBank: AAS55855.1 and ABF06654.1, respectively) and a putative transcriptional regulator gene that might be related to its regulation were amplified by using specific primers designed from Lreu_1860 and Lreu_1859 of ATCC53608 strain. The structural genes and potential promoter and terminator sequences have been identified. Although *L. reuteri* CRL1101 are recalcitrant to electroporation, the potential isolation of *mdh* mutants, using pJP042 and p29cat232Small plasmids, is currently being evaluated.

MI-P42.
FUNCTIONAL ANALYSES OF IMMUNITY PROTEINS FROM BACTERIOCINS PRODUCED BY *Enterococcus mundtii*

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Mundticin CRL1656 (mun CRL1656) and enterocin CRL35 (ent CRL35) are two subclass IIa bacteriocins. The entire biosynthetic cluster of ent CRL35 was previously characterized, and that from mun CRL1656 was identified in the recently sequenced genome. *In silico* analysis of both clusters revealed one ORF encoding a putative immunity protein (*munC*) being part of an operon structure related to the bacteriocin production. In class II bacteriocins that use components of the mannose phosphotransferase system of susceptible cells as targets, immunity proteins form a complex with the receptor and the bacteriocin and prevent cells from being killed. Another mechanism has been described for some class II bacteriocins, which relies on the activity of a multidrug transporter protein. In this work a functional analysis of both MunC proteins was performed. Primers were designed to amplify both *munC* genes. Expected size fragments were cloned into pNF8 vector downstream GFP gene and under P*dlt*. The recombinant plasmids were introduced into *Listeria monocytogenes* and *L. innocua* 7(Li7). Only recombinant cells of Li7 were recovered. These clones grew in the presence of cell free supernatant (CFS) from *E. mundtii* CRL35, CRL1656 and synthetic ent CRL35. Li7 plus pNF8 and Li7 were sensitive to all CFS. These results demonstrated that MunC is actively expressed in *Listeria*.

MI-P43.
PHENOTYPIC RESISTANCE AND BETALACTAMASE PRODUCTION IN ENTEROBACTERIA ISOLATED FROM BOVINE MASTITIS I

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Escherichia coli is a common cause of environmental intramammary infection in dairy cattle. Antimicrobials are an important tool in mastitis control programs. The surveillance of antimicrobial resistance is important to ensure optimal results of antimicrobial use and minimize the risk for selection and spread of antimicrobial resistance. So, the purpose of this survey was to investigate the *E.coli* antimicrobial panorama associated with bovine mastitis in Brazilian and Argentine dairy cattle's, and the presence of distinct phenotypes of betalactamases. The Brazilian milk samples, were from five dairy cattle farms located in three different towns of the State of Rio de Janeiro. Five farms from Córdoba were also selected. Most *E.coli* were susceptible to all antimicrobial tested and this highest sensibility was observed in 73,68% from Argentina and 58,82% from Brazil strains. A total of 15,79% of argentine strains were resistant to any betalactams antimicrobial while this number raised up to 64.71% in Brazil. No previous data were found in the literature on phenotypic studies on betalactamases in *E. coli* strains isolated from bovine mastitis in Latin America. The present findings represent the first report on ESßL-producing *E. coli* from the milk of cow affected by mastitis in Latin America.

MI-P44.
FASR, A NOVEL POSITIVE REGULATOR OF THE *FAS* GENE IN MYCOBACTERIA

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M. tuberculosis remains a major human public health threat. The success of this pathogen largely stems from its remarkable capacity to survive within the infected host, being its unusual cell wall a key factor in this survival. Mycobacteria cell wall biosynthesis involves two fatty acid synthase systems, FAS-I and FAS-II. We have previously characterized a novel regulatory protein of the main *fasII* operon named MabR. Our leading studies on the regulation of this operon provided strong evidences of the existence of a sophisticated coordination of the two mycobacterial FAS systems at the transcriptional level. Recently, we were able to identify and purify from *M. smegmatis* crude extracts, a new transcriptional regulator of the *fas* gene, named FasR. This regulatory protein specifically recognizes two repeated sequences in the *fas* gene promoter region (P*fas*). Beta-galactosidase and EMSA assays indicated that FasR is a positive regulator of *fas* and that its binding to P*fas* is impaired by long chain acyl-CoAs. Construction and characterization of a *fasR* conditional mutant in *M. smegmatis* demonstrated that this regulatory protein is an essential component of the regulatory network involved in maintaining lipid homeostasis, therefore an attractive target for the development of new and specific antimycobacterial drugs.

MI-P45.
CHARACTERIZATION OF BETA-OXIDATION PATHWAY IN *Streptomyces coelicolor*
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Whilst the vast majority of bacteria synthesize polyhydroxyalkanoates as a carbon storage compound, actinomycetes have the almost unique capability of accumulate triacylglycerol (TAGs) in the form of lipid droplets. Utilization of stored TAGs involves the beta-oxidation of long chain fatty acids to generate acetyl-CoA. Nevertheless, there is no substantial information regarding genes involved in this process in actinomycetes. By means of methodical bioinformatics approaches using the genes *fadA* and *fadB* (coding for the fatty acid beta-oxidation complex) from *E. coli* as probe, we identified three genes coding for putatives *fadB*, each one associated to a putative *fadA* gene, suggesting the existence of three possible operons in *Streptomyces coelicolor* genome. We tested the capability of simple *fadA* and *fadB knockout* mutants to grow on fatty acids of different chain lengths as sole carbon and energy source and we observed no changes in growth rates compared to the wild type strain. However, there is a strong difference in TAG content in submerged liquid culture. While the wild type strain presents a marked depletion of the pool of TAGs at late-stationary phase of growth, this depletion is nearly inexistent in the mutant strains. Further construction and analysis of double and triple *fadA/B* mutants and analysis of expression profiles constitutes experimentation currently in course.

MI-P46.
CHARACTERIZATION OF A TRANSCRIPTIONAL REGULATOR OF MYCOLIC ACID BIOSYNTHESIS IN MYCOBACTERIA
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Mycolic acids, the dominant feature of the *Mycobacterium tuberculosis* outer membrane, are essential for the survival, virulence, and antibiotic exclusion of this human pathogen. Mycobacteria, unlike most bacteria, have two fatty acid synthases (FAS-I and II). Both of them are involved in the biosynthesis of mycolic acids. Our research group identified MabR as a new transcriptional regulator that controls the expression of *fasII* operon genes, by binding specifically to the *fasII* promoter region. The construction and characterization of a MabR conditional mutant in *M. smegmatis*, allowed us to demonstrate that this protein modulates the expression of *fasII* genes *in vivo*. We also confirmed that MabR is essential to the regulatory network involved in maintenance of lipid homeostasis and that such regulation is modulated by long chain acyl-CoAs or long chain acyl-ACPs that appear to be the signal molecules recognized by this protein. In order to understand the molecular bases of the MabR interaction we set up to obtain the crystal structure of this protein. Laminar crystals were obtained using crystallization kits based on sitting drop vapor diffusion. Currently, we are optimizing the crystallization conditions in order to obtain a suitable crystal for structural function studies. MabR might become an excellent new target for the development of conceptually new antimycobacterium compounds.

MI-P47.
IN VITRO* RECONSTITUTION OF THE COMPLETE MYCOLIC ACID CONDENSATION SYSTEM OF *Mycobacterium tuberculosis
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Mycolic acids are essential for the survival, virulence and antibiotic resistance of the human pathogen *Mycobacterium tuberculosis*. In mycobacteria, the acyl-CoA carboxylases (ACCases) provide carboxyacyl-CoAs, the building blocks for *de novo* fatty acid and mycolic acid biosynthesis. Malonyl-CoA is produced by ACCase 6 and is necessary for both fatty acid and mycolic acid biosynthesis. Mycolic acid is synthesized by the condensation of a long-chain carboxyacyl-CoA and a meromycolil-AMP by Pks13. So far, there are no conclusive results on the subunit composition of the ACCase responsible to generate the long-chain carboxyacyl-CoA. To solve this issue, we performed an *in vitro* assay in which we mixed different ACCase subunits together with the enzymes FadD32 and Pks13, and looked for the condensation products. We found that the subunits AccA3, AccD4, AccD5 and AccE5 form the active ACCase that generates the long-chain carboxyacyl-CoA. Furthermore, by generating an *accD5* conditional mutant in *M. smegmatis* we demonstrated that *accD5* is essential in this bacterium, and is necessary for optimal levels of mycolic acid biosynthesis. These results propose AccD5 as a good target for the development of novel antimycobacterial drugs.

MI-P48.
ALLANTOIN METABOLISM IN *Streptomyces coelicolor*: CROSSTALK WITH ANTIBIOTIC PRODUCTION
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Here we studied the allantoin metabolism in the biotechnologically important strain *Streptomyces coelicolor*. This bacterium is characterized by a complex life cycle, including programmed cell death. In submerged cultures of *S. coelicolor*, growth arrest corresponds to a transition phase from a first vegetative mycelium to a second differentiated mycelium. Remarkably, antibiotics are produced by this second mycelium and depend on growth condition such us cell density and probably assimilation of cell components released by cell lysis. Our results have shown that the catabolism of allantoin in *S. coelicolor* causes a decrease of antibiotic production. We have identified and characterized proteins involved in the allantoin pathway including allantoinase, allantoicase and malate syntase. Genetic studies allowed us to validate this as the only pathway to metabolize allantoin in *S. coelicolor*. Phenotypic analysis of allantoinase or allantoicase mutant strains showed that the catabolism of allantoin and release of ammonium is probably the cause of the decreased antibiotic production. Furthermore, metabolic and proteomic studies revealed a downregulation of nitrogen uptake enzymes, accumulation of urea and several aminoacids, suggesting that in these conditions nitrogen is in excess. These observations link the impairment of antibiotic production with an unbalance in nitrogen metabolism.

MI-P49.
SOLUBLE FACTORS OF *Lactobacillus reuteri* CRL1098 MODULATE THE IMMUNE RESPONSE OF LPS–CHALLENGED RAW 264.7 CELLS
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Soluble factors produced by probiotic bacteria such as lactobacilli can modulate the immune system responses. Previous studies in our laboratory showed the immunomodulatory capacity of *Lactobacillus reuteri* CRL 1098 and its supernatant on peripheral blood mononuclear cells. In this work we investigated the nature of the soluble factors responsible of immune modulation and their effect on lipopolysaccharide–treated RAW 264.7 cell line. *L. reuteri* survival and extracellular protein production in RPMI 1640 medium were evaluated. The cytotoxicity of the bacterial culture supernatant was determined by MTT assay. The cytokines and nitric oxide (NO) produced by RAW 264.7 cells were measured by ELISA and Griess assay respectively. *L. reuteri* was able to survive 8 h at 37ºC in RPMI 1640 medium producing 12 µg/ml of protein at exponential growth phase. A dilution of 1:3 of the bacterial supernatant does not exert toxic effects on RAW 264.7 cells after 24 h of incubation at 37 ºC. A sub–cytotoxic dose of *L. reuteri* supernatant was pre–incubated with $1x10^6$ cells before LPS challenge; results showed that the levels of TNF–α, IL–6 and NO were significantly lower (34, 65 and 58% respectively) than the LPS control cells, while IL–10 release was not affected. A peptide ‹10 kDa and ›3 kDa is responsible of immunomodulatory effect.

MI-P50.
MICRORNAS IN CESTODE PARASITES
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Objective: *Echinococcus spp* are endemic cestode parasites of sanitary importance and constitute an interesting model for developmental studies. Our objective is to study the mechanisms of development of *Echinococcus spp* focusing on the role of small RNAs, in particular microRNAs (miRNAs), in this process.
Methodology and results: We showed the presence of miRNAs in *E. granulosus* by construction and bioinformatic analysis of a small RNAs library and Northern blot validation. According to homology and RT-qPCR analyses, these miRNAs are also present in *E. multilocularis*. Furthermore, we found miRNAs stage specific expression, suggesting they play a role in development. We also analyzed the possible miRNAs´ target genes by miRNA binding sites prediction and observed that they would be involved in the regulation of development, homeostasis and host-parasite interaction. We also identified the miRNA biogenesis machinery components in *Echinococcus spp*. genomes by *in silico* analyses and found Ago proteins specific for parasitic flatworms.
Conclusions: Gene expression regulation by miRNAs opens a new field to study the molecular bases of development of parasite cestodes, which is of main importance in biomedicine. Now, we are designing high throughput strategies to analyse the complete repertoire of small RNAs and also silencing protocols to analyse their role in development.

MI-P51.
TRANSLATIONAL STRATEGY OF NEW WORLD ARENAVIRUSES: ROLE OF THE VIRAL NUCLEOPROTEIN
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Translation of viral proteins is a key factor for viruses, which are obligate intracellular parasites and entirely dependent on the cellular translation machinery to synthesize their proteins. The aim of this work was to analyze the initial steps of protein translation during Junin (JUNV), Tacaribe (TACV) and Pichinde (PICV) virus infection. Results showed that silencing of eIF4E by a siRNA had not effect in viral protein synthesis of JUNV, TACV and PICV, respectively, suggesting an eIF4E independent translation mode. Involvement of eIF4GI, was evaluated using an specific siRNAs against this factor and by the expression of the poliovirus 2A protease that cleaves this factor. Both strategies showed a significant reduction in JUNV protein synthesis. Similar results were obtained for eIF4A, in which a significant reduction in JUNV virus replication was observed by treatment with the inhibitor hippuristanol. On the other hand, microscopy studies showed co-localization of JUNV nucleoprotein (N) with eIF4GI strongly suggesting that this viral protein would be part of the translation complex of viral mRNAs. Additionally, the presence of JUNV-N in eIF4GI and eIF4A, but not in eIF4E immune-precipitated complexes was observed. Finally JUNV-N was able to bind to a 7-methyl cap column, strongly suggesting that N could be a cap-binding factor that replaces eIF4E.

MI-P52.
IDENTIFICATION OF A NOVEL LIPOPOLYSACCHARIDE BIOSYNTHESIS GENE IN *Bordetella bronchiseptica*
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Bordetella bronchiseptica is a respiratory pathogen of different mammals, including humans. The lipopolysaccharide (LPS) of this pathogen plays an important role in bacterial host interaction not only as adhesin but also in protection. Here we describe one particular gene BB3394 and confirmed its function as a glycoyltransferase involved in the biosynthesis of the *B. bronchiseptica* core oligosaccharide. By insertionally inactivating this gene and studying the resulting LPS structure, we showed that BB3394 is involved in core lateral substitution with a GalNA. The MALDI-MS spectrum of the mutant BB3394 strain showed the highest mass species at m/z 1259.589 consistent with the core fragment (Hep)GlcN-Glc-(GlcNGlcAHep)Hep. The lack of higher m/z species would indicate the absence of the GalNA unit. The lateral position of this sugar explains previously described absence of differences in SDS PAGE profiles of the mutant compared to wild type LPS.
With the description of Bb3394 only two more glucosiltransferases remain to be discovered in order to know all enzymes involved in *B. bronchiseptica* LPS synthesis. Knowledge of this information is necessary to manipulate LPS structure in order to determinate the role of this important molecule in *Bordetella* pathogenesis.

MI-P53.
PHENOTYPIC SWITCHING AND HYPERMUTABILITY IN *Pseudomonas aeruginosa* ADAPTABILITY
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During biofilm growth *P. aeruginosa* diversifies into different niche specialists. Small Colony Variant (SCV) is one of these adapted phenotypes which have been characterized as hyperadherent and highly biofilms producer cells. When SCV are grown on solid media, the wild type-like phenotype emerges from the edges of the colony indicating a switch between two phenotypic phases. However, molecular basis underlying SCV conversion and reversion remain unknown. We have previously obtained evidences for mutator-driven short-term adaptive evolution in biofilms, associated with increased rates of phenotypic diversification. Here, we explore the *P. aeruginosa* adaptability in long-term evolutionary experiments, by analyzing mutators mismatch deficient and WT strains subjected to controlled, successive and repeated rounds of SCV conversion/reversion by exposing bacteria to alternating cycles of biofilm growth (conversion) and growth in solid media (reversion). Our results suggest that SCV phenotypic switching is based on mutational events and that a high mutation rate do not alter the capacity of mutators to phenotypically switch, even maintaining the high capability of phenotypic diversification throughout the successive rounds of evolution. Whole genome sequencing of ancestral and final clones is being performed in order to evaluate those mutations which are involved in this adaptive process.

MI-P54.
ROLE OF THE MISMATCH REPAIR SYSTEM (MRS) DEFICIENCY ON THE EVOLUTION OF *Pseudomonas aeruginosa*
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Hypermutation may accelerate bacterial evolution in the short-term. However, it is not yet certain how much mutators contribute to adaptive genetic variation in natural systems. Here, we explore how *P. aeruginosa* (PA) mutators evolve throughout cystic fibrosis chronic airway infections (CAI). We choose CAI as a model system since (i) they are characterized by a high prevalence of mutators, mainly driven by a defective MRS (ii); they are typically established from a single persistent PA clone, which suffers adaptive radiation leading to a phenotypically diverse population. We performed a longitudinal and a cross-sectional study coupled with whole-genome sequencing of different isolates obtained from 2 CF patients (CFA and CFD) infected by single PA clones. Whole genomic sequencing in CFA includes 13 mutator isolates spanning 6 years and CFD 14 isolates spanning 20 years of evolution. Most isolates showed hypermutable phenotype. Hence, all analyzed genomes hold mutations in mutator genes, although coexistence of different lineages was observed, with one being the persistent and dominant. Accordingly, mutational spectra skew towards transitions and small indels. Most SNPs favored the support of single phylogenetic trees only leaving few homoplastic sites. However, a negative selection for most accumulated mutations was observed with a strong signature for genetic drift (dN/dS ratios <1)

MI-P55.
GLOBAL TRANSCRIPTIONAL ANALYSIS OF *Salmonella* ADAPTATION TO INCREASED LEVELS OF COPPER AND ZINC
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Salmonella is a highly prevalent food-borne pathogen. The understanding of how *Salmonella* modulate its gene expression repertoire to survive and replicate within host tissues is of particular relevance for human health. Transition metals such as copper and zinc are essential due to their requirement as cofactors in different metabolic processes. However, they also can be toxic even at low concentration, and bacteria have developed homeostatic systems to control their uptake and release. Recent studies have suggested that this control plays a critical role in virulence. We investigated the transcriptional response of *Salmonella* to these metal ions both in rich and minimal medium, using a customized whole-genome tiling arrays analysis. Groups of genes were clustered according to their behavior in the presence or absence of these metal ions. Regulation of expression of each individual gene was confirmed by both, RT-qPCR and *lacZ*-reporter assays, and minimum inhibitory concentration tests were performed to investigate their role in metal-ion homeostasis. Overall, these analyses allowed us to identify novel copper and zinc regulated genes and to uncover an effect of these metal ions on other previously described stimulons. This information reveals a complex regulatory interplay between different metal-ions and their homeostasis in bacteria.

MI-P56.
THE *IN VIVO* TRYPANOCIDAL EFFECT OF THE DITERPENE 5-EPI-ICETEXONE
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T. cruzi is the causative agent of Chagas´ disease. When cultured, this parasite is found mainly as epimastigote and, at lesser extent, as trypomastigote. Recently, we have found that 5-epi-icetexone (ICTX), obtained from a native plant, affects the growth of epimastigotes and amastigotes. In this study, we evaluated the effect of this compound on parasitic forms in Albino Swiss infected. The distribution of parasites in the tissues and parasitaemia were evaluated at different times after the infection. Parasitemia was measured by counting of parasites in blood. Thirty five days after infection, when parasitaemia declined, the animals were sacrificed and some organs were removed, fixed and processed for microscopy. Thin sections from each tissue were stained with hematoxylin–eosin and observed with light microscope. We found that parasitemia decreased when 0.3 mg of ICTX is administered. This effect was lower than that of Benznidazole, although ICTX was administered at lower doses and by a different via. We also observed some nests of parasites in the heart of either controls or treated with DMSO alone, while they were not observed in the mice treated with ICTX. In addition, inflammatory infiltrates caused by parasites were not observed in the heart of treated animals. We conclude that this compound is effective against *T. cruzi* even if they reside in a host.

MI-P57.
CGPA IS REQUIRED FOR LAG-LOG PHASE TRANSITION AT LOW TEMPERATURES IN *Brucella abortus*

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Our laboratory has previously identified through an N-terminal CAT-fusion screening, a set of secreted and/or periplasmic proteins in *B. abortus*. Serendipitously, we observed that a mutant in one of these genes (BAB1_1280) had a growth defective phenotype at 23°C, but grew as the wild type strain at 37°C. For this reason we named this gene *cgpA*, for Cold Growth Protein A. Sequence and biochemical analysis indicated that CgpA is a small outer membrane associated 9.8 KDa protein present in most alpha-proteobacteria. Our results indicate that the Δ*cgpA* growth defective phenotype is due to a lower replication rate rather than a diminished low temperature resistance. This is likely to be the result of a defective metabolic activity at low temperatures. In fact, our results from liquid culture experiments strongly suggest a role for CgpA on the transition from lag phase to log phase at low temperatures.
In order to identify other genes that participate in the same cold adaptive pathway, we generated a library of random transposon-inserted mutants using the Δ*CgpA* mutant strain as the acceptor and selected for growth at 23°C. Using this approach we isolated a set of phenotypic revertants and identified the gene affected in each one. Future characterization of these mutants and the genes affected will provide valuable information about *cgpA*-dependent low temperature growth in *B. abortus*.

ST-P01.
EXTRACELLULAR SPHINGOSINE-1-PHOSHATE STIMULATES NF-κB PATHWAY IN CELLS LACKING FILAMIN A

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Sphinghosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that regulates several physiological and pathological events, including inflammation and cancer progression. S1P might act as an intracellular second messenger or in an autocrine or paracrine manner through binding to five G-protein-coupled receptors named $S1P_{1\text{-}5}$. Hence, the biological response to extracellular S1P depends on which receptors are expressed in the surface. In many solid tumors, the inflammatory microenvironment stimulates NF-κB activation, converting this pathway in a critical link between inflammation and cancer. We have previously demonstrated the importance of intracellular S1P in NF-κB activation triggered by tumor necrosis factor (TNF) in melanoma cell lines that express Filamin A (FlnA), an actin-binding protein. Here, we show that extracellular S1P induces the activation of NF-κB only in cells lacking FlnA. To examine the NF-κB pathway, we analyzed IKK phosphorylation and IκBa degradation. Moreover, by using inhibitors and siRNA, our preliminary data suggest that PKCd might be involved in the pathway. To definitely address the role of FlnA, we silenced the protein in FlnA-expressing A7 cells and we are currently evaluating both TNF and S1P induced NF-κB activation. Also, further investigations are necessary to determine which S1PR is involved in the proposed mechanism.

ST-P03.
EXTRACELLULAR ATP REGULATES FOXO TRANSCRIPTION FACTORS AND CELL CYCLE PROGRESSION IN MCF-7 CELLS

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Forkhead box-O (FoxO) transcription factors regulate the expression of genes involved in DNA damage repair, apoptosis and cell cycle. Therefore, they play an important role in tumor suppression. Released nucleotides can regulate intracellular signaling pathways through membrane-bound purinergic receptors to promote or prevent malignant cell transformation. In this work, we studied the role of extracellular ATP in the modulation of FoxO transcription factors and in cell cycle progression in MCF-7 breast cancer cells. Western blot analysis showed that ATP induced the phosphorylation of FoxO1/3a, and also reduced the expression of FoxO1. The PI3K inhibitor Ly294002 and a siRNA against the serine/threonine kinase Akt demonstrated that these effects are mediated by the PI3K/Akt signaling pathway. In addition, ATP increased the expression of the cyclins D1 and D3 and diminished the inhibitory proteins p21Cip1 and p27Kip1, in a PI3K/Akt dependent manner as revealed by the use of Ly294002 and a siRNA against Akt. Flow cytometry analysis showed that ATP increases the number of cells in the S phase of cell cycle, effect that was significantly reduced in the presence of Ly294002 and bortezomib, a proteasome inhibitor. Our results suggest that extracellular ATP can play a pivotal role in breast cancer cell proliferation inducing cell cycle progression through the PI3K/Akt/FoxO pathway.

MI-P02.
THE ROLE OF KRUPPEL-LIKE FACTOR 6 IN H-RAS^{G12V}-INDUCED TRANSFORMATION

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KLF6 is a member of the Krüppel-like family of transcription factors which have diverse roles in the regulation of cell physiology including proliferation, signal transduction and apoptosis. Mutations or downregulation of *klf6* gene have been described in several human malignancies, suggesting a tumor suppressor function of KLF6.
We previously examined the effects of KLF6 on proliferation and transformation of NIH3T3 fibroblasts stably transduced with a constitutively activated form of H-Ras (G12V) whose expression is induced by tetracycline. Induction H-Ras^{G12V} increased the KLF6 protein level in a c-Jun N-terminal Kinases (JNK)-dependent manner. Also, ectopic expression of KLF6 following activation of Ras induced a G1 cell cycle arrest and decreased cell proliferation. Furthermore, constitutive expression of KLF6 was able to revert some transformed phenotypes typically induced by activated Ras, like density-dependent growth inhibition and colony formation in soft agar.
Here we show that KLF6 transcript levels are significantly raised in oncogenic H-Ras-transformed NIH3T3 cells correlating with increased levels of the p21 cyclin-dependent kinase inhibitor and E-cadherin, a suppressor of cellular invasion. However, no significant changes were detected on p53 levels. These results support a tumor suppressor function of KLF6 upon oncogenesis triggered by activated Ras.

ST-P04.
PERK SIGNALLING ACTIVATED BY GM2 ACCUMULATION

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The accumulation of misfolded proteins into the Endoplasmic Reticulum (ER) activates a signal transduction cascade called Unfolding Protein Response (UPR), which attempts to restore homeostasis in the organelle. (PKR)-like-ER kinase (PERK) is an early stress response element that attenuates protein synthesis. We demonstrated that Calcineurin (CN) associates with PERK, enhancing inhibition of protein translation and cell viability (Plos One 5:8e11925).
Nevertheless deregulation of UPR or prolonged ER stress promotes apoptotic cell death. PERK signalling, including proapoptotic transcription regulator Chop induction, persists under prolonged ER stress.
Chronic UPR is proposed to contribute to the pathology of many neurodegenerative diseases. GM2-gangliosidosis are characterized by a progressive neurodegeneration. However, the mechanisms that determine how GM2 accumulation triggers neuronal cell death remain unknown. We hypothesized that PERK activation participates in the pathogenesis of GM2-gangliosidosis. Here, we show a significant uptake of GM2 in N2a neurons loaded with exogenous ganglioside. In addition, thin layer chromatography and immunocytochemistry approaches revealed a pool of intracellular GM2 localized in the ER. Moreover, the abnormal ganglioside accumulation induces PERK activation, and provokes up-regulation of either CN or CHOP, at different time points

ST-P05.

VDR EXPRESSION IS REQUIRED IN 1a,25(OH)2-VITAMIN D3- RAPID ACTIONS IN SKELETAL MUSCLE CELLS

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1a,25(OH)2-vitamin D3 [1,25D] is a steroid hormone that produces biologic effects via both genomic and nongenomic pathways in skeletal muscle cells. The genomic responses occur through its binding with the vitamin D receptor (VDR). However, there is no information about the molecules involved in the initiation of rapid-nongenomic events. In our laboratory, we have previously demonstrated the stimulation of MAPKs through c-Src by 1,25D in skeletal myoblasts. Moreover, we reported that caveolae components mediate the fast effects of the hormone in the skeletal muscle cell line C2C12. In this work we obtained data indicating that the VDR is required in the non-genomic action of 1,25D. We knocked down (80%) vitamin D receptor expression transfecting C2C12 cells with 2 shRNAs against VDR. In these cells (VDR-KD) Western blot analysis show that 1,25D-induced Src activation and p38 phosphorylation are suppressed. In addition, ERK1/2 and Akt activation are also dependent on VDR expression in C2C12 skeletal muscle cells. These data evidence for first time that the VDR is involved in rapid events triggered by 1a,25(OH)2D3 in skeletal muscle cells, providing relevant information on the mechanism of initiation of the non-genomic hormone signal.

ST-P06.

GABA INDUCTION OF THE *Saccharomyces cerevisiae* UGA4 GENE DEPENDS ON THE QUALITY OF THE CARBON SOURCE

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The quality of the carbon source modulates the expression of UGA4 gene that encodes a permease capable of transporting gamma-aminobutyric acid into the cells. In the presence of a fermentable carbon source, UGA4 expression is induced by GABA while in the presence of a non-fermentable carbon source this expression is GABA-independent. The aim of this work was to study the mechanisms responsible for the differences in the profiles of UGA4 expression in both growth conditions. We found that the activity of the main transcription factors responsible for UGA4 induction by GABA varies depending on the quality of the carbon source. In glucose, the negative GATA factor Dal80 binds to UGA4 promoter; only after the addition of the inducer, the positive factors Uga3, Dal81 and Gln3 interact with the promoter removing Dal80 leading to gene induction. In contrast, in acetate the negative GATA factor remains bound to UGA4 promoter under all the conditions assayed, the positive factors are not detected bound in any conditions and in consequence, UGA4 is not induced. We also established the participation of Tor1 and Snf1 kinases on UGA4 expression and the subcellular localization of Gln3, a key positive GATA factor in this regulation. Using FT-IR spectroscopy we detected that gene expression changes induced subtle modifications in metabolic profiles.

† In memory of Pr. Gérard Déléris

ST-P07.

INTERPLAY BETWEEN TRANSCRIPTION FACTORS ACTING ON *Saccharomyces cerevisiae* UGA GENE

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Gamma-aminobutyric acid (GABA) transport and catabolism in *Saccharomyces cerevisiae* are subject to a complex transcriptional control that depends on the nutritional status of the cells. The expression of the genes that form the UGA regulon is inducible by GABA and sensitive to Nitrogen Catabolite Repression (NCR). GABA induction of these genes is mediated by Uga3 and Uga35/Dal81 transcription factors whereas GATA factors are responsible for NCR. Here we show that all members of the UGA regulon share the activation mechanism. Our results show that both Uga3 and Uga35/Dal81 interact with UGA genes in a GABA-dependent manner, and that they depend on each other for the interaction with their target promoters and the transcriptional activation. The typical DNA-binding domain Zn(II)2-Cys6 of Uga35/Dal81 is unnecessary for its activity and Uga3 acts as a bridge between Uga35/Dal81 and DNA. The trans-activation activity of the GATA factor Gln3 is exerted by its interaction with UGA promoters in response to GABA, indicating that Uga3, Uga35/Dal81 and Gln3 all act in concert inducing the expression of UGA genes. So, some interplay between the factors responsible for GABA induction and those responsible for NCR in the regulation of the three UGA genes is here proposed.

ST-P08.

COUNTING MOLECULES AROUND THE CIRCADIAN CLOCK WITH TARGETED PROTEOMICS

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Circadian clocks allow organisms to coordinate their behavioural and physiological rhythms in a daily cyclic environment. A fascinating phenomenon related to this clock is that the period of the rhythms is temperature compensated but the mechanisms responsible for this are poorly understood. The molecular mechanisms underlying circadian rhythms have been studied extensively and it was shown that the transcriptional-translational feedback loops formed by a set of core circadian genes control the regulation of circadian physiology. A major challenge in circadian biology is to derive models of gene regulatory networks that predicts the dynamic transcriptional events underlying gene expression. A major drawback of existing models is that little quantitative data has been used in their calibration. For example, little is currently known about the absolute molecular abundance of core clock mRNAs and particularly proteins, which is of central importance to understand most biochemical processes. I will combine molecular biology and state-of-the-art proteomics with computational biology to derive quantitative models of circadian gene regulation. The quantitative approach will be invaluable in developing circadian gene regulatory models to accurately predict the behaviour of regulatory networks underlying the circadian clock

ST-P09.
ALBUMIN OVERLOAD UP-REGULATES MKP-1 IN RENAL TUBULAR CELLS

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In renal tubular cells, albumin overload promotes both the transient activation of MAP kinases (ERK1/2, JNK1/2 and p38) and reticulum stress (RS). MAP kinase phosphatases (MKPs) are induced by several stimuli to inactivate MAPKs. MKP-1 is a nuclear MKP that dephosphorylates all MAPKs. Here we analyzed the effect of albumin (BSA, 20 mg/ml) in a proximal tubule-derived cell line of opossum (*Didelphis virginiana*) origin (OK cells). Western blot analysis showed that BSA promoted MKP-1 accumulation after 1 h of stimulation (2-fold). Since the MKP-1 genome sequence of opossum is not completely characterized, we designed oligonucleotides based on the sequence described for the highly related *Monodelphis sp*, to isolate the MKP-1 cDNA from OK cells by RT-PCR. Sequence homology between species of the isolated cDNA was 98%. Next, using specific oligonucleotides, we evaluated the effect of BSA on MKP-1 mRNA levels. BSA transiently increased mRNA levels (2-fold after 1h), an effect blocked by actinomycin D, which suggests that BSA activates MKP-1 gene transcription. BSA also increased mRNA levels of GRP78 protein, an RS marker, and an inhibitor of ERK1/2 activation (PD98059, 50 µM), prevented this effect. Collectively, our data indicate that MKP-1 is induced by BSA at a transcriptional level and suggest that it may contribute to turn off ERK-dependent events triggered by BSA.

ST-P10.
YEAST PKA CATALYTIC SUBUNITS LOCALIZE ON STRESS AND PROCESSING FOCI UNDER HEAT STRESS

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Various stresses cause a fast and transient redistribution of nontranslated mRNAs into SGs (stress granules) and processing granules (PBs) in response to environmental injuries. In *Saccharomyces cerevisiae*, sphingolipids mediate the formation of PBs during heat stress. PKA regulatory subunit is encoded by the BCY1 gene, and the catalytic subunits are encoded by three genes: TPK1, TPK2 and TPK3. Here we report that during middle heat stress (37ºC) Tpk2-GFP re-localizes to the cytoplasm while Tpk1 and Tpk3 require severe heat stress (46ºC) for relocalization. Bcy1 shows an unresponsive nucleo-cytoplasmic distribution at both temperatures. In addition, at 37ºC only Tpk3 shows foci accumulation whereas at 46ºC Tpk2 also accumulates in foci. Inhibition of the synthesis of sphingolipids previous to heat stress impairs the Tpk cytoplasmic re-localization and reduces Tpk2 and Tpk3 foci accumulation, suggesting that a sphingolipid-dependent pathway is involved in Tpk localization. Co-localization experiments in cells expressing Tpk2-GFP or Tpk3-GFP and Dcp1-RFP (PB marker) or Rpg1-RFP (SG marker) during heat stress revealed that Tpk2 and Tpk3 are associated with Pbs and SGs. We have previously described that unstressed *tpk3*Δ strain contains foci that resemble SG induced by heat shock. Here we show that SG granules accumulated by TPK3 deletion have a protective function against heat stress injury.

ST-P11.
IMPLICATIONS OF PHOSPHORYLATION ON SER^{179} OF THE C SUBUNIT ISOFORM TPK1 FROM YEAST PKA

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Many protein kinases are themselves phosphoproteins, and their biological function and activity are frequently regulated by phosphorylation. PKA regulatory subunit is encoded by the BCY1 gene, and the catalytic subunits are encoded by three genes: TPK1, TPK2 and TPK3. Previously we have reported that following cAMP-PKA pathway activation, Tpk1 changes its phosphorylation status toward more phosphorylated isoforms by an intramolecular phosphorylation mechanism. Tpk1 increases its specific activity toward kemptide. Using mass spectrometry and array peptides derived from Tpk1 we identified mainly Ser^{179} as a putative target residue. Here we report the *in vivo* role of Ser^{179} phosphorylation of Tpk1 by several readouts analysis of PKA. We have constructed strains containing Tpk1wt or Tpk1^{S179A} or Tpk1^{S179D} as sole source of PKA activity. Our results show that Tpk1^{S179D} strain is deficient on non-fermentable carbon source growth, glycogen content, tolerance to heat stress, growth on rapamycin containing medium and shows a reduced life span on stationary phase. Tpk1-GFP and Tpk1^{S179D}-GFP show a nucleo-cytoplasmic distribution in glucose growing cells whereas Tpk1^{S179A}-GFP was localized in the nucleus. Our results suggest that the unphosphorylated state of Ser^{179} reduces the Tpk1 kinase activity improving the respiratory metabolism, survival to stress and life span of the yeast cells.

ST-P12.
NUCLEAR IMPORT OF PKA SUBUNITS REQUIRES ß IMPORTIN-DEPENDENT PATHWAY

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In *Saccharomyces cerevisiae* PKA subunits exhibit nuclear-cytoplasmic localization regulated by carbon source and growth stage. PKA contains three catalytic subunits, Tpk1, Tpk2 and Tpk3 and one regulatory subunit Bcy1. Previously, we have shown that Tpk1 and Tpk2 can differentially regulate transcriptional activity in response to several stress conditions, such as NaCl and H_2O_2. However, the mechanism by which PKA subunit enters the nucleus and whether its localization is regulated by osmotic and oxidative stress remains unknown. Here we assessed GFP-tagged PKA subunits localization in response to NaCl or H_2O_2 of exponentially glucose cells. Tpk1 showed nuclear accumulation upon stress whereas Tpk2, Tpk3 and Bcy1 localization remained unchanged. PKA subunits showed an ATP-dependent nuclear accumulation suggesting an active transport mechanism. Using strains in which each of ß-karyopherins was defective at a time we determined that Bcy1 is a KAP95 cargo for glucose growing cells. Tpk1 nuclear import required KAP114/KAP123 both pre- and post- stress stimulus. Tpk2 required KAP108 for its nuclear import in exponentially glucose growing cells but still shows nuclear import in response to stress in *kap108*Δ ?strain. Thus, nuclear accumulation of each PKA subunit by different ß-karyopherin-dependent pathway could control kinase activity in both compartments.

ST-P13.
TRANSCRIPTION REGULATION OF PKA SUBUNITS IN *Saccharomyces cerevisiae* UNDER HEAT SHOCK
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Yeast respond to environmental changes by transduction cascades that in general allow regulating gene expression. The aim of this work is to contribute to the knowledge of the cAMP/PKA pathway in *S.cerevisiae*, studying the expression regulation of PKA catalytic and regulatory subunits, TPK1, TPK2 and TPK3, and BCY1, under stress conditions. Using TPKs-LacZ and BCY1-LacZ fusion genes, we found that the activity level of TPK1 and TPK3 promoters occurred near the diaxic-shift. In contrast, TPK2 and BCY1 promoters activities were higher at the beginning of the log phase. We also demonstrated that there is a mechanism of negative autoregulation on PKA subunits expression that involves a PKA signaling in an isoform specific way. Under heat and saline stress conditions TPK1 showed an increment in its promoter activity, while TPK2, TPK3 and BCY1 remained constant. Studies with several deletion mutants involved in the stress signal transduction pathway showed differences among promoters. TPK1 and BCY1 were positively regulated by Rim15 and Gis1, TPK1 was also positively regulated by Yak1 and Msn2/4. Results of ChIP assays showed the presence of Msn2 and Gis1, in the TPK1 promoter transcriptionally active. Any of these factors are regulators of TPK2 and TPK3 promoters. These results were confirmed by mRNAs quantification.

ST-P14.
ACROSOMAL EXOCYTOSIS IS INHIBITED BY A PHOSPHOMIMETIC NSF MUTANT (NSF-Y83E)
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Acrosomal exocytosis (AE) is a regulated secretion essential for fertilization in mammals. A member of the fusion machinery, NSF, is inactivated by tyrosine phosphorylation in resting sperm. In consequence, SNARE proteins are assembled in *cis* complexes instead of cycling between this and monomeric configurations. NSF is derepressed when AE triggers activate PTP1B, a phosphatase that dephosphorylates it. NSF does not bind directly to SNARE complexes but it is bridged to them via α-SNAP. Here, we report that the phosphomimetic NSF-Y83E mutant blocks Ca^{2+}-induced exocytosis in streptolysin-O permeabilized human sperm. By means of functional and indirect immunofluorescence assays we found that NSFY-83E does not disassemble SNARE complexes as wild type NSF does. We performed in vitro binding assays and found no diminished affinity of the mutant for α-SNAP. Furthermore, NSF-Y83E dissociated α-SNAP from syntaxin as efficiently as did the wild type. We determined in functional assays that NSF-Y83E's inhibitory effect was reversed by recombinant PTP1B. We suggest that NSFY-83E inhibits secretion because it sequesters endogenous PTP1B, preventing the latter from dephosphorylating endogenous NSF. These data lend support to the notion that NSF activity is not constitutive in all systems. Furthermore, it provides an explanation for the ongoing puzzle about why this mutant inhibit secretion.

ST-P15.
$1,25(OH)_2D_3$ AND TX 527 SUPPRESS THE GROWTH OF KAPOSI SARCOMA CELLS BY INHIBITION OF THE NFκB PATHWAY
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The Kaposi Sarcoma-associated herpes virus G protein-coupled receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi Sarcoma (KS). Persistent expression and activity of vGPCR is required for tumor maintenance. It has been recently reported that NFkB gene is increased in experimental and human KS and that vGPCR potently activates the NFκB pathway. In this work we investigated if 1,25(OH)2D3 and its less calcemic analog TX 527 exert its growth inhibitory effects by modulation of the NFκB pathway in endothelial cells transformed by vGPCR (SVEC-vGPCR). Cell proliferation studies demonstrated that both 1,25(OH)2D3 and TX 527, similarly to bortezomib, a proteasome inhibitor that suppressed the activation of NFκB, reduced both proliferation and NFκB activity of SVEC-vGPCR. Time-response studies showed that the hormone and TX 527 significantly decreased NFκB and increased IkBa mRNA and protein levels. The increase of IkBa was accompanied by a reduction in p65/NFκB translocation to the nucleus. These responses were reversed when vitamin D receptor (VDR) expression was blocked by a shRNA against VDR. In parallel with NFκB inhibition induced by TX 527, there was a down-regulation of inflammatory genes such as IL-6, MIP3a, and MCP. Altogether, these results suggest that the antiproliferative effects of 1,25(OH)2D3 and TX 527 on Kaposi sarcoma cells occurred by modulation of the NFκB pathway.

ST-P16.
DIFFERENTIAL ROLE OF PTH AND PTHRP ON SURVIVAL/APOPTOTIC RESPONSE OF HUMAN INTESTINAL CACO-2 CELLS
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We previously demonstrated that parathyroid hormone (PTH) induces apoptosis in human Caco-2 intestinal cells. To evaluate the effect of its tumoral analog PTHrP, the cells were treated with the hormone (10-8 M, 24-72h) in a free serum media and then analyzed by flow citometry. Additionally, mitochondrial and nuclear integrity was evaluated by fluorescence microscopy. Neither the absence of serum nor PTHrP induce apoptosis. To investigate whether PTHrP presents a protective effect under apoptotic conditions, Caco-2 cells were incubated with the PTH analog for 24h and then exposed to H_2O_2 (0.5 mM, 4.5h). Cell viability was measured by MTS assay and the activation of Akt and the MAP kinases ERK1/2, JNK and p38 was evaluated by Western blot analysis. H_2O_2 decreases cell viability (50%) and also activates Akt and MAPKs, suggesting that these signaling pathways are activated under stress situation to mediate a cell survival response. The employment of specific inhibitors evidenced that MAPKs do not participate in Caco-2 cells response to H_2O_2-induced apoptosis. Furthermore, PTHrP is not able to prevent the cellular death induced by oxidative stress in Caco-2 cells. In conclusion, differently to PTH pro-apoptotic effect on Caco-2 intestinal cells, PTHrP is unable to induce apoptosis and may not present a protective effect under oxidative stress.

ST-P17.
VITAMIN D RECEPTOR AGONISTS INDUCE APOPTOSIS IN KAPOSI'S SARCOMA CELLS

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We previously showed that 1,25(OH)2-Vitamin D3 [1,25(OH)2D3] and its less calcemic analog TX 527 inhibited the proliferation of endothelial cells transformed by the viral G protein-coupled receptor associated to Kaposi sarcoma (vGPCR) involving the inhibition of the NFkB pathway. In this work we further explore the mechanism of action of both vitamin D compounds studying whether they induce cell cycle arrest and subsequent apoptosis of endothelial cells (SVEC) and transformed by vGPCR (SVEC-vGPCR) through the vitamin D receptor (VDR). Cell cycle analysis of SVEC and SVEC-vGPCR treated with 1,25(OH)2D3 (10 nM, 48 h) reveled that the hormone induced G0/G1 and reduce S phase. Moreover, S phase in SVEC-vGPCR was higher than in SVEC due to vGPCR expression. TX 527 has similarly effects on SVEC-vGPCR cell cycle. These effects were suppressed when VDR expression was block by stable transfection of shRNA against VDR. Annexin V-PI stain showed that apoptosis take place in both SVEC and SVEC-vGPCR under 1,25(OH)2D3 and TX 527 treatment (10 nM, 24 h), being the effect VDR dependent. Cleavage caspase-3 detected by western blot was increased in SVEC more than in SVEC-vGPCR, and this effect was blocked when VDR was knockdown. Taking together these results suggest that 1,25(OH)2D3 and TX 527 inhibit the proliferation of SVEC and SVEC- vGPCR and induce apoptosis by a mechanism that involves the VDR

ST-P18.
THE PI 3-KINASE REGULATOR TCVPS15 PARTICIPATES IN AUTOPHAGY IN *Trypanosoma cruzi*

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The phosphatidylinositol 3-phosphate has been shown to be important for several membrane trafficking pathways. Previously, we have characterized the first class III PI3K in *Trypanosoma cruzi*, named TcVps34, which has a role in vital processes such as osmoregulation, acidification and endocytosis. In other organisms, it has been shown that Vps34 forms a complex with the Ser–Thr protein kinase Vps15. In this work, we biochemically characterized the regulatory kinase TcVps15 in *T. cruzi*, and provide evidence that this protein participates in autophagy. Analysis of the recombinant protein indicated that it is a catalytically active kinase, with a Km value between 1,5 and 3 nM, showing a cation preference for Mn^{+2}. Moreover, parasites overexpressing TcVps15 showed an increase in their kinase activity comparing to wild type cells. Regarding to the functional role of TcVps15, immunofluorescence studies demonstrated that under starvation conditions TcVps15 colocalize with Atg8, an autophagosome marker in *T. cruzi*. Furthermore, assays using acridine orange, which is indicative of the lysosomal activity that is associated with autophagy, showed a higher level of acidification in TcVps15 transgenic parasites under nutritional stress compared to wild type controls. Taken together, our results unveil a previously unknown function for TcVps15 as a positive regulator of autophagy in *T. Cruzi*.

ST-P19.
PARACRINE FACTORS INVOLVED IN DIABETIC INTESTINAL DYSFUNCTION

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Diabetic intestinal dysfunction is a well-established complication of diabetes mellitus. Bone morphogenetic protein-7 (BMP-7) and TGF-ß1 are growth and differentiation factors which belongs to the TGF-ß superfamily of proteins. Despite, little is known about this pathway in the muscle layer of normal and diabetic adult intestine. Using an experimental model of diabetes in rodents, we explored the hypothesis that diabetic intestinal disfunction, could be consequence in part of a defect in the TGF family members homeostasis. The principal change in diabetes was an up-regulated TGF-ß/Smad signaling in the large intestine. TGF-ß1 and TGF-RII receptor were increased in the diabetic muscle layer at mRNA and protein level. p-Smad2/3 protein was distributed throughout the muscle, but the highest levels of active protein were associated with myenteric cells. Our analysis showed down-regulated BMP-7 protein expression in diabetic muscle layer. We also observed that diabetes environment upregulates extracellular matrix deposition in the intestinal muscle layer. An increased synthesis of fibronectin and type III collagen in smooth muscle cells was also observed. Diabetes causes an imbalance in TGF-ß1/BMP-7 signalling at the intestinal muscle layer leading to a fibrotic process at early stage of the disease.

ST-P20.
INCORPORATION OF MEMBRANE- PERMEABLE cAMP SPONGE PROTEIN IN HUMAN SPERM

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Acrosomal reaction (AR) is a type of regulated exocytosis that leads to the release of the acrosomal granule content, a key event in fertilization. Sperm are transcriptionally and translationally inactive; hence, overexpression or silencing experiments cannot be performed. An alternative method to overcome this limitation is the use of membrane-permeant proteins. We have designed a cAMP sponge that possesses the sequence of the C-terminal domain of human PKA-RIß (AA 133-380) coupled to a TAT peptide that confers membrane permeability and a His6-tag for purification and detection. We analyzed incorporation into human sperm by indirect immunofluorescence and found a high number of cells stained. cAMP-induced tyrosine phosphorylation is a hallmark of sperm capacitation. When we capacitated human sperm in the presence of the sponge, we observed a decrease in tyrosine phosphorylation. To evaluate the effect of cAMP depletion in the AR, we performed functional assays on intact and streptolysin-O permeabilized sperm. The sponge inhibited the AR elicited by progesterone, A23187 and calcium. These results indicate that the protein can transduce into cells and cause a decrease in intracellular cAMP, reflected in tyrosine phosphorylation diminution and AR inhibition. The development of permeant versions of proteins brings new perspectives to the study of sperm physiology.

EN-P01.
IMPROVED A. TUMEFACIENS GLYCOGEN SYNTHASE BY ADDITION OF AN SBD FROM *Arabidopsis thaliana* STARCH SYNTHASE III
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Starch synthase III (SSIII) from *Arabidopsis thaliana* is a SS isoform with a particular primary structure organization; the C-terminal domain, highly conserved in other SS isoforms, is preceded by a unique specific domain (SSIII-SD) which contains three in tandem starch binding domains (SBDs, named D1, D2 and D3) characteristic of degrading enzymes. These N-terminal SBDs have a probed regulatory role in SSIII activity, showing starch binding ability and modulating the catalytic properties of the enzyme.
To further investigate the functional role of *A. thaliana* SSIII-SD, three His tagged chimeric proteins were constructed combining the SBDs from *A. thaliana* with the glycogen synthase (GS) from *Agrobacterium tumefaciens*, which lacks SBDs. Recombinant chimeric proteins were expressed and purified to homogeneity from *Escherichia coli* in order to kinetically characterize them. Furthermore, chimeric enzymes, capability to restore *in vivo* glycogen biosynthesis in *E. coli* cells was also tested. The results obtained showed that the D3-GS enzyme showed increased capacity of glycogen synthesis *in vivo* with minor changes in their *in vitro* kinetic parameters.

EN-P02.
CHARACTERIZATION OF THIOREDOXIN SYSTEM FROM *Leptospira interrogans*
Sasoni N, Reinoso AM, Arias DG, Iglesias AA, Guerrero SA.
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During tissue invasion, *Leptospira interrogans* (the causative agent of leptospirosis) is exposed to elevated amounts of exogenous reactive oxygen and nitrogen species, which are highly toxic for the bacteria. However, this pathogen is able to establish a persistent infection in its human host. The metabolic pathways for redox homeostasis in this organism are poorly understood. In this work, we present the cloning, recombinant expression and functional characterization of the thioredoxin system (thioredoxin, TRX and thioredoxin reductase, TRXR) from *L. interrogans*. The *Lin*TRXR (a low molecular mass TRXR) was evaluated in its ability to catalyse the NADPH dependent reduction of DTNB and *Lin*TRX. *Lin*TRX system could be assayed as a functional redox pair that, together with 2Cys typical peroxiredoxin (*Lin*2CysPrx) mediates the NADPH-dependent reduction of hydroperoxides. Moreover, *Lin*2CysPrx was able to accept electrons from heterologous thioredoxin system and it was shown to be sensitive to overoxidation by peroxide substrate. This work strongly supports the occurrence of TRX system in *L. interrogans*, adding value to the genome project information of this pathogen.
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EN-P03.
FUNCTIONAL CHARACTERIZATION OF METHIONINE SULFOXIDE REDUCTASE B FROM *Trypanosoma cruzi*
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Methionine is an amino acid susceptible to be oxidized to methionine sulfoxide (MetSO). Reduction of MetSO to methionine is catalyzed by methionine sulfoxide reductase (MSR), an enzyme present in almost all organisms. In trypanosomatids, the study of antioxidant systems has been mainly focused on the involvement of trypanothione, a specific redox component in these organisms. However, poorly information is available concerning their mechanisms for repairing oxidized proteins, which would be relevant for the survival of these pathogens in the various stages of their life cycle. Recently, we characterized two A-type MSR proteins from *T. cruzi* and *T. brucei*. In this work, we report the molecular cloning of a gene encoding a putative B type MSR. The gene was expressed in *E. coli*, and the corresponding recombinant protein was purified and functionally characterized. The enzyme was specific for L-Met(*R*)SO reduction, using *T. cruzi* TXNI, TXNII and TRX as the reducing substrates. In addition, we found that *Tc*MSRB could compensate for MSR deficiency in yeast mutant strain lacking both MSRA and MSRB genes. The protein presented redox-dependent change in monomer/dimer oligomerization states. The results support the occurrence of a metabolic pathway in *T. cruzi* involved in the critical function of repairing oxidized macromolecules.
PIP 112 2008-01-02519, PICT´07 668 & PICT´08 1754.

EN-P04.
DNA STRUCTURE/SEQUENCE REQUIREMENTS FOR *Pseudomonas aeruginosa* MUTL ENDONUCLEASE ACTIVITY
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The hallmark of the mismatch repair system (MRS) in bacterial and eukaryotic organisms devoid of MutH, is the presence of a MutL homolog containing endonuclease activity. Our group has recently described that *Pseudomonas aeruginosa* MutL (PaMutL) has a cation-dependent endonuclease activity. In this study, DNA structure/sequence requirements of PaMutL activity were analyzed. The results showed that PaMutL generated incisions on supercoiled plasmids, but not on homoduplex or heteroduplex linear DNA molecules. Furthermore, PaMutL displayed the same endonuclease activity on highly negative supercoiled and on relaxed plasmids. The fact that PaMutL was able to generate incisions on plasmids independently of its supercoiling degree, but not on linear DNA, suggests that PaMutL requires fixed-ends DNA molecules for its *in vitro* endonuclease activity. In addition, the analysis of the incision sites generated on supercoiled plasmids indicated that PaMutL, as well as *Bacillus thuringiensis* MutL homolog, has no sequence specificity.
These results indicate that contrary to MutH, which specifically recognizes and cleaves GATC sequences, MutL homologs with endonuclease activity do not require a specific sequence to make an incision. As a consequence, the MRS in these organisms could be more efficient than in those with MutH, considering that GATC distribution can affect the MRS efficiency.

EN-P05.
RECOMBINANT EXPRESSION AND PRELIMINARY CHARACTERIZATION OF THE HUMAN GLYCOGEN BRANCHING ENZYME

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Glucose is the principal source of energy for most cells. In mammals, glucose is stored as glycogen, the branched polymer formed by linear α-1,4 oligoglucan chains linked by α-1,6-glucosidic bonds. Three enzymes are mainly responsible for the *de novo* biosynthesis of glycogen. First, glycogenin autoglucosylation produces a protein-bound oligoglucan that serves as a primer for the other two enzymes, then glycogen synthase elongates the chains, and the glycogen branching enzyme catalyzes the cleavage of a linear segment and transfers this chain to the 6-position of a non-terminal glucosyl unit.
Glycogen branching enzyme (GBE) is the least studied of the three enzymes. It has been isolated and characterized in some bacteria, rabbit and rat. The human enzyme has been mainly studied in clinical cases of glycogen storage disease type IV caused by deficiencies in GBE. Phosphoproteome and acetylome analysis over human cell lines suggests the possibility of a regulation for branching activity in humans by phosphorylation on tyrosine 173 and acetylation on lysine 68. In this study we report the recombinant production of human glycogen branching enzyme in *Pichia pastoris*, and in insect cells using the baculovirus expression system, and the activity analysis of the recombinant enzymes produced in the two expression systems.

EN-P06.
COMPARATIVE SUBSTRATE SPECIFICITY ANALYSIS OF M32 METALLOCARBOXYPEPTIDASES IN TRYPANOSOMATIDS

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Metallocarboxypeptidases (MCP) of the M32 family of peptidases have been identified in a number of prokaryotic organisms but they are absent from eukaryotic genomes with the exception of those of trypanosomatids. The genome of Trypanosoma brucei, the causative agent of Sleeping Sickness, encodes one MCP which displays 72% identity to the characterized TcMCP-1 from T. cruzi. As its orthologue, T. brucei MCP is a cytosolic enzyme expressed in both major stages of the parasite but it displays a different substrate specificity with respect to P1 position. To further explore the enzyme specificity, we employed 4 positional-scanning synthetic combinatorial libraries of fluorescence resonance energy transfer peptides. These peptides, with general sequences AbzGXXRZK(Dnp)OH; AbzGXXZXK(Dnp)OH; AbzGXZRXK(Dnp)OH; and AbzGZXRXK(Dnp)OH (where Z was successively occupied with one of the 19 amino acids with the exception of Cys and X is a random residue), contain the ortho-aminobenzoyl (Abz) and 2,4-dinitrophenyl (Dnp) as a donor/acceptor pair and permitted to study the P1, P2, P3, P4 substrate preference of recombinant MCPs. Our results indicate that TbMCP-1 had a more restricted selectivity for Phe in P1 position compared to TcMCP-1, which presented a wide range of substrate utilization. On the other hand, the S2, S3 and S4 subsites of both MCPs could accommodate a broad range of residues.

EN-P07.
STUDY OF *Leptospira interrogans* HEME OXYGENASE, A KEY ENZYME IN THE IRON ACQUISITION

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Leptospira interrogans, the pathogenic spirochete that caused leptospirosis, acquires its essential nutrient iron from the host hemoglobin during the infection. To scavenge the metal contained within the heme the bacterium utilizes an enzyme known as heme oxygenase (LepHO). Heme breakdown is a complex reaction that requires the input of seven electrons and three molecules of oxygen to release iron, biliverdin and carbon monoxide. Even though the mechanism of heme cleavage is broadly conserved between HOs from most organisms, the source of reducing equivalents is highly variable. So far there is no evidence of which proteins support the catalytic activity of LepHO by delivering the reducing power needed for the acquisition of iron. We have expressed the gene encoding LepHO and the recombinant product was purified by affinity chromatography as a soluble protein with a molecular weight of 26 kDa. The enzyme was able to bind its substrate heme whit high affinity displaying a typical soret absorption peak of the complex at 402 nm. We also studied the catalytic system of heme degradation *in vitro* by optical absorption spectroscopy and we established the physiological electron-donating partner of LepHO. Our results suggest that the plastidic type ferredoxin-$NADP^+$ reductase found in this bacterium efficiently delivers the electrons needed by LepHO to oxidize heme into biliverdin.

EN-P08.
STRUCTURAL AND BIOCHEMICAL STUDIES OF RIBOFLAVIN AND LUMAZINE SYNTHASES FROM *Brucella abortus*

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The last two steps in the biosynthesis of riboflavin are sequentially catalyzed by Lumazine Synthase (RibH) and Riboflavin Synthase (RibE). Certain Gram-negative pathogenic bacteria like *Brucella spp.* lack a riboflavin uptake system and are dependent on its endogenous biosynthesis. Additionally, mammals lack the riboflavin pathway enzymes and must obtain this vitamin from their dietary. Therefore, inhibitors of riboflavin biosynthesis will display selective cytotoxicity for pathogenic microorganisms as opposed to human cells. For these reasons RibH and RibE have been identified as promising targets for the development of new antimicrobial agents for the treatment of Brucellosis. In this work we performed a screening of a library of inhibitors on RibH2 and RibE of *B. abortus* and we identified two putative inhibitors. By binding assays we demonstrate that these compounds are able to displace riboflavin from de RibE and RibH active sites. On the other hand, enzymatic activity assays indicate that RibE possesses non-Michaelian kinetics. Furthermore we crystallized RibE as a free enzyme and in complex with riboflavin, roseoflavin and NRP (a putative inhibitor). These newly determined structures and the biochemical characterization of RibE may be useful for the rational design of novel inhibitors with activity against *Brucella*.

EN-P09.
KINETIC AND STRUCTURAL STUDY OF GALU AND GALF PROTEINS FROM *Escherichia coli*
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UDPGlc, a key glycosyl donor for carbohydrate metabolism, is produced from UTP and Glc1P by UDPGlc pyrophosphorylase (GalU). Previously, we demonstrated that in *E. coli* another protein (GalF) can catalyze the reaction. We compared the kinetic and structural properties of both enzymes, and constructed a GalF mutant (M15TH16R) which exhibited a partial "resurrection" of the activity. In this work we complemented an *E. coli* strain deficient in the galU gene with constructions that allow overexpression of the enzymes (pGALU, pGALF and pM15TH16R). In accordance with in vitro results, transformed cells with pGALU were able to ferment galactose within the first 24 h, cells complemented with pM15TH16R after 190 h, whereas cells carrying pGALF needed more than 240 h. We also produced the analogous T20MR21H GalU mutant, which had a V_{max} three orders of magnitude lower and a $S_{0.5}$ for Glc1P 60-fold higher than the wild type. Results support key roles for kinetics of critical residues present in GalU and absent in GalF. In addition, we hypothesize that differences between enzyme activities would, in part, be due to oligomerization status of the respective protein. In fact, production of monomeric GalU gave an enzyme with similar affinity for substrates but with 20-fold lower V_{max} than the tetramer. We discuss about a putative in vivo function of GalF and its possible interaction with GalU.

EN-P10.
STRUCTURE-GUIDED ENGINEERING OF GLUCITOL DEHYDROGENASE COSUBSTRATE SPECIFICITY
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Glucitol (Gol) is the major photosynthetic product in plants from the Rosaceae family (which includes peach, apple, and pear, among others). NAD^+-dependent Gol dehydrogenase (GolDHase, EC 1.1.1.14) is the enzyme responsible for metabolizing this polyol in heterotrophic tissues of these plants. In contrast to its metabolic relevance, few studies deal with this enzyme and no structures have been obtained for any plant GolDHase. In this work, we built a homology model for the enzyme from peach (*Prunus persica*) fruits using GolDHases from *Homo sapiens* and *Bemisia argentifolii* as templates. With this model we determined the NAD^+-binding pocket and found that Asp^{216} might be involved in cosubstrate specificity. Site saturation mutagenesis at position 216 and a high-throughput method for analyzing the resulting clones led us to identify several mutants capable of using $NADP^+$. DNA sequencing of these clones showed an Ala or a His at position 216, as key residues for determining the dinucleotide specificity. Engineering of substrate specificity on GolDHase is important due to its potential biotechnological applications, and our methodology is feasible to obtain enzymes with the desired characteristics and to reach key designs to improve the affinity for $NADP^+$ and other polyols.

EN-P11.
ROLE OF PPGALNAC-TS LECTIN DOMAINS ON MUCIN-TYPE O-GLYCOSYLATION INITIATION
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ppGalNAc-Ts (polypeptide GalNAc transferases) are involved in the first step of mucin-type O-glycosylation. ppGalNAc-T2 and T3 are members of this extended enzyme family. They are mammalians type II transmembrane proteins with a Golgi lumenal region that contains a catalytic domain with glycosyltransferase activity. Particularly, they are the only glycosyltransferases having a C-terminal "ricin-like" lectin domain. ppGalNAc-Ts and the lectin domains were expressed as soluble recombinant proteins in Sf9 insect cells. Constructs contain 6xHis and T7 tags. Recombinant proteins were purified to homogeneity using Co++ affinity chromatography. We evaluated enzyme activity of ppGalNAc-T2 and T3 in presence ppGalNAc-T3 and T4 lectin domains with MUC1 and MUC2 as peptide acceptors. Kinetics parameters were measured by using a colorimetric assay. We found the lectin domains have an inhibitory effect on these ppGalNAc-Ts activity and inhibitory constants (Ki) were measured. Binding assays showed the recognition of lectin domains to ppGalNAc-Ts and we were able to characterize the type of inhibition. The influence of ppGalNAc-T lectin domains on mucin-type O-glycosylation is also appreciated in *in vivo* assay. These results indicate that lectin domains could have an important role in regulation of mucin-type O-glycosylation

EN-P12.
SMC02309, A NOVEL LOW MOLECULAR WEIGHT PHOSPHOTYROSINE PHOSPHATASE FROM *Sinorhizobium meliloti*
Medeot DB[1], Rivero MR[1], Cendoya E[1], Ferrari W[1], Rossi F[1], Contreras-Moreira B[2], Fischer SE[1], Jofré E[1].
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In Gram-negative bacteria tyrosine phosphorylation has been shown to play a role in polysaccharide production. The predicted protein product of open reading frame Smc02309 from *Sinorhizobium meliloti 2011* possesses significant similarity with known low molecular weight protein tyrosine phosphatases (LMW-PTP). KM and Vmax parameters for SMc02309 towards p-nitrophenyl phosphate are similar to those of other biochemical characterized bacterial LMW-PTP. Using a combination of immunodetection, mass spectrometric analysis and bioinformatics approaches, it was shown that SMc02309 is a protein phosphatase. Moreover, preliminary results provide evidence that the phosphatase SMc02309 can utilize the protein tyrosine kinase ExoP as an endogenous substrate. Bioinformatics approaches also evidence that, in contrary that happen in other bacterial species, in the *S. meliloti 2011* genome these both proteins present a unique location. Altogether, these results suggest the occurrence of a novel regulatory mechanism connected with protein phosphorylation on tyrosine in *S. meliloti*. The identification and functional characterization of this novel phosphotyrosine-protein phosphatase will increase our understanding of key aspects of polysaccharides biosynthesis not only in bacteria of agricultural significance but also in polysaccharides-producing proteobacteria with human and animal health relevance.

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