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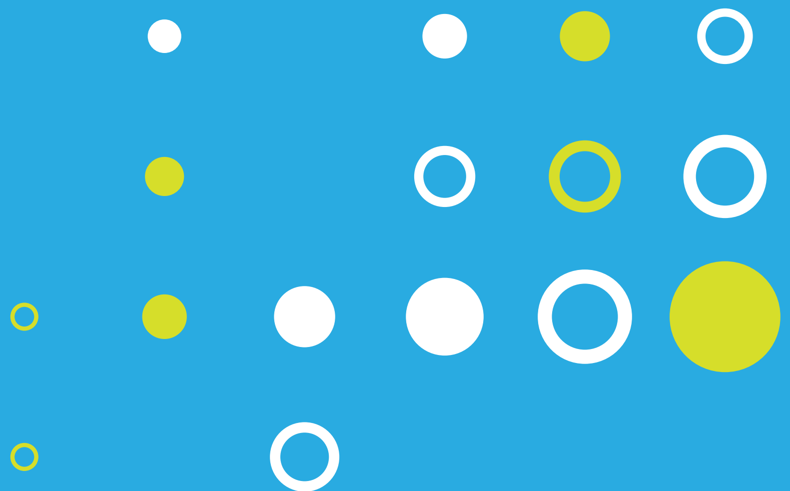

SAIB

Sociedad Argentina de
Investigaciones en Bioquímica
y Biología Molecular

From the cover:

A vascular bundle in a thin cross-section from the stem of a *Dracaena* plant. This specimen was stained with three dyes which stain cell-wall components differentially. This type of preparation is generally used for brightfield microscopy. In this case, however, colors were generated by sequential excitation of the dyes with 4 different laser lines from a Zeiss LSM 510 Meta confocal microscope and the resulting images were composited to beautifully illustrate different cell wall types. Relatively thin primary cell walls of the parenchyma and phloem are green, thick secondary cell walls of the xylem are red, and the thickest supporting cell walls of the fibres are blue.

Image courtesy of Dr. John Runions - Oxford Brookes University, Oxford, UK

- SAIB -
51 Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

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

November 3 - November 6, 2015
Mar del Plata, República Argentina

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INBIOSUR-CONICET, Universidad Nacional del Sur, Bahía Blanca

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-Cell Biology-
Laura Morelli
IIBBA – CONICET

-Lipids-
Ana Ves Losada
INIBIOLP - CONICET. Universidad Nacional de La Plata

-Microbiology-
Viviana Rapisarda
INSIBIO - CONICET. Universidad Nacional de Tucumán

-Plant Biochemistry and Molecular Biology-
Jorgelina Ottado
IBR - CONICET. Universidad Nacional de Rosario

-Signal Transduction-
Alejandro Colman Lerner
IFIBYNE–CONICET, Universidad de Buenos Aires

ABSTRACT EVALUATION COMMITTEE

Alejandro Colman-Lerner, Mónica Delgado, María F. Drincovich, Verónica González Pardo, Mario Guido, Laura Morelli, Jorgelina Ottado, Viviana Rapisarda, and Ana Ves Losada

ACKNOWLEDGMENTS

The following Institutions supported the organization of the LI SAIB Meeting:

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Tuesday, November 3	Wednesday, November 4	Thursday, November 5	Friday, November 6
	9:00 - 11:00 **Symposia** *Room A (Nereidas Sur):* **Cell Biology** *Room B(Los Corales):* **Signal Transduction**	9:00 - 11:00 **Symposia** *Room A (Nereidas Sur):* **Microbiology** *Room B (Los Corales):* **Plants**	9:00 - 11:00 **Symposia** *Room A (Nereidas Sur):* **Translational Science** *Room B (Los Corales):* **Lipids**
	11:00-11:30 Coffee break	11:00-11:30 Coffee break	11:00-11:30 Coffee break
	11:30-12:30 **Plenary Lecture** *Thomas Jentsch* *Room A (Nereidas Sur)*	11:30-12:30 **Plenary Lecture** *Barry Rosen* *Room A (Nereidas Sur)*	11:30-12:30 **Plenary Lecture** *Michael Knop* *Room A (Nereidas Sur)*
	12:30-13:00 **Short presentation** *Biological Science in Argentine* *Room A (Nereidas Sur)*	12:30 Lunch	12:30 Lunch
14:00 Registration	13:00 Lunch 15:00-17:00 **Oral Communications** *Room A (Nereidas Sur):* **Cell Biology (CB-C01 to CB-C06)** *Room B (Los Corales):* **Microbiology (MI-C01 to MI-C06)** *Room C (Las Arenas):* **Structural Biology (SB-C01 to SB-C03) and Lipids (LI-C01 to LI-C03)**	14:30-15:50 **Oral Communications** *Room A (Nereidas Sur):* **Cell Biology (CB-C07 to CB-C10)** *Room B (Los Corales):* **Microbiology (MI-C07 to MI-C10)** *Room C (Las Arenas):* **Biotechnology (BT-C01 and BT-C02) and Enzymology (EN-C01 and EN-C02)** 16:00-16:45 **Short Talk** *Hugo L. Mónaco* *Room A (Nereidas Sur)*	14:30-16:30 **Oral Communications** *Room A (Nereidas Sur):* **Cell Biology (CB-C11 to CB-C16)** *Room B (Los Corales):* **Plants (PL-C01 to PL-C05) and Biotechnology (BT-C03)** *Room C (Las Arenas):* **Signal Transduction (ST-C01 to ST-C06)**
17:00 - 19:00 **Round Table: Plataformas Tecnológicas MINCYT** *Room A (Nereidas Sur)*	17:00-18:00 **"Alberto Sols" Lecture** *Xosé Bustelo* *Room A (Nereidas Sur)*	17:00-18:00 **"Ranwel Caputto" Lecture** *Armando J. Parodi* *Room A (Nereidas Sur)*	16:30 Coffee break 16:30-18:00 **Posters Section** *Nereidas Norte* **BT-P31 to BT-P45** **CB-P40 to CB-P56** **MI-P53 to MI-P71** **LI-P15 to LI-P23** **PL-P31 to PL-P39** **EN-P01 to EN-P07**
19:00 - 19:15 **Opening Ceremony**	18:00 Coffee break 18:00 – 20:00 **Posters Section** *Nereidas Norte* **BT-P01 to BT-P15** **CB-P01 to CB-P20** **MI-P01 to MI-P28** **NS-P01 to NS-P10** **PL-P01 to PL-P15** **ST-P01 to ST-P12** **SB-P01 to SB-P10**	18:00 Coffee break 18:00-20:00 **Posters Section** *Nereidas Norte* **BT-P16 to BT-P30** **CB-P21 to CB-P39** **MI-P29 to MI-P52** **LI-P01 to LI-P14** **PL-P16 to PL-P30** **ST-P13 to ST-P25**	18:00-19:00 **Closing Lecture** **"EMBO" Lecture:** *Chris Bowler* *Room A (Nereidas Sur)*
19:15 - 20:15 **Opening Lecture "Héctor Torres"** *Lutz Birnbaumer* *Room A (Nereidas Sur)*			19:00-19:30 **Closing Ceremony & Awards** *Room A (Nereidas Sur)*
20:30 Cocktail		20:00 **SAIB General Assembly** *Room A (Nereidas Sur)*	22:00 Closing Dinner

SAIB 2015

TUESDAY, November 3, 2015

14:00 **REGISTRATION**

17:00-19:00 ***ROUND TABLE***

Chairpersons: Laura Morelli and Luis Mayorga

PLATAFORMAS TECNOLOGICAS (MINCYT): unidades de apoyo a la investigación, al desarrollo y a la innovación tecnológica

- Carlos E. A. Cassanello
Director General del FONCyT, Agencia Nacional de Promoción Científica y Tecnológica

- Plataforma argentina de Biología Estructural y Metabolómica
- Carlos Bertoncini

- Consorcio argentino de Tecnología Genómica
- Norma Paniego

- Centro de Estudios Químicos y Biológicos por Espectrometría de Masas
- Silvia Moreno

- Plataforma de Células Madre reprogramadas humanas
- Valeria Roca

19:00-19:15 ***OPENING CEREMONY***

- Carlos S. Andreo
SAIB President
CEFOBI, CONICET - Universidad Nacional de Rosario, Argentina

19:15-20:15 ***OPENING LECTURE "HECTOR TORRES" LECTURE***

- Lutz Birnbaumer
Instituto de Investigaciones Biotecnológicas (IIB-INTECH, UNSAM-CONICET), Argentina
"Mechanisms of Ca^{2+} entry into cells mediated by ORAI and TRPCS activated by the ER Ca^{2+} sensor STIM1"

Chairperson: Ernesto Podestá

20:30 ***COCKTAIL***

WEDNESDAY, November 4, 2015

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

Room A

CELL BIOLOGY SYMPOSIUM

Chairpersons: María Isabel Colombo and Laura Morelli

- Rafael Radi

Universidad de la República, Uruguay

"Mitochondrial dysfunction, oxidant production and mitochondrial-targeted redox-based therapeutics"

- Sergio Lavandero

Advanced Center for Chronic Diseases, Universidad de Chile & UT Southwestern Medical Center, Dallas

"Interorganelle communication in cardiomyocytes: control of nuclear Ca^{2+} *by the IGF-1 receptors"*

- George Perry

Texas University, San Antonio, USA

"Oxidative damage is correlated with mitochondrial autophagy"

- Gyorgy Hajnoczky

Thomas Jefferson University, Philadelphia, USA

"Mitochondria-ER communication in cell signaling and dynamics"

Room B

SIGNAL TRANSDUCTION SYMPOSIUM

Chairpersons: Silvia Moreno and Alejandro Colman-Lerner

- Gabriela Amodeo

Instituto de Biodiversidad y Biología Experimental, FCEN, UBA, Argentina

"Cytosolic pH as a gating signal for plant plasma membrane aquaporins"

- Pablo Wappner

Instituto Leloir, Argentina

"Mechanisms of cell adaptation to hypoxia"

- Carla V Finkielstein

Virginia Polytechnic Institute and State University, USA

"Clock proteins: new mediators of DNA-damage signaling"

- Edith Kordon

IFIBYNE, UBA-CONICET, Argentina

"The mRNA binding protein Tristetraprolin contributes to lactation by reducing local levels of TNF"

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

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

- Thomas Jentsch

Leibniz-Institut für Molekulare Pharmakologie, Germany

"Molecular identification and role in physiology of the volume-regulated anion channel VRAC"

Chairperson: Guillermo Spitzmaul

12:30-13:00 *SHORT PRESENTATION: Biological Science in Argentine*

- Nora Calcaterra and Carlos Argaraña

Informe documento elaborado a pedido de la Academia Nacional de Ciencias y la Academia Nacional de Ciencias Exactas, Físicas y Naturales, Argentina

13:00 ***LUNCH***

15:00-17:00 ***ORAL COMMUNICATIONS***

Room A: Cell Biology (CB-C01 to CB-C06)

Room B: Microbiology (MI-C01 to MI-C06)

Room C: Structural Biology (SB-C01 to SB-C03) and Lipids (LI-C01 to LI-C03)

Room A

Cell Biology (CB-C01 to CB-C06)

Chairpersons: Gerardo De Blas y Claudio Fader

15:00-15:20

CB-C01

***Shigella* BLOCKS INTRACELLULAR TRAFFICKING IN HOST CELLS BY A SUBSET OF EFFECTORS**

Ferrari M, Malardé V, Sansonetti P, Sauvonnet N. Institut Pasteur, Unité de Pathogénie Microbienne Moléculaire, Paris, FranceE-mail: mariana.ferrari@pasteur.fr

15:20-15:40

CB-C02

THE ROLE OF N-β-ALANYL DOPAMINE SYNTHASE IN THE INNATE IMMUNE RESPONSE OF INSECTS

Pérez M[1,3], Turdera L[2,3], Schachter J[2,3], Lopez Lastra C[4], Quesada-Allué L[1,2]. [1]IIBBA-CONICET, [2]FCEyN-UBA, [3]Inst. Leloir. (C.P. 1405), C.A.B.A. 4CEPAVE 120 y 61, La Plata. E-mail: mperez@leloir.org.ar

15:40-16:00

CB-C03

G-QUADRUPLEXES CONTROL THE EXPRESSION OF DEVELOPMENTALLY REGULATED GENES *IN VIVO*

David AP, Margarite E, Banchio C, Calcaterra NB, Armas P. IBR-CONICET. FCByF-UNR. Ocampo y Esmeralda, Rosario, Santa Fe, Argentina. E-mail: david@ibr-conicet.gov.ar

16:00-16:20

CB-C04

OVEREXPRESSION OF CNBP RESCUES MORPHANT PHENOTYPE IN ZEBRAFISH MODEL OF TREACHER COLLINS SYNDROME (TCS)

Porcel de Peralta M, Mouguelar V, Carnevale M, Coux G, Calcaterra NB. IBR (CONICET/UNR). Esmeralda y Ocampo - Predio CCT. Rosario, Santa Fe – Argentina. E-mail: porceldeperalta@ibr-conicet.gov.ar

16:20-16:40

CB-C05

GENE EXPRESSION REGULATION DURING MELANOCYTE DIFFERENTIATION: SOX10, MITFA AND DICER ROLES

Weiner AM[1], Kelsh RN[2], Calcaterra NB[1]. [1]IBR, UNR-CONICET, Rosario, Argentina. [2]Centre for Regenerative Medicine, Univ. of Bath, Bath, UK. E-mail: weiner@ibr-conicet.gov.ar

16:40-17:00

CB-C06

PI3K SIGNALING IS IMPLICATED IN BRADYKININ-INDUCED COLLECTIVE MIGRATION OF URETERIC BUD (UB) CELLS

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

Room B

Microbiology (MI-C01 to MI-C06)

Chairpersons: Mónica Delgado and Christian Magni

15:00-15:20

MI-C01

DEVELOPMENT OF A HIGH THROUGHPUT ASSAY FOR THE SCREENING OF ACYL-COA CARBOXYLASE INHIBITORS

Bazet Lyonnet, B; Diacovich, L; Gago, G; Gramajo, HC. Instituto de Biología Molecular y Celular de Rosario (IBR), Argentina. E-mail: bazetlyonnet@ibr.gov.ar

15:20-15:40

MI-C02

TREHALOSE-6-PHOSPHATE SYNTHESIS IN *Streptomyces* IS RELATED TO A NEW GDP-GLUCOSE PYROPHOSPHORYLASE

Asencion Diez, M[1,2]; Syson, K[2]; Miah, F[2]; Bornemann, S[2]; Iglesias, AA[1][1]Lab. de Enzimología Molecular -IAL-CONICET- Argentina.[2]John Innes Centre, Norwich, United Kingdom. E-mail: masencion@fbcb.unl.edu.ar

15:40-16:00

MI-C03

ELECTROCHEMISTRY OF EXTRACELULAR CYTOCHROME OMC FROM THE ELECTRO-ACTIVE BACTERIA *G. sulfurreducens*

Ordóñez, MV[1]; Schrott, GD[1]; Infossi, P[2]; Lojou, E[2]; Busalmen, JP[1]. [1]Lab. Bioelectroquímica, INTEMA, CONICET-UNMdP. [2]BIP, CNRS-Marseille, Francia.E-mail: mvordone@fi.mdp.edu.ar

16:00-16:20

MI–C04

PHOB MODULATION BY POLYPHOSPHATE LEVELS IN *Escherichia coli* AND ITS INVOLVEMENT IN BIOFILM FORMATION

Grillo-Puertas, M; Rintoul, MR; Rapisarda, VA. INSIBIO (CONICET-UNT) and Inst. de Qca.Biol. “Dr. BernabéBloj” (FBQF, UNT). Tucumán, Argentina.E-mail: marianagrillo24@gmail.com

16:20-16:40

MI-C05

ROLE OF INTERSPECIFIC INTERACTIONS IN THE EVOLUTION OF MUTATOR BACTERIA

Luján, A[1,2]; Madsen, L[3]; Marvig, R[3]; Sharma, MD[2]; Smania, AM[1]; Krogh Johansen, H[3]; Molin, S[3]; Buckling, A[2]
[1]CIQUIBIC,CONICET UNC. [2]Biosciences-ESI, Univ. of Exeter, England.[3]Novo Nordisk Foundation, DTU DK
E-mail: adem.lujan@gmail.com

16:40-17:00

MI-C06

HEPATITIS E VIRUS ORF3 PROTEIN IS A HUB PROTEIN WITH REGULATORY FUNCTIONS

Osterman, A[1]; Stellberger, T[2]; Nitschko, H[1]; Uetz, P[3]; Baiker, A[2]; Vizoso Pinto, MG[4]. [1]MvP Inst, LMU, Munich. [2]LGL, Oberschl.Germany.[3]CSBC, VCU, USA.[4]INSIBIO, CONICET-UNT, Arg.E-mail: guadalupevizoso@yahoo.com

Room C

Structural Biology (SB-C01 to SB-C03) and Lipids (LI-C01 to LI-C03)

Chairpersons: Susana Pasquaré and Nicolás Favale

15:00-15:20

SB-C01

DETAILED ANALYSIS OF THE CATALYTIC MECHANISM OF HUMAN GLUTAMINE SYNTHETASE

Issoglio FM[1]; Campolo N[2]; Zeida A[1]; Radi R[2]; Bartesagui S[2]; Estrin DA[1] .[1]DQIAyQF and INQUIMAE CONICET, FCEN UBA, [2]CEINBIO, UdelaR, Montevideo, Uruguay. E-mail: fedeissoglio@gmail.com

15:20-15:40

SB-C02

BA41, A NOVEL TPM-DOMAIN ATPASE FROM THE ANTARTIC FLAVOBACTERIUM *Bizionia argentinensis*

Cerutti ML[2]; Otero LH[2]; Smal CA[1]; Pelliza L[1]; Goldbaum FA1; Arán M1; Klinke S[2]. [1]Fundación Instituto Leloir, IIBBA-CONICET, Buenos Aires, Argentina. [2]PLABEM E-mail: mcerutti@leloir.org.ar

15:40-16:00

SB-C03

CRYSTAL STRUCTURE OF THE NTRX RESPONSE REGULATOR AND ANALYSIS OF ITS DNA BINDING ACTIVITY

Fernández I[1]; Cornaciu I[2]; Hoffmann G[2]; Sieira R1; Carrica MC[1]; Márquez JA[2]; Goldbaum FA[1]. [1]Fundación Instituto Leloir - IIBBA (CONICET), [2]EMBL - Outstation Grenoble E-mail: ifernandez@leloir.org.ar

16:00-16:20

LI-C01

NUCLEAR LIPID METABOLISM IS DIFFERENTLY REGULATED BY POLYUNSATURATED FATTY ACIDS DURING AGING

Gaveglio VL; Pascual AC; Giusto NM; Pasquaré SJ

Instituto de Investigaciones Bioquímicas de Bahía Blanca, CCT – Bahía Blanca, UNS-CONICET. E-mail: vgaveglio@criba.edu.ar

16:20-16:40

LI-C02

SK1 AS KEY G1-G0 TRANSITION MODULATOR IN RENAL EPITHELIAL CELL

Udovin LD; Santacreu BJ; Sterin de Speziale NB; Favale |NO

Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. IQUIFIB – CONICET. E-mail: lucas2304@hotmail.com

16:40-17:00

LI-C03

CIRCADIAN REGULATION OF CLOCK GENE EXPRESSION AND PHOSPHOLIPID BIOSYNTHESIS IN GLIOBLASTOMA CELLS

Sosa-Alderete L; Wagner PM; Gorné LD; Guido ME

CIQUIBIC-CONICET, Dept Biol Chemistry, School of Chemistry. Natl University of Cordoba. Argentina. E-mail: lsosa@exa.unrc.edu.ar

17:00-18:00 ***"SOLS" LECTURE***

- Xosé Bustelo

Universidad de Salamanca-CSIC, España

"Genetic analysis of the role of the VAV Oncoprotein family in health and disease"

Chairperson: Omar Coso

18:00 ***COFFEE BREAK***

18:00-20:00 ***POSTERS SECTION***

BT-P01 to BT-P15	**CB-P01 to CB-P20**
MI-P01 to MI-P28	**NS-P01 to NS-P10**
PL-P01 to PL-P15	**ST-P01 to ST-P12**

SB-P01 to SB-P10

THURSDAY, November 5, 2015

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

Room A

MICROBIOLOGY SYMPOSIUM

Chairpersons: Angeles Zorreguieta and Viviana Rapisarda

- Andrea Smania
CIQUIBIC, Córdoba, Argentina
"Unravelling the linkage between adaptative evolution and hypermutability in Pseudomonas aeruginosa"

- Claudia Studdert
IIB, Mar del Plata, Argentina
"Chemotaxis and beyond: diversity of chemosensory signal transduction pathways"

- Carlos Jerez
Universidad de Chile, Chile
"Role of the inorganic polyphosphate in copper and oxidative stress resistance in the thermoacidophilic Sulfolobus solfataricus"

- Sara Soto
Universitat de Barcelona, ISGlobal, Spain
"New strategies to combact biofilms"

Room B

PLANT SYMPOSIUM

Chairpersons: Laura De la Canal and Jorgelina Ottado

- Pablo Cerdan
Instituto Leloir, Argentina
"CYP integrates light and temperature signaling by regulating auxin responses in Arabidopsis"

- Claudia Casalongué
Instituto de Investigaciones Biológicas, Mar del Plata, Argentina
"Auxin and miRNA393: towards a better understanding on mechanisms of developmental plasticity"

- Vanesa Tognetti
Central European Institute of Technology, Czech Republic

"ROS and auxin crosstalk during plant development and stress adaptation"

- Bernd Mueller-Roeber

University of Potsdam and Max Planck Institute of Molecular Plant Physiology, Germany

"Unravelling the GRNs of senescence- and stress-associated NAC transcription factors"

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

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

- Barry Rosen

Florida International University, USA

"Pathways of organoarsenical biosynthesis and detoxification"

Chairperson: Viviana Rapisarda

12:30 ***LUNCH***

14:30-15:50 ***ORAL COMMUNICATIONS***

Room A: Cell Biology (CB-C07 to CB-C10)

Room B: Microbiology (MI-C07 to MI-C10)

Room C: Biotechnology (BT-C01 and BT-C02) and Enzymology (EN-C01 and EN-C02)

Room A

Cell Biology (CB-C07 to CB-C10)

Chairpersons: Carolina Touz and Julio Caramelo

14:30-14:50

CB-C07

SUMO CONJUGATION TO SPLICEOSOMAL PROTEINS MODULATES SPLICEOSOME ASSEMBLY AND FUNCTION

Pozzi B, Bragado L, Mammi P, Risso G, Srebrow A. IFIBYNE-CONICET-FBMC-FCEN-UBA. E-mail: bertapozzi@fbmc.fcen.uba.ar

14:50-15:10

CB-C08

PROTEIN TRAFFICKING IN *Giardia lamblia*

Zamponi N[1], Svard S[2], Touz MC[1]. [1]Instituto M&M Ferreyra INIMEC-CONICET-UNC, Córdoba, Argentine [2]BMC, Uppsala University, Sweden. E-mail: nzamponi@immf.uncor.edu

15:10-15:30

CB-C09

DENGUE VIRUS HOST ADAPTATION IS ASSOCIATED TO A DYNAMIC PATTERN OF SUBGENOMIC RNA ACCUMULATION

Filomatori CV[1]; Carballeda* JM[1]; Villordo SM[1]; Aguirre S[2]; Fernández-Sesma A[2]; Gamarnik AV[1]. [1]Fundación Instituto Leloir, CONICET, CABA, Argentina [2]Mount Sinai School of Medicine, NY, USA. E-mail: cfilomatori@leloir.org.ar*

15:30-15:50

CB-C10

DENGUE VIRUS NS5 PROTEIN INTEGRATES THE SPLICEOSOME AND MODULATES CELLULAR SPLICING DURING INFECTION

De Maio FA[1], Risso G[2], Iglesias NG[1], Shah P[3], Krogan N[3], Andino R[3], Srebrow A[2], Gamarnik AV[1]. [1]Fundación Instituto Leloir – CONICET. [2]FCEyN UBA – CONICET. [3]University of California, USA. E-mail: fdemaio@leloir.org.ar

Room B

Microbiology (MI-C07 to MI-C10)

Chairpersons: Martha Dardanelli and Silvia Altabe

14:30-14:50

MI-C07

THE *Salmonella typhimurium* RcsCDB SYSTEM IS INVOLVED IN THE *std* OPERON REGULATION

Farizano, JV[1]; García-Pastor, L[2]; Casadesús, J[2]; Delgado, MA[1]. [1]INSIBIO-CONICET/UNT, Tucumán-Argentina. [2]Dpto de Genética-Universidad de Sevilla, Sevilla-España. E-mail: jfarizano@fbqf.unt.edu.ar

14:50-15:10

MI-C08

***Brucella* MONOMERIC ADHESINS: VARIABILITY, FUNCTIONALITY AND POSSIBLE PSEUDOGENIZATION**

Bialer, MG[1]; Posadas, DM[1]; Ferrero, MC[2]; Ruiz-Ranwez, V[1]; Delpino, MV[2]; Baldi, PC[2]; Zorreguieta, A[1]. [1]Fundación Instituto Leloir, IIBBA CONICET, Argentina. [2]IDEHU (CONICET/UBA), FFyB, UBA, Argentina. E-mail: mbialer@leloir.org.ar

15:10-15:30

MI-C09

INSIGHTS INTO THE ROLE OF RAPA, A RHIZOBIAL EXTRACELLULAR LECTIN INVOLVED IN BIOFILM FORMATION

Abdian, PL; Vozza, NF; Russo, DM; Caramelo, JJ; Zorreguieta, A. Fundación Instituto Leloir, IIBBA-CONICET, Av. Patricias Argentinas 435, Buenos Aires, Argentina. E-mail: pabdian@leloir.org.ar

15:30-15:50

MI-C10

IMPACT ON THE SOIL BACTERIAL COMMUNITIES BY THE HERBICIDES USED BY THE *Lotus tenuis* PROMOTION

Nieva, SA[1]; Bailleres, MA[2]; LLames, ME[1]; Menendez, AB[3]; Ruiz, OA[1]. [1]IIB-INTECH.CONICET-UNSAM, [2]CEICH.MAA-INTA.[3]FCEN.UBA. E-mail: susanna_nieva@hotmail.com

Room C

Biotechnology (BT-C01 and BT-C02) and Enzymology (EN-C01 and EN-C02)

Chairpersons: Verónica González Pardo and Alberto Iglesias

14:30-14:50

BT-C01

NANOBODY-BASED IMMUNOCAPTURE ASSAY FOR DETECTION OF SHIGA TOXIN-PRODUCING *Escherichia coli*

Melli L[1], Hiriart Y[2], Lauche C[2], Pardo R[2], Chinen I[3], Rivas M[3], Zylberman V[2], Ugalde J[1], Comerci D[1], Ciocchini A[1]. [1]IIB-UNSAM-CONICET [2]Inmunova S.A. [3]Servicio de Fisiopatogenia, ANLIS-Malbrán. E-mail: lmelli@iibintech.com.ar

14:50-15:10

BT-C02

BIODIESEL PRODUCTION USING NANOSTABILIZED BIOCATALYSTS

Cappa VA, Rivero CW, Ares MN, Trelles

JA. Laboratorio de Investigaciones en Biotecnología Sustentable, Universidad Nacional de Quilmes. E-mail: jatrelles@gmail.com

15:10-15:30

EN-C01

KINETIC AND DYNAMICAL CHARACTERIZATION OF AN ANTIOXIDANT SYSTEM FROM *Mycobacterium tuberculosis*

Zeida A[1], Reyes AM[2], Lichtig P[1], Santos J[3], González Flecha FL[3], Radi R[2], Trujillo M[2], Estrin DA[1]. [1]DQIAyQF, INQUIMAE-CONICET, FCEN-UBA [2]CeInBio, UdelaR, Uruguay [3]DQB, IQUIFIB-CONICET, FFBQ-UBA.E-mail: azeida@qi.fcen.uba.ar

15:30-15:50

EN-C02

A SINGLE MUTATION OF *Leptospira interrogans* HEME OXYGENASE GREATLY IMPAIRS ENZYME ACTIVITY

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

16:00-16:45 ***SHORT TALK***

- Hugo L. Mónaco

Biocrystallography laboratory, University of Verona, Italy

"Structures of antineoplastic lectins isolated from edible mushrooms"

Chairperson: José Luis Bocco

17:00-18:00 ***"RANWEL CAPUTTO" LECTURE***

- Armando J. Parodi

Instituto Leloir, Argentina

"The endoplasmic reticulum as the subcellular site that (almost) decides our fate"

Chairperson: Carlos Argaraña

18:00 ***COFFEE BREAK***

18:00-20:00 ***POSTERS SECTION***

BT-P16 to BT-P30	**CB-P21 to CB-P39**
MI-P29 to MI-P52	**LI-P01 to LI-P14**
PL-P16 to PL-P30	**ST-P13 to ST-P25**

20:00 **SAIB GENERAL ASSEMBLY**

FRIDAY, November 6, 2015

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

Room A

TRANSLATIONAL SCIENCE SYMPOSIUM

Chairpersons: Gustavo Chiabrando and Omar Coso

- Hugo Luján
CEDEI, Universidad Católica de Córdoba, Argentina
"Efficacy of an oral vaccine platform based on surface proteins of the intestinal parasite Giardia lambia*"*

- Juliana Cassataro
Instituto de Investigaciones Biotecnológicas IIB-INTECH, UNSAM-CONICE, Argentina
"A bacterial protease inhibitor protects oral vaccines from digestion while triggers immune responses"

- Uri Saragovi
Lady Davis Institute-Jewish General Hospital, McGill University, Canadá
"Academic translational medicine: From target discovery and validation, to experimental therapeutics, to clinic"

- Carlos Davio
ININFA (UBA-CONICET) y Cátedra de Química Medicinal (FFYB, UBA), Argentina
"MRP4/ABCC4-mediated cAMP extrusion as a new potential target for cancer therapy"

Room B

LIPID SYMPOSIUM

Chairpersons: María del Carmen Fernández Tome and Ana Ves Losada

- Gabriela A. Salvador
INIBIBBB, Bahía Blanca, Argentina
"PLD and PI3K signaling in neuronal oxidative stress"

- Marcelo D. Costabel
IFISUR, Bahía Blanca, Argentina
"Insights into the mechanism of interaction between FABPS and biological membranes. A computational approach"

- James Ntambi
University of Wisconsin-Madison, USA
"Role of the monounsaturated fatty acids in liver-adipose tissue cross-talk and metabolic regulation"

- Adrian Salic
Harvard Medical School, USA
"Lipids and hedgehogs"

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

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

- Michael Knop
University of Heidelberg, Germany
"Probing the network topology of the ubiquitin-proteasome system for protein degradation in yeast using functional high throughput strategies"

Chairperson: Alejandro Colman-Lerner

12:30 ***LUNCH***

14:30-16:30 ***ORAL COMMUNICATIONS***

Room A: Cell Biology (CB-C11 to CB-C16)
Room B: Plants (PL-C01 to PL-C05) and Biotechnology (BT-C03)
Room C: Signal Transduction (ST-C01 to ST-C06)

Room A

Cell Biology (CB-C01 to CB-C06)
Chairpersons: Nora Calcaterra and Carlos Arregui

14:30-14:50
CB-C11
ROLE OF N-GLYCOSYLATION IN THE CONFORMATIONAL MATURATION OF SECRETORY PATHWAY PROTEINS
Diez AL, Couto PM, Labanda MS, Caramelo JJ, Labriola CA. Fundación Instituto Leloir-IIBBA-CONICET
E-mail: clabriola@leloir.org.ar

14:50-15:10
CB-C12
***Mycobacterium bovis* REQUIRES P27 TO ARREST PHAGOSOME MATURATION AND SURVIVE IN BOVINE MACROPHAGES**
Vázquez CL, Bianco MV, Blanco FC, Forrellad MA, Bigi F. Instituto de Biotecnología, CICVyA-INTA, Dr. N. Repetto y De Los Reseros, Hurlingham, Argentina. E-mail: vazquez.cristina@inta.gob.ar

15:10-15:30
CB-C13
CELL ADHESION MOLECULE AND SMALL GTPASE T3-REGULATED GENES IN *Xenopus laevis* GUT EPITHELIA
Galetto CD, Izaguirre MF, Casco VH. Laboratorio de Microscopia Aplicada a Estudios Moleculares y Celulares, FIUNER. OV, ER, Argentina. E-mail: cgaletto@bioingenieria.edu.ar

15:30-15:50
CB-C14
***Staphylococcus aureu* INDUCES A REORGANIZATION OF ENDOCYTIC MEMBRANES CAUSING THE FORMATION OF DYNAMIC TUBULES**
Lopez de Armentia MLA, Colombo MI. Instituto de Histología y Embriología Mendoza, CONICET, Facultad de Cias Médicas, UN Cuyo, Mendoza. E-mail: milagrosarmentia@gmail.com

15:50-16:10
CB-C15
ACYL-COA SYNTHETASE-4, A NEW POTENTIAL THERAPEUTIC TARGET IN HORMONE-RESISTANT BREAST CANCER
Castillo AF, Orlando UD, Dattilo MA, Solano AR, Maloberti PM, Podestá EJ. INBIOMED UBA-CONICET, Department of Biochemistry, School of Medicine, University of Buenos Aires. E-mail: castillofernanda@yahoo.com

16:10-16:30

CB-C16

CYTOPLASMIC FRA-1 AND C-FOS: POTENTIAL TARGETS FOR SPECIFIC BREAST CANCER THERAPY

Racca AC, Prucca CG, Caputto BL. Departmento de Química Biológica, Universidad Nacional de Córdoba. CIQUIBIC-CONICET. Argentina. E-mail: aracca@fcq.unc.edu.ar

Room B

Plants (PL-C01 to PL-C05) and Biotechnology (BT-C03)

Chairpersons: Mariana Saigo and Ana Laxalt

14:30-14:50

PL-C01

LIPID PROFILING OF PEACH CULTIVARS WITH DIFFERENT SUSCEPTIBILITY TO CHILLING INJURY

Bustamante C[1], Brotman Y[2], Monti L[1], Gabilondo Budde JC[3], Andreo C[1], Lara MV[1], Fernie A[2], Drincovich MF[1]. [1]CEFOBI-UNR. [2]MPI für Molekulare Pflanzenphysiologie. [3]INTA-EE San Pedro. E-mail: bustamante@cefobi-conicet.gov.ar

14:50-15:10

PL-C02

A MULTI-LEVEL REGULATION OF *Arabidopsis* FUMARASES REVEALS NOVEL EMERGENT PROPERTIES OF C4 METABOLISM

Zubimendi JP, Drincovich MF, Andreo CS, Tronconi MA. CEFOBI-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. E-mail: zubimendi@cefobi-conicet.gov.ar

15:10-15:30

PL-C03

LIGHT, CHROMATIN AND ALTERNATIVE SPLICING

Godoy Herz MA, Kubaczka MG, Petrillo E, Kornblihtt AR. IFIBYNE-UBA-CONICET. E-mail: mica.gh@fbmc.fcen.uba.ar

15:30-15:50

PL-C04

IS THE *Arabidopsis* ELECTRONIC SHUTTLE ADXR-ADX-P450 INVOLVED IN STEROID SYNTHESIS AND DEVELOPMENT?

Distéfano AM, Bellido AM, Fiol DF, Zabaleta EJ, Pagnussat GC. Instituto de Investigaciones Biológicas-CONICET-UNMdP. E-mail: adistefa@mdp.edu.ar

15:50-16:10

PL-C05

CONTRASTING THE BIOCHEMICAL AND PHYSIOLOGICAL RESPONSE OF *Lotus* ECOTYPES SUBJECTED TO COLD STRESS

Calzadilla PI[1], Signorelli S[2], Maiale SJ[1], Monza J[2], Ruiz OA[1]. [1]IIB-INTECh (CONICET-UNSAM). Chascomús. Argentina [2] UDELAR. Montevideo. Uruguay. E-mail: pablo_calza@hotmail.com

16:10-16:30

DEVELOPMENT OF IMMOBILIZED BIOCATALYST WITH POLYGALACTURONASE ACTIVITY FOR JUICE CLARIFICATION

Ramírez Tapías YA, Rivero CW, Britos CN, Trelles JA. Laboratorio de Investigaciones en Biotecnología Sustentable. Universidad Nacional de Quilmes. E-mail: jatrelles@gmail.com

Room C

Signal Transduction (ST-C01 to ST-C06)

Chairpersons: Paula Portela and Silvia Rossi

14:30-14:50
ST-C01
AKT POSTTRANSLATIONAL AND SUBCELLULAR LOCALIZATION PROFILING: TOWARDS AN AKT FINGERPRINT
Blaustein M[1]; Bush A[1]; Clemente J[1]; Riggio M[2]; Novaro V[2]; Colman-Lerner AA[1]. [1]IFIBYNE-CONICET, DFBMC-FCEyN-UBA, [2]IBYME. mblaustein@fbmc.fcen.uba.ar.

14:50-15:10
ST-C02
CELL-TO-CELL VARIABILITY IN LIGAND-RECEPTOR BINDING DYNAMICS
Vasen G; Bush A; Colman-Lerner A. IFIBYNE-CONICET and DFBMC, FCEN, UBA. E-mail: gvasen@fbmc.fcen.uba.ar.

15:10-15:30
ST-C03
ROBUSTNESS TO VARIATION IN RECEPTOR ABUNDANCE IN A CANONICAL GPCR SIGNAL TRANSDUCTION SYSTEM
Bush A; Patop IL; Colman-Lerner A. IFIBYNE (UBA-CONICET). E-mail: abush@fbmc.fcen.uba.ar.

15:30-15:50
ST-C04
TETRAMERIC STRUCTURE AND INTERACTORS OF THE N-TERMINUS OF BCY1, THE YEAST PKA REGULATORY SUBUNIT
Tofolón E[1]; González Bardeci N[1]; Valacco P[12]; Fernández G[2]; Neme Tauil R[2]; Rossi S[1]; Moreno S[12]. [1]Depto Química Biológica y [2]CEQUIBIEM, FCEN, UBA e IQUIBICEN/CONICET. E-mail: enzotofolon@qb.fcen.uba.ar.

15:50-16:10
ST-C05
NOVEL STRESS GRANULE REGULATORS IDENTIFIED IN AN RNAI SCREEN IN DROSOPHILA
Pérez-Pepe M[1]; Loschi M[1]; Arán M[1]; Thomas MG[1]; Wolosiuk R[1]; Cooke A[2]; Henzte M[2]; Boccaccio GL[1]. [1]Fundación Instituto Leloir, IIBBA CONICET, Argentina [2]EMBL, Heidelberg, Alemania. E-mail: maperez@leloir.org.ar.

16:10-16:30
ST-C06
CROSS-REGULATION BETWEEN BACILLUS SUBTILIS TWO COMPONENT SYSTEMS DESK-DESR AND YVFT-YVFU
Fernández P; Porrini L; Albanesi D; de Mendoza D; Mansilla MC. Instituto de Biología Molecular y Celular de Rosario (CONICET). Fac. de Cs. Bioq. Y Farm. UNR. E-mail: pfernandez@ibr-conicet.gov.ar.

16:30 ***COFFEE BREAK***

16:30-18:00 ***POSTERS SECTION***

BT-P31 to BT-P45	**CB-P40 to CB-P56**
MI-P53 to MI-P71	**LI-P15 to LI-P23**
PL-P31 to PL-P39	**EN-P01 to EN-P07**

18:00-19:00 *CLOSING LECTURE* ***"EMBO" LECTURE***

- Chris Bowler
Institut de Biologie de l'EcoleNormaleSupérieure (IBENS), Paris, France
"TARA OCEANS: Eco-systems biology at planetary scale"

Chairperson: Jorgelina Ottado

19:00 ***CLOSING CEREMONY AND AWARDS***

- José Luis Bocco
SAIB Vice-President
CIBICI, Universidad Nacional de Córdoba, Argentina

22:00 ***CLOSING DINNER***

- ABSTRACTS:

- Lectures
- Lectures L01 to L08

- Symposia
- Cell Biology: CB-01 to CB-04
- Lipids: LI-01 to LI-04
- Microbiology: MI-01 to MI-04
- Plant Biochemistry and Molecular Biology: PL-01 to PL-04
- Signal Transduction: ST-01 to ST-04
- Translational Science: TS-01 to TS-04

- Oral Communications:
- Biotechnology: BT-C01 to BT-C03
- Cell Biology: CB-C01 to CB-16
- Enzymology: EN-C01 and EN-C02
- Lipids: LI-C01 to LI-C03
- Microbiology: MI-C01 to MI-C10
- Plant Biochemistry and Molecular Biology: PL-C01 to PL-C05
- Structural Biology: SB-C01 to SB-C03
- Signal Transduction: ST-C01 to ST-C06

Posters:
- Biotechnology: BT-P01 to BT-P45
- Cell Biology: CB-P01 to CB-P56
- Enzymology: EN-P01 to EN-P07
- Lipids: LI-P01 to LI-P23
- Microbiology: MI-P01 to MI-P71
- Neuroscience: NS-P01 to NS-P10
- Plant Biochemistry and Molecular Biology: PL-P01 to PL-P39
- Structural Biology: SB-P01 to SB-P10
- Signal Transduction: ST-P01 to ST-P25

LECTURES

L-01
MECHANISMS OF Ca^{2+} ENTRY INTO CELLS MEDIATED BY ORAI AND TRPCS ACTIVATED BY THE ER Ca^{2+} SENSOR STIM1

Birnbaumer, L
Instituto de Investigaciones Biotecnológicas (IIB-INTECH UNSAM-CONICET), San Martín, Pcia de Buenos Aires.

Excitable and non-excitable cells possess a mechanism for admitting Ca^{2+} from the extracellular milieu activated secondarily to stimulation by agonists that promote mobilization of phosphoinositides with the production of IP3 and diacylglycerols. This Ca2+ serves to insure sustained intracellular signaling while cells are under the influence of the agonist and is responsible for changes in metabolic activities and gene expression that are not only essential for life, but which, under pathologic situations, can lead to cell and tissue damage. The molecular nature and structural features of the channels admitting Ca2+, formed by ORAI and TRPCs will be presented and commonalities and differences in their activation by the endoplasmic reticulum (ER) calcium sensor STM1 will be discussed.

L-02
MOLECULAR IDENTIFICATION AND ROLE IN PHYSIOLOGY OF THE VOLUME-REGULATED ANION CHANNEL VRAC

Jentsch, TJ
Leibniz-Institut für Molek. Pharmakol. (FMP) und Max-Delbrück-Centrum (MDC), Berlin, Alemania

Cells need to regulate their volume in response to osmotic stress, during cell division, growth and migration, and during apoptosis. A crucial player in regulatory volume decrease (RVD) is the volume-regulated anion channel VRAC that has been known from physiological and biophysical studies for more than two decades. VRAC is normally closed and can be opened by cell swelling or other stimuli, for during apoptosis. The mechanisms of VRAC activation remain very poorly understood. There is also a controversy whether VRAC conducts organic osmolytes such a taurine or glutamate, or whether they use a distinct channel named VSOAC. Many 'candidates' were proposed to be VRAC, but all of them crashed. Using a genome-wide siRNA screen that used YFP fluorescence quenching iodide as read-out, we recently identified LRRC8A as an essential VRAC component. We showed that LRRC8A needs heteromerization with at least one other LRRC8 subunit (B-E) for yielding swelling-activated anion currents. The specific LRRC8 subunit composition determines the inactivation properties of VRAC, demonstrating that LRRC8 protein directly participate in channel formation. Swelling-induced taurine and glutamate efflux also depend on LRRC8 heteromers, suggesting that a strict distinction of VRAC and VSOAC is not warranted. A weak homology of LRRC8 proteins with pannexins suggest that VRACs may be hetero-hexamers.

L-03
GENETIC ANALYSIS OF THE ROLE OF THE VAV ONCOPROTEIN FAMILY IN HEALTH AND DISEASE

Bustelo, XR
Centro de Investigación del Cáncer, CSIC-University of Salamanca, Campus Unamuno s/n, 37007 Salamanca, Spain

Evolution has favored the emergence of a large number of GDP/GTP exchange factors (GEFs) that, in addition to mediate the rapid activation of Rho GTPases in stimulated cells, provide enough functional plasticity to adapt the stimulation step to different cell types, receptors, and downstream effectors. The study of GEFs is therefore of interest to unveil the mechanistics of GTPase activation in physiological conditions as well as their participation in the development and/or malignant progression of disease states. However, the large number of them also raises the issue of whether the inactivation of a small subset of them could provide any therapeutic advantage and, if so, the negative collateral effects induced by such an inactivation on healthy tissues.

In this talk, we will use the phosphorylation-dependent Vav GEF family as paradigm to address the above issues using genetic, animal model-based approaches. Specifically, we will provide information about hitherto unknown regulatory steps for these GEFs, their complex multifunctional roles in tumorigenic processes, and the *pros* and *cons* for considering them potentially interesting therapeutic targets.

This work is supported by grants from the Spanish Ministry of Economy and Competitiveness (SAF2012-31371, RD12/0036/0002), Worldwide Cancer Research (14-1248), and the Solórzano and Ramón Areces foundations.

L-04
PATHWAYS OF ORGANOARSENICAL BIOSYNTHESIS AND DETOXIFICATION

Rosen BP
Department of Cellular Biology and Pharmacology, Florida International University Herbert Wertheim College of Medicine Miami, FL USA, brosen@fiu.edu, http://medicine.fiu.edu/about-us/directory/profile.php?id=1062

Life arose in an alien and hostile environment. Before the atmosphere became oxidizing, the concentrations of dissolved metal ions in primordial oceans were undoubtedly considerably higher than today. An initial challenge of the earliest cells would have been the ability to detoxify toxic metals and metalloids such as arsenic, the most pervasive environmental toxic substance and carcinogen in nature. The presence of arsenic resistance (*ars*) genes in the genome of nearly every living organism sequenced to date illustrates first that ars genes must be ancient and second that arsenic must still be ubiquitous in the environment, providing the selective pressure that maintains them in present-day organisms. Without arsenic detoxification systems, life would not exist. Nearly every bacterial species has one or more *ars* operons, such as the *arsRDABC* operon of *E. coli* plasmid R773. Since life evolved in a neutral atmosphere where reduced As(III) predominated, the first microbes would have evolved detoxification mechanisms to cope with trivalent As(III), and the only mechanism required to cope with pentavalent As(V) was reduction to As(III). Recently a variety of additional genes have been identified in various *ars* operons, including *arsH*, *arsI*, *arsM* and *arsP*, which we show encode new pathways of organoarsenical biosynthesis, degradation and detoxification. ArsM is an As(III) S-adenosylmethione methyltransferase that produces extremely toxic MAs(III). ArsH is an NADPH-FMN oxidoreductase that oxidizes MAs(III) to relatively nontoxic MAs(V). ArsI is C-As lyase that that cleaves the C-As bond. ArsP is a permease that confers resistance to MAs(III) by active efflux from the cell. Some members of microbial communities synthesize methylarsenite (MAs(III)) and use this extremely toxic organoarsenical as an antimicrobial against neighboring bacteria. Other members of microbial communities have evolved resistance mechanisms against both MAs(III) and even more toxic synthetic organoarsenicals such as roxarsone, a growth promoter used poultry and swine. We hypothesize that microbes use ArsM to produce a primordial antibiotic, MAs(III), and ArsH, ArsI and ArsP evolved as antibiotic resistances to detoxify MAs(III). Supported by NIH grants R37 GM55425 and R01 ES023779.

L-05
STRUCTURES OF ANTINEOPLASTIC LECTINS ISOLATED FROM EDIBLE MUSHROOMS

Monaco, HL
Biocrystallography laboratory, Department of Biotechnology, University of Verona, Verona, Italy. hugo.monaco@univr.it

Lectins are defined as proteins of non-immune origin that selectively recognize and reversibly bind carbohydrates without modifying them enzymatically. In general ligand binding precedes the fulfilment of an important biological function, that requires as the triggering event the recognition of the carbohydrate by the protein. Although initially characterized in plants, lectins are ubiquitous in nature and have been identified in most living species, from viruses to man. Their sugar selectivity has been shown to be very useful and they are widely used in both basic and applied science. Tumour associated carbohydrate structures are commonly exposed in many types of human cancers. They are broadly expressed and often specific of tumors and thus potentially very useful for diagnosis and therapy. Three different lectin folds present in novel antineoplastic mushroom lectins will be described. The lectins have anti-proliferative effects on human cancer cells without any apparent cytotoxicity on normal cells, an activity due to the recognition of specific tumor associated carbohydrate structures. The possibility of using one of these folds in human theranosis will also be discussed.

L-06
THE ENDOPLASMIC RETICULUM AS THE SUBCELLULAR SITE THAT (ALMOST) DECIDES OUR FATE

Parodi AJ
Laboratorio de Glicobiología. Fundación Instituto Leloir, Buenos Aires

How proteins are first coated with saccharides in the endoplasmic reticulum lumen, how then saccharides are transformed as glycoproteins thus formed get older and how the transformed sugars provide cells with the information required for dealing with the glycoprotein hazardous folding processes as well as with how to dispose of the irreparably misfolded species will be presented as a long trip from naive work performed with rat liver microsomes and live trypanosomes to more sophisticated experiments employing molecular biology and biophysical methodology.

L-07
PROBING THE NETWORK TOPOLOGY OF THE UBIQUITIN-PROTEASOME SYSTEM FOR PROTEIN DEGRADATION IN YEAST USING FUNCTIONAL HIGH THROUGHPUT STRATEGIES

Knop M
Group leader in the DKFZ-ZMBH Alliance, 69120 Heidelberg, Germany

Protein homeostasis denotes the summary of all cooperative, competing and integrated processes and pathways in cells that regulate the synthesis, folding, transport, turnover and proteolytic degradation of proteins within and outside the cell. Central to this is the ability of cells to remove proteins upon 'request': Proteolysis can occur as a function of a cellular regulatory process, e.g. during cell division, or it can occur upon damage or misfolding of a protein, and can target individual proteins, protein species, protein-ensembles (e.g. aggregates or complexes), larger structures including entire organelles, depending on the needs, the state of the cell and the cellular processes. The ubiquitin-proteasome system (UPS) plays a pivotal role in the regulation of selective protein degradation and it is assumed that approx. 10% of all genes in the eukaryotic genome are involved in some of its processes. In this talk I will explain how we employ a novel type of fluorescent protein timer, the so-called tandem fluorescent protein timer (tFT) to visualise protein dynamics and turnover by fluorescence microscopy as well as high throughput plate assays, and how this enables us to systematically map the involvement of every component of the UPS in the degradation of the cellular proteome, with single protein resolution. I will discuss our results about the overall topology of the UPS with respect to the specificity of different E2, E3 and DUBs, and about a new ER associated protein quality control pathway.

L-08
TARA OCEANS: ECO-SYSTEMS BIOLOGY AT PLANETARY SCALE

Bowler, C *and the Tara Oceans Consortium*
Ecology and Evolutionary Biology Section, Institut de Biologie de l'EcoleNormaleSupérieure (IBENS), Paris, France

The ocean is the largest ecosystem on Earth and yet we know very little about it. This is particularly true for the plankton that drift within. Although these organisms are at least as important for the Earth system as the forests on land, most of them are invisible to the naked eye and thus are largely uncharacterized, even though they form the base of marine food webs. To increase our understanding of this underexplored world, a multidisciplinary consortium, *Tara* Oceans, was formed around the 110-ft research schooner *Tara,* which sampled plankton at more than 210 sites and multipledepth layers in all the major oceanic regions during expeditions from 2009-2013 (Karsenti et al. Plos Biol., 2011). The seminar will describe the first foundational resources from the project (based on a first data freeze from 579 samples at 75 stations; seeScience special issue May 22, 2015) and their initial analyses, illustrating several aspects of the *Tara*Oceans' eco-systems biology approach. The project provides unique resources for several scientific disciplines, capturing biodiversity of a wide range of organisms that are rarely studied together, exploring interactions between them and integrating them with environmental conditions to further our understanding of life in the ocean and beyond in the context of ongoing climate changes.

SYMPOSIA

CB-01
MITOCHONDRIAL DYSFUNCTION, OXIDANT PRODUCTION AND MITOCHONDRIAL-TARGETED REDOX-BASED THERAPEUTICS

Radi, R
Department of Biochemistry, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay

Mitochondrial dysfunction is increasingly recognized as a key process in the pathophysiology of a variety of genetic-borne and acquired disease conditions in human pathology. In many of these cases, enhanced levels of mitochondrial-derived free radicals and oxidants mediate alterations in cellular bioenergetics, calcium homeostasis and trigger cell death. Recent research efforts have been directed to unambiguously demonstrate and connect mitochondrial dysfunction to enhanced oxidant formation and how these processes impact in cell and tissue homeostasis. Moreover, novel interventions are represented by the development of mitochondrial-targeted therapeutics, either by pharmacological intervention with redox active molecules that are bioactivated and/or concentrated inside mitochondria or by the induction, *via* transcription of nuclear genes, of mitochondrial enzyme antioxidant systems. In

the presentation, I will analyze some of the biochemical events that link mitochondrial dysfunction with cellular oxidative stress and provide evidence for the effective intervention with mitochondrial-targeted redox based therapeutics in vascular and neurodegenerative disease models *in vitro* and *in vivo*.

CB-02
INTERORGANELLE COMMUNICATION IN CARDIOMYOCYTES: CONTROL OF NUCLEAR CA^{2+} BY THE IGF-1 RECEPTORS

Lavandero, S
Advanced Center for Chronic Diseases, Universidad de Chile & UT Southwestern Medical Center, Dallas

Eukaryotic cells contain membrane contact sites formed upon juxtapositioning of the respective organelles. These contacts facilitate both signaling and the passage of molecules from one cellular compartment to another. In cardiomyocytes, T-tubules confer the necessary compartmentation of Ca^{2+} signals but whether there is a similar structure tunneling extracellular stimulation to control nuclear Ca^{2+} signals locally has not been explored. We show that IGF-1 triggers a fast and independent nuclear Ca^{2+} signal in rat and human cardiomyocytes. This fast and localized response is achieved by activation of IGF-1R signaling complexes present in perinuclear invaginations of the plasma membrane. The perinuclear IGF-1R pool connects extracellular stimulation to local activation of nuclear Ca^{2+} signaling and transcriptional upregulation through the perinuclear IP3 production, nuclear Ca^{2+} release, and activation of MEF2C. Genetically engineered Ca^{2+} buffers—parvalbumin—with cytosolic or nuclear localization demonstrated that the nuclear Ca^{2+} handling system is physically and functionally segregated from the cytosolic Ca^{2+} signaling machinery. Our data reveal the existence of an IP3–dependent nuclear Ca^{2+} toolkit located in direct apposition to the cell surface, which allows the local control of rapid and independent activation of nuclear Ca^{2+} signaling in response to an extracellular ligand. FONDAP 15130011- CONICYT, Chile.

CB-03
OXIDATIVE DAMAGE IS CORRELATED WITH MITOCHONDRIAL AUTOPHAGY

Perry G[1], *Perry EA[2], Moreira PI[3], Correia S[3], Castellani RJ[4], Wang X[5], Lee H-g[5], Zhu X[5]*
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Alzheimer disease (AD) and aging are marked by oxidative damage and mitochondrial abnormalities. Since mitochondria can play a critical role in oxidative damage, we conducted this study to identify the relationship of oxidized RNA, 8-hydroxyguanosine (8OHG), and mitochondrial DNA (mtDNA) accumulation in AD and aging individuals. Abnormalities were examined by using densitometry of hippocampal pyramidal neurons: mtDNA accumulation as a marker of mitophagy and oxidative damage by 8OHG. Among aging individuals, oxidative damage and mtDNA were highly correlated (r2 = 0.86). While both 8OHG and mtDNA were at higher levels in AD individuals, they were uncorrelated (r2 = 0.06). In contrast, as we found before, oxidative damage was inversely correlated with amyloid-β; it was unrelated in normal aging individuals. These results suggest that oxidative damage is directly related to mitophagy in aging individuals. With the onset of AD, amyloid-β plays a strong antioxidant role. These findings indicate that the onset of AD is marked by a pleotrophic change in oxidative stress, one characterized by a change from mitochondria to amyloid-β dependency.

CB-04
MITOCHONDRIA-ER COMMUNICATION IN CELL SIGNALING AND DYNAMICS

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The function and organization of “synapses” among intracellular organelles have been a topic of broad interest in recent years. Compelling evidence has been presented that the mitochondria-ER interface is central to calcium signaling, organellar dynamics and lipid biosynthesis. The ER and mitochondrial membranes also host sources and targets of reactive oxygen species (ROS) but their local dynamics and relevance remained elusive since measurement and perturbation of ROS at the interface has proven difficult. Employing drug-inducible synthetic ER-mitochondrial linkers we overcame this problem and present real-time measurements of calcium and H_2O_2 nanodomains at the ER-mitochondrial interface. We have also studied the origin of these nanodomains and their relevance in signal transduction and mitochondrial and ER dynamics. The results give new clues to inter-organelle communication, regulating organellar and cellular activities.

LI-01
PLD AND PI3K SIGNALING IN NEURONAL OXIDATIVE STRESS

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Lipid signaling cascades have important roles in the regulation of cellular fate. Our studies provide new insights into the regulation and physiological role of lipid messengers during neuronal oxidative stress (OS). Specifically, we have studied neuronal signal events derived from phosphatidylcholine (PC) and phosphatidylinositol (PI).
Synaptic OS triggers phospholipase D (PLD) activation and, consequently, a rise in phosphatidic acid and diacylglycerol (DAG) generation from PC. These lipid messengers activate downstream signaling cascades as ERK1/2 and conventional PKCs and regulate glutamate transport in the synaptic cleft of adult rat brains. Studies in aged brains reveal an increased synaptic susceptibility to OS and an impairment in the DAG-mediated signaling pathways. Tyrosine phosphorylation associated with PI phosphorylation and phosphoinositide 3 kinase (PI3K) activation are stimulated in OS-exposed hippocampal neurons and synapses. PI3K activation and its downstream effector kinase, Akt, trigger pleiotropic neuroprotective mechanisms against OS by suppressing FOXO3A transcriptional activity, inhibiting GSK3β and upregulating glutathione metabolism.
In summary, we have characterized signaling events elicited by PLD and PI3K activation, which produce lipid messengers that control smart strategies for preventing neuronal death triggered by OS.

LI-02
INSIGHTS INTO THE MECHANISM OF INTERACTION BETWEEN FABPS AND BIOLOGICAL MEMBRANES. A COMPUTATIONAL APPROACH

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The role of fatty acid binding proteins (FABPs) as intracellular fatty acid (FA) transporters may require their interaction with membranes; not only to deliver long-chain fatty acids to target surfaces, but also to remove them from the membranes. In vitro studies have shown that different FABPs, transfer FA to or from membranes by two different mechanisms. Liver FABPs do it by a supposed aqueous phase diffusion ("diffusional" mechanism); but, in marked contrast, a larger number of FABPs, including adipocyte, intestinal, brain, and heart/muscle types, transfer their FAs by directly interacting with a membrane ("collisional" mechanism). However, the process in which both kind of proteins adsorbs to the membrane remains to be elucidated. Based on computational analysis, we confirmed that recruitment to membranes is facilitated, in a first step, by electrostatic interactions; and this analysis can quantitatively differentiate among the mechanisms of membrane association proposed, and determinate the most energetically favorable configuration for the membrane-associated states of different FABPs. Moreover, we have identified the aminoacids putatively responsible for both, collisional and diffusional mechanisms; and, also, we pointed out how the structures could be punctually modified to adapt their function. Finnally, using molecular dynamics simulations we studied the interactions of apoFABPs with lipid membranes of anionic and zwitterionic phospholipids, even for FABPs of unknown behavior, using molecular modeling methods as predictive tools in biophysics.

LI-03
ROLE OF MONOUNSATURATED FATTY ACIDS IN LIVER-ADIPOSE TISSUE CROSS-TALK AND METABOLIC REGULATION

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The maintenance of metabolic health requires complex regulation of metabolic processes in several tissues. The coordination of this metabolic regulation involves extensive crosstalk among tissues. Signaling factors that are secreted into the circulation and impart systemic metabolic effects include molecules such as hepatokines, adipokines and lipokines. Many of these factors regulate lipid metabolism, including de novo lipogenesis. Stearoyl-CoA desaturases (SCDs) catalyze the delta 9-desaturation of the saturated fatty acids palmitate and stearate to the monounsaturated fatty acids (MUFAs) palmitoleate (16:1n7) and oleate (18:1n9), respectively. These MUFAs, mainly oleate, are predominant components of cellular and circulating free fatty acids, triglycerides, cholesterol esters and membrane phospholipids. In mice, genetic ablation of SCD-1 isoform reduces hepatic de novo lipogenesis (DNL) and protects against high carbohydrate diet-induced adiposity and liver steatosis. To understand the mechanism by which hepatic MUFA production influences adiposity, we created two liver-specific transgenic mouse models in the SCD1 global knockout that express either human SCD5 or mouse SCD3, that synthesize 18:1n9 and 16:1n7

respectively. We demonstrated that hepatic de novo synthesized oleate, but not palmitoleate, stimulated hepatic lipid accumulation and adiposity. We showed more importantly, that the endogenously synthesized hepatic oleate was associated with suppressed DNL and fatty acid oxidation in white adipose tissue. The data suggested an extra hepatic mechanism where endogenous hepatic oleate regulates lipid homeostasis in adipose tissues.

LI-04
LIPIDS AND HEDGEHOGS

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The Hedgehog cell-cell signaling pathway is critical for animal development, and its misregulation is implicated in birth defects and in cancer. In unstimulated cells, the tumor suppressor membrane protein Patched suppresses Hedgehog pathway activity, by inhibiting the seven-spanner protein Smoothened. The pathway is triggered by the Hedgehog ligand, a secreted protein covalently modified with two lipids, palmitate and cholesterol. Due to lipidation, Hedgehog is very insoluble, and its secretion requires at least two dedicated factors: the membrane protein Dispatched and the secreted chaperone Scube. Once secreted, Hedgehog reaches the surface of the responding cell, where it binds Patched and inhibits it, leading to Smoothened activation, which sets in motion the downstream events in Hedgehog signal transduction. I will discuss our findings on the essential role that lipids play in Hedgehog secretion and transport, in Patched inhibition, and in Smoothened activation. Our results elucidate important lipid-dependent mechanisms in Hedgehog signal transduction, and suggest strategies for blocking oncogenic Hedgehog signaling.

MI-01
UNRAVELLING THE LINKAGE BETWEEN ADAPTIVE EVOLUTION AND HYPERMUTABILITY IN *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a remarkable versatile bacterial species that proliferates in diverse habitats, including the human body. It constitutes an important opportunistic pathogen, being the main cause of chronic airway infections and mortality in cystic fibrosis patients (CF). We are currently focused on understanding the molecular basis underlying its adaptive capability. In this sense, we have long studied the systems by which cells can increase the mutation rate and acquire hypermutator states. Our main interest is to decipher the role of hypermutability in the occurrence of particular phenotypic switches related to chronic airway infections, as well as in more complex processes such as biofilm formation. In the last years, we unravelled the way in which the combined activities of the mismatch repair protein MutS with the error-prone DNA polymerase IV (Pol IV) and DNA simple sequence repeats (SSRs) can drive the mutagenesis of genes underlying specific adaptive processes. Moreover, we observed that *P. aeruginosa* mutators exhibit an enhanced morphotypical diversification in biofilms which correlates with an increased competitiveness. By carrying out a genomic approach we revealed the genetic evolution of mutators during the course of CF chronic infection, which showed an extensive within-patient diversification and populations structured in different coexisting subpopulations.

MI-02
CHEMOTAXIS AND BEYOND: DIVERSITY OF CHEMOSENSORY SIGNAL TRANSDUCTION PATHWAYS

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The signaling pathway involved in bacterial chemotaxis is remarkably well conserved both in Bacteria and Archaea. Chemoreceptors detect a variety of stimuli and transmit the signal to the flagella via associated proteins that include a dedicated two-component system pair. Along evolution, variations in this pathway have added complexity yet keeping the essence of the signal transduction mechanism. In our lab, we study the molecular bases of signal transduction in the well-characterized chemotaxis pathway in *E. coli*. In this context, some new insights about the role of the coupling protein CheW in the signaling complex will be discussed. On the other hand, we also try to understand how variations in this system have given origin to independent though related signaling pathways, and their physiological significance. As an approach to this topic, experimental demonstration of how two different chemotaxis complexes can operate independently in the same cell will be showed. Besides, the chemotaxjs-related chemosensory pathways in an environmental *Halomonas sp*. strain will be described. Our main goal is to understand

how the basic module of chemotaxis-like pathways can be adapted to different physiological or ecological needs within the bacterial world.

MI-03
ROLE OF INORGANIC POLYPHOSPHATE IN COPPER AND OXIDATIVE STRESS RESISTANCE IN THE THERMOACIDOPHILIC *Sulfolobus solfataricus*

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Inorganic polyphosphates (polyP) are linear polymers present in all organisms and perform a wide variety of regulatory functions. It has been postulated that the hydrolysis of polyP by an exopolyphosphatase (PPX) and the formation and transport of metal–phosphate complexes could act as an additional heavy metal resistance mechanism in acidophilic microorganisms. To further study this defense system a recombinant *S. solfataricus* strain unable to accumulate polyP by overexpression of its endogenous *ppx* gene was used. This polyP (-) strain had a 3-fold decrease in its MIC value compared to the wild type, demonstrating the involvement of polyP in Cu resistance in this archaeon. In response to copper, polyP (-) cells increased expression of *copA* earlier than the wt, possibly to compensate for the lack of polyP.
Quantitative proteomics (ICPL) was used to assess the global impact of copper. Increased expression of proteins such as superoxide dismutase, peroxiredoxins and heat shock proteins amongst several other metabolic changes strongly suggests the generation of oxidative stress response and energy depletion due to polyP deficiency. Therefore, polyP may provide mechanistic alternatives in tuning microbial fitness for the adaptation under stressful environmental situations amongst extremophiles. Supported by FONDECYT grants 1110214 and 1150791

MI-04
NEW STRATEGIES TO COMBACT BIOFILMS

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Biofilm formation is especially important in implants and catheters related infections. Although some of these microorganisms colonizing not cause infection, they can promote an immune reaction resulting in the inflammation of the underlying tissue. This fact finally causes a release of the implant that should be removed and replaced by a new one. Bacteria that form biofilms are difficult to eradicate because the antimicrobial resistance conferred by the matrix and persistent cells. This, bacteria embedded in a biofilm could be 1,000-folds more resistant to antibiotics than planktonic counterparts. Due to the importance of biofilm related infections worldwide, the search for new therapeutic tools become very necessary. Currently, new therapeutic options are being investigated as an alternative to existing treatments with antibiotics in order to not only prevent biofilm formation, but also the emergence of resistant bacterial populations in the surrounding tissues. Among these new approaches, we can find nanoparticles containing or not antibiotics, hydrogels, iontophoresis, biofilm enzyme inhibitors, liposomes, bacterial interference, bacteriophages, quorum sensing inhibitors, low energy surface acoustic waves, and antiadhesion agents.

PL-01
CYP INTEGRATES LIGHT AND TEMPERATURE SIGNALING BY REGULATING AUXIN RESPONSES IN ARABIDOPSIS

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As sessile organisms, plants have developed precise mechanisms to rapidly acclimate to environmental changes. Light and temperature are two of the most important cues. Under certain conditions, plants face resource allocation dilemmas: two environmental variables may promote different responses and resources may not be not sufficient for. How is then that plants “decide” which response is more important for survival?
Here we present data supporting a new connection between light and temperature signaling. The responses to shade and to high temperatures have common partners. The response to shade is mediated by phytochromes, mostly phytochrome B (phyB). Low levels of active phyB are produced under shade or at higher plant densities and promote auxin synthesis leading to elongation of stems and petioles. Higher temperatures also activate auxin synthesis leading to similar phenotypes. However, at lower temperatures, the effects of phyB are limited.
Using a transcriptomic approach we found several genes showing genotype x temperature interaction. Auxin response genes were enriched in this group as expected. However, we also found a cytochrome P450 (CYP) of unknown

function which is regulated by both light and temperature cues. Our expression and genetic analysis led to the conclusion that CYP is important to regulate auxin responses at lower temperatures and in this way modulates the responses to shade mediated by phyB.

PL-02
AUXIN AND miRNA393: TOWARDS A BETTER UNDERSTANDING ON MECHANISMS OF DEVELOPMENTAL PLASTICITY

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One of the most striking traits of plant plasticity is the modulation of growth and development in response to environmental changes. The aim of this project is to contribute towards a better understanding on mechanisms that modulate plant plasticity to abiotic stress. The phytohormone auxin regulates several key processes by activating gene expression through direct physical interaction with TIR1/AFB F-box receptor proteins. Phenotypic characterization of *tir1/afb2* double mutants resulted in enhanced tolerance to oxidative and salt stresses. Further, we demonstrated that induction of miR393 is required for down regulation of auxin signaling and auxin-mediated physiological responses during salinity. Under stress, *mir393ab* mutant that is unable to down-regulate TIR1-dependent signaling showed reduced inhibition of emergent and number of lateral roots. Additionally, *mir393ab* mutant plants showed higher level of ROS and reduced ascorbate peroxidase activity compared with wild-type plants. miR393 regulation could be a critical checkpoint between auxin signaling and redox-associated components in order to coordinate tissue and time-specific growth responses. We also conclude that miR393-mediated down regulation of auxin signaling represents a gateway of stress signals into the development program.

PL-03
ROS AND AUXIN CROSSTALK DURING PLANT DEVELOPMENT AND STRESS ADAPTATION

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Understanding the mechanisms that control adaptation of plants to their environment are amongst the most mysterious and fascinating open questions in biology. There are now clear research evidences that reciprocal interaction between reactive oxygen species (ROS) and auxin signalling pathway modulates plant stress adaptation responses. Auxin and its distribution rates play key roles in many aspects of plant growth and development, which largely depend on the spatiotemporal control of auxin homeostasis. Upon stress, increased ROS production affects auxin homeostasis and can lead to a reorientation of growth in order to attenuate deleterious effects of stress. On the other hand, environmental factors primary affect photosynthesis, compromising plant growth and yield. Although ROS-auxin interplay is being intensively studied, the mechanisms underlying their crosstalk are poorly understood. In this respect, molecular insight into the nature of ROS-auxin crosstalk remains scarce. The presentation will draw on our current strategies to shed some light on the mechanisms that integrate environmental and developmental signals by ROS-auxin crosstalk and leading to modulation of photosynthesis and stress-induced growth reorientation, vital for plant survival.

PL-04
UNRAVELLING THE GRNS OF SENESCENCE- AND STRESS-ASSOCIATED NAC TRANSCRIPTION FACTORS

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Plants develop in a fluctuating environment where conditions for growth are often suboptimal or even harmful if not counteracted properly by adaptive physiological responses or changes in developmental programs. Abiotic stresses imposed by e.g. soil salinity, high temperature or drought limit plant growth and typically lead to precocious tissue degeneration and senescence, a process through which nutrients from photosynthetic organs are recycled for the formation of flowers and seeds to secure reaching the next generation. Although some important aspects of the gene regulatory networks underlying senescence in plants have been unravelled in recent years, it is currently not well understood through which molecular mechanisms senescence is connected to developmental programs and the various abiotic stress response pathways. We identified several NAC transcription factors as central transcriptional regulators that coordinate developmental programs with stress-related inputs from the environment. Recent studies in

our group uncovered the cellular involvement of such regulators and identified their target genes and GRNs, providing new insights into the complex and multiply intertwined molecular networks of senescence. Recent findings will be presented.

ST-01
CYTOSOLIC pH AS A GATING SIGNAL FOR PLANT PLASMA MEMBRANE AQUAPORINS

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Many studies have highlighted the intracellular pH dependence of the activity of membrane proteins such as receptors, transporters, ion channels and enzymes. This list can now be extended to certain aquaporins. Plant plasma membrane holds one of the largest groups of aquaporins -known as PIPs- that are described for their capacity to sense cytosolic pH. Biophysical evidence has led to propose a gating mechanism in PIP aquaporins whereas a close state of the channel seems to prevail under cytosolic acidification. Here we summarize evidence consistent not only with pH stimuli as a key regulatory mechanism but also how this response can increase in complexity. Our working hypothesis is that cytosolic pH matters as much as which type of PIPs is present in the membrane and this will reflect the whole membrane water permeability (P_f). Our experimental approach includes i) designing mutants to alter aquaporin water transport capacity; ii) tracking aquaporin localization -at internal structures or expressed at the level of the plasma membrane-; iii) analyzing plasma membrane water transport capacity and iv) analyzing our results in the context of its physiological relevance. We propose that the outcome of the crosstalk between sensing cytosolic pH and regulatory mechanisms (protein trafficking, homo vs heterotetramerization) not only determines final P_f but also broadens the membrane versatility to modulate water exchange.

ST-02
MECHANISMS OF CELL ADAPTATION TO HYPOXIA

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Cells and whole organisms adapt to low oxygen conditions (hypoxia) through specific modifications in their transcription and translation profiles. The Hypoxia Inducible Factor (HIF), a transcription factor conserved in all animal phyla, plays a central role in this adaptation. We have carried out genetic screens in Drosophila melanogaster to define mechanisms that mediate survival in hypoxia. An RNAi based genome-wide screen performed in Drosophila S2 cells rendered 21 novel regulators of HIF, including Argonaute1 and other components of the miRNA machinery. An over-expression screen of all the miRNAs encoded in the Drosophila genome, carried-out in vivo in transgenic fly lines, led to the identification of several MIRs that mediate inhibition of negative regulators of HIF, thereby enhancing molecular and biological responses to hypoxia. Finally, we report that, following exposure of flies to hypoxia, HIF induces the expression of the translational inhibitor 4E-BP, which, by shutting-down general CAP-dependent protein synthesis and favouring translation of a specific subset of transcripts, is essential to prevent accumulation of reactive oxygen species (ROS) and rapid lethality. Our results have revealed a novel network of interactions essential for cell adaptation to hypoxia in vivo

ST-03
CLOCK PROTEINS: NEW MEDIATORS OF DNA-DAMAGE SIGNALING

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Unlike single-cell organisms with self-contained timekeeping systems, multicellular organisms partition their oscillators among different cell types and depend on more complex molecular networks to sense signals and coordinate effective responses. We found that the core circadian clock protein Period 2 (Per2) directly interacts with the checkpoint regulatory component p53, promoting its stabilization and controlling p53 transcriptional activity. Remarkably, circadian phases of Per2 and p53 are anti-phase in the cytoplasm and in-phase in the nucleus, posing new questions about the extent to which Per2 association modulates p53 distribution. Because of the relevance of hp53 in checkpoint signaling, we hypothesize that hPer2 association with hp53 acts as a regulatory module that

influences hp53's downstream response to genotoxic stress. Quantitative transcriptional analyses of hp53 target genes demonstrated that hPer2 dissociation from the hPer2/hp53 complex was absolutely required for activation of the DNA-damage response. Our results provide evidence of the mode by which the circadian tumor suppressor hPer2 modulates hp53 signaling in response to genotoxic stress.

ST-04
THE mRNA BINDING PROTEIN TRISTETRAPROLIN CONTRIBUTES TO LACTATION BY REDUCING LOCAL LEVELS of TNF

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A rapid switch from survival to death occurs in the mammary gland after weaning, when there is a rapid increase of inflammatory cytokines, as IL6 and LIF, which induce Stat3 activation that leads to apoptosis. In addition, IKK2, either alone or through NF-κB, is a proapoptotic factor in this scenario. Tristetraprolin (TTP) is a RNA-binding protein that induces degradation of mRNA coding for inflammatory cytokines and invasiveness-related proteins. We have shown that TTP is specifically induced during lactation. To determine TTP´s physiological role, WAP-Cre recombinase mice, in which the enzyme is expressed uniquely in the lactating mammary tissue, were crossed with lox-P TTP transgenic animals. Mammary glands of bitransgenic females showed clear signs of involution at mid-lactation, as abundant apoptotic cells, high levels of cleft caspase-3, phosphorylated STAT3 and increased levels of LIF, IL6 and TNFα, which is, reportedly, TTP´s most important target. Therefore, we blocked the activity of this cytokine by treating bitransgenic females with its soluble receptor during lactation. This procedure inhibited precocious cell death without affecting LIF and IL6 high levels. These observations indicate that TTP significantly contributes to lactation maintenance, mainly by reducing TNF levels. This prevents apoptosis by avoiding caspase 8 and IKK2 activation.

TS-01
EFFICACY OF AN ORAL VACCINE PLATFORM BASED ON SURFACE PROTEINS OF THE INTESTINAL PARASITE *Giardia lamblia*

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Despite the impact of world-wide vaccination, there is still a great necessity to develop cheap and safe innovative vaccination strategies inducing long-lasting immunity. 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 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 parasite *Giardia lamblia* expresses at its surface variant-specific surface proteins (VSPs) that are extremely resistant to the low pH of the stomach and to intestinal proteases, allowing the parasite to survive in the harsh environmental conditions of the small intestine. We thus hypothesized that the expression onto virus-like particles (VLPs) of *Giardia* VSPs should shield these particles for oral administration. To obtain a proof of principle and, simultaneously, to develop a potential vaccine candidate, we used Influenza Hemagglutinin (HA) as a vaccinal antigen. 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 vaccine platform should have a broad application to different infectious diseases.

TS-02
A BACTERIAL PROTEASE INHIBITOR PROTECTS ORAL VACCINES FROM DIGESTION WHILE TRIGGERS IMMUNE RESPONSES

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We report that a bacterial protease inhibitor from *Brucella* spp. called U-Omp19 behaves as an important component of vaccine formulations against infectious diseases. When co-delivered orally with an antigen (Ag), U-Omp19: i) can bypass the harsh environment of the gastrointestinal tract by inhibiting stomach and intestine proteases and consequently increases the half-life of the co-delivered Ag at immune inductive sites: Peyer's Patches and mesenteric lymph nodes while ii) it induces the recruitment and activation of antigen presenting cells (APCs) and increases the

amount of intracellular Ag inside APCs. Besides, U-Omp19 reduces the amount of digested Ag within APCs at inductive sites. Therefore, mucosal as well as systemic Ag-specific immune responses, antibodies, Th1, Th17 and $CD8^+$ T cells are enhanced when U-Omp19 is co-delivered with the Ag orally. Finally, this bacterial protease inhibitor in oral vaccine formulations confers mucosal protection against CT-induced diarrhea, and reduces bacterial or parasite loads after oral challenge with virulent *Salmonella or Toxoplasma gondii*.

TS-03
ACADEMIC TRANSLATIONAL MEDICINE: FROM TARGET DISCOVERY AND VALIDATION, TO EXPERIMENTAL THERAPEUTICS, TO THE CLINIC

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Research funding has shifted to a model that emphasizes "medicinal translation" and progression of discoveries from academia to industrial R&D. However, academics often misunderstand the meaning of "medicinal translation", and are usually not trained to establish a research program that is meant to yield results that are translatable to the clinic.
We have had success in applying academic research that have lead to discovery and validation of novel targets etiological to disease states, and the development of novel compounds acting as agonists or as antagonists of these targets. Small molecules and monoclonal antibodies that originated in our laboratory passed Phase 3 clinical trials in humans and received approval. Specifically I will show specific examples of our work on neurotrophin receptors TrkA, TrkB, TrkC, and p75NTR. These receptors regulate neuro-glia-vascular interactions in health and in disease states. The targets are relevant (and validated) to pathologies ranging from cancer to neurodegeneration to vascular dysfunction, with predictable mechanisms of action that facilitates translation to the clinic. Each receptor target plays a specific role at a specific stage of pathological progression in disease. I will present experimental therapeutics of Diabetic Retinopathy and ALS; which comprise neurodegeneration, inflammatory, and vascular components during the evolution of disease. We can modulate the function of each target using pharmacological agonists and antagonists.

TS-04
MRP4/ABCC4-MEDIATED CAMP EXTRUSION AS A NEW POTENTIAL TARGET FOR CANCER THERAPY

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G-protein coupled receptors (GPCRs) are the major membrane receptors that mediate extracellular signal transduction. These key signaling mediators are targeted by 25% of the therapeutic agents currently used in clinical treatments. Although diverse extracellular signals activate GPCRs leading to an increase in cAMP, signal specificity results from accurate adjustments at different levels of the cAMP dependent pathway. Cyclic AMP was the first second messenger reported and since then numerous studies have shown its participation in many physiological and/or pathophysiological processes. Cancer is one of the main causes of death worldwide and most of the drugs used in cancer therapy are highly toxic and/or lack specificity. In this context searching for novel specific targets to define new therapeutic strategies is crucial. Our results, provide solid evidence as to propose MRP4-mediated cAMP extrusion as a new target for cancer therapy. In the present lecture the paradigmatic second messenger cAMP and its complex signal transduction network will be addressed with the aim to define therapeutic targets for the development of safer and more efficacious drugs to be used in cancer clinical therapies. Current knowledge is challenging the main actors of the Signal Transduction Play, starring cAMP. Unknown actors emerge as potential main characters with a promising future on stage.

ORAL COMMUNICATIONS

Biotechnology

BT-C01
NANOBODY-BASED IMMUNOCAPTURE ASSAY FOR DETECTION OF SHIGA TOXIN-PRODUCING *Escherichia coli*

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Human infection with Shiga toxin-producing *Escherichia coli* (STEC) is a major cause of postdiarrheal hemolytic uremic syndrome (HUS), a life-threatening condition characterized by hemolytic anemia, thrombocytopenia and acute renal failure. Shiga toxins (Stx1 and/or Stx2) are the most important virulence factor of STEC strains and all the strains express one or both toxins independently of the serotype. Therefore, Shiga toxins detection assays are considered the most suitable methods for detection of STEC and HUS diagnosis. In this work, we have exploited nanobody technology for the development of a double nanobody sandwich ELISA for Stx2 detection. A panel of 13 anti-Stx2 nanobodies, obtained from a variable-domain repertoire library isolated from a llama immunized with the recombinant protein BLS-Stx2B (Brucella Lumazine Synthase-B subunit of Stx2), was evaluated in 130 capture-detection pair combinations. Based on this analysis two nanobodies were selected. Using this combination, we were able to detect the recombinant protein BLS-Stx2B (detection limit, 0.05 ng/ul) as well as the native toxin obtained from culture supernatants of STEC strains expressing Stx2. The nanobody-based immunocapture assay is a novel diagnostic tool that allows a sensitive and specific detection of Stx2 in broth cultures and may be of great value for HUS diagnosis from stool samples.

BT-C02
BIODIESEL PRODUCTION USING NANOSTABILIZED BIOCATALYSTS

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Biodiesel is produced from vegetable oils by chemical or enzymatic transesterification. The enzymatic process, in which the lipases are the most widely used, offers several advantages over chemical routes, including the minimization of waste and byproducts generation, promoting the use of renewable raw materials. Is noteworthy that biocatalysts immobilization favors their stabilization and reusability, enabling the design of sustainable industrial bioprocesses. Additionally, the incorporation of nanocomposites, as montmorillonite, modifies the structure of polymeric matrices to improve its mechanical properties. This enhance the operative stability of biocatalysts increasing its productivity. In this work, *Candida Rugose* lipase immobilization in montmorillonite with yields above 80% was achieved. These immobilized derivatives were then entrapped in alginate, obtaining a mixed biocatalyst which was stable for more than 150 h. This allowed the design of a bioreactor with airlift configuration mode for bioprocess scale-up. Therefore, a continuous sustainable bioprocess was developed which was able to produce 140 g/l of biodiesel.

BT-C03
DEVELOPMENT OF IMMOBILIZED BIOCATALYST WITH POLYGALACTURONASE ACTIVITY FOR JUICE CLARIFICATION

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Biocatalyst design refers to development of processes and technologies for the sustainable manufacture. Also, protein immobilization is a promising method to improve enzyme properties as stability, activity, specificity and selectivity. In this regard, polygalacturonases (PG) are pectinolytic enzymes and their most common application is in food industry for mash treatment, juice clarification and extraction of plant tissues. In this work, an immobilized PG from *S. halstedii* ATCC 10897 was applied in fruit juice clarification. Bioprocess design included culture medium

formulation, optimization of fermentation conditions for PG production and protein immobilization. Designed culture medium contains 15 g/L soy peptone that provided carbon and nitrogen for microbial growth and highest PG production. Besides, pH 8, 28 °C and 200 rpm during 12 h enhanced fermentation yield. Enzyme purification by ultrafiltration allowed to obtain a 48 kDa protein was obtained. Activated agarose supports were evaluated for enzyme immobilization. PG immobilized in Glyoxyl-agarose was selected due to highest conversion (3.12 mg/mL), operational (90 h) and storage stability (120 d). The kinetic parameters Km and Vmax were 76.613 mg/mL and 0.047 min-1, respectively. Finally, application of biocatalyst in grape and plum juices was feasible achieving good features in turbidity and viscosity.

Cell Biology

CB-C01
Shigella BLOCKS INTRACELLULAR TRAFFICKING IN HOST CELLS BY A SUBSET OF EFFECTORS

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Shigella is an enteroinvasive bacterium that delivers effectors inside host cells through a type 3 secretion apparatus. *Shigella* induces Golgi fragmentation and a reorganization of the endocytic recycling compartment, provoking inhibition of secretion, retrograde transport and recycling of host cell molecules. We are interested in the interplay between the subset of *Shigella* effectors involved in host cell secretion blockage. We showed by a synchronized secretion assay (RUSH) that invasion of cells by *Shigella* abrogates the secretion of the adhesion molecule E-cadherin and the cytokine TNFα. Intriguingly, cells infected with either *ΔvirA* or *ΔipaJ* strains did not recover the trafficking of TNFα reporter to the plasma membrane. However, cells infected with a *ΔvirA ipaJ* strain recovered completely the TNFα trafficking. These data suggest a synergistic effect of VirA and IpaJ effectors, blocking the secretory pathway of the host cells at different levels. Moreover, these effectors are also involved in transferrin receptor recycling blockage. We are now evaluating the dynamics of VirA and IpaJ on the Golgi-mediated trafficking pathway and on receptor recycling. In addition, we are studying the consequences of *Shigella* invasion on the secretion of apical and basolateral proteins in the context of polarized epithelial cells, and the impact in epithelial homeostasis and barrier function.

CB-C02
THE ROLE OF N-β-ALANYL DOPAMINE SYNTHASE IN THE INNATE IMMUNE RESPONSE OF INSECTS

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Insects trigger a multifaceted innate immune response to fight microbial infections. The first line of response to microbial entry is mediated by the rapid action of phenoloxidases present in the integument and in the hemolymph which generate quinones that lead to localized melanization and cross-linking, eventually encapsulating invaders. We ascertained if N-β-alanyldopamine (NBAD), which is the main sclerotization precursor of insect brown cuticles involved in the innate immune response. Insects were injected with microorganisms (bacteria, yeast), polysaccharides or spores of the entomopathogenic fungus *Beauveria bassiana* and the activation of NBAD-synthase was assessed. Antimicrobial properties of NBAD were also determined. We show that septic injures induce, in *Tenebrio molitor* and *Ceratitis capitata*, the synthesis of NBAD. We demonstrated in cell-free extracts that NBAD synthase is induced in the epidermis and exhibit the same properties of the ecdysone-induced enzyme expressed only at the time of cuticle molt. Significantly, NBAD showed antimicrobial properties in vitro. These results indicate, for the first time, that synthesis of NBAD is a novel aspect of the overall innate immune response in insects, which likely reinforces the action of the well-known antibacterial peptides.

CB-C03
G-QUADRUPLEXES CONTROL THE EXPRESSION OF DEVELOPMENTALLY REGULATED GENES *IN VIVO*

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Genomic DNA may transiently fold as G-quadruplex (G4), a non-canonical structure associated with gene expression control and genome integrity. Although G4 formation and function was demonstrated *in vitro* and *in cellulo*, *in vivo* biological relevance of this structure is still elusive. To approach this, we used zebrafish embryonic development as an *in vivo* model to assess the role of G4 on the transcription of conserved developmentally regulated genes. In previous studies we found three developmentally regulated genes containing conserved putative quadruplex sequences (PQS) within their promoters (-1000 bp) that formed G4 *in vitro*. Here we present *in cellulo* data obtained by cloning human and zebrafish PQS upstream a basal promoter controlling firefly luciferase transcription in neuro2A cell line, which reveal a transcriptional enhancer role for all tested G4. Furthermore, the *in vivo* role of these G4 was analyzed by specific disruption microinjecting antisense oligonucleotides in zebrafish embryos. This strategy led to transcriptional reduction of the analyzed genes measured by RT-qPCR and morphological changes previously reported for the loss of function of these genes. Overall, this study indicates that G4 display a function *in vivo* and may act as conserved gene expression fine-tuning elements during embryonic development.

CB-C04
OVEREXPRESSION OF CNBP RESCUES MORPHANT PHENOTYPE IN ZEBRAFISH MODEL OF TREACHER COLLINS SYNDROME (TCS)

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TCS is a mandibulofacial dysostosis due to mutations in TCOF1 in which patients show variable expressivity in phenotype. Recent studies suggest that genetic background contribute to these clinical variations. Among the proteins involved in craniofacial development that are affected in the pathology is CNBP (cellular nucleic acid binding protein). We developed an alternative to the murine model of the disease by injecting zebrafish embryos with morpholinos against the TCOF1 ortholog (nolc1). The purpose of this study was: a) to validate zebrafish TCS-like model with respect to the murine model of the syndrome; b) to study the link between Nolc1 and Cnbp. In TCS-like zebrafish embryos we found, in agreement with the reported findings in Tcof1+/- mouse, a reduction of pre-RNAr 47S levels, Tp53 stabilization and an induction of apoptosis markers. Besides, we evaluated the effect of different Cnbp abundance (using fish lines with high and low over-expression of cnbp) on the expression of nolc1 and the craniofacial phenotype of TCS-like embryos. Our findings suggest that Cnbp acts as a transcriptional activator of nolc1 and its over-expression rescues cranial cartilage defects. In conclusion, the TCS-like model in zebrafish replicates the results described in Tcof1+/- mouse. More importantly, embryonic basal levels of Cnbp should play a significant role in TCS expressivity.

CB-C05
GENE EXPRESSION REGULATION DURING MELANOCYTE DIFFERENTIATION: SOX10, MITFA AND DICER ROLES

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Melanocytes derive from neural crest stem cells and are an excellent model to understand gene expression regulation during cell differentiation. Recently, it was reported a gene regulatory network (GRN) for melanocyte differentiation in zebrafish that elucidated new players and gene interactions, as well as integrated novel information with previously reported data. In this GRN, sox10 expression is essential for triggering melanocyte differentiation since it activates the master gene mitfa. However, sox10 has to be sharply depleted because it inhibits the expression of enzymes responsible for melanin synthesis. As Mitf regulates Dicer expression in murine and human melanocytes, we speculate that sox10 abundance is controlled by miRNAs. By RT-qPCR and whole mount in situ hybridization on dicer, mitfa and sox10 mutant zebrafish lines we assessed dicer participation in melanocyte differentiation. Comparing to wild-types, dicer mutant showed lower pigmentation and aberrant expression of sox10 while lower abundance of dicer-mRNA was detected in mitfa mutant. Besides, *in silico* analyses revealed the presence of putative mitfa binding sites in dicer promoter and several predicted miRNA target sites in sox10-3'UTR. All these preliminary

results suggest that dicer is involved in the GRN. Further studies will allow us to incorporate a miRNA family in the GRN of melanocyte differentiation.

CB-C06
PI3K SIGNALING IS IMPLICATED IN BRADYKININ-INDUCED COLLECTIVE MIGRATION OF URETERIC BUD (UB) CELLS

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Previously, we reported that bradykinin (BK) favors UB cell association to form migratory colonies through B2 receptor (B2R) activation. Now, we investigated the mechanisms involved in this phenomenon. We examined the expression of B2R in primary culture UB cells from seeded up to 48 hs by immunofluorescence. In early culture time, most of the cells expressed B2R and were DBA+ (an UB marker). When cultures acquired a higher cell compaction, denoted by E-cadherin immunostaining in adherens junctions, B2R expression decreased. Also, primary cultures of adult CD exhibited very few B2R+ cells. An increase in the protrusive activity denoted by extension of ruffling-lamelipodium was observed after BK stimulation by time-lapse analysis. Pretreatment with LY294002 (PI3K inhibitor) impaired BK-induced cell-cell adhesion and membrane ruffles formation. Immunoblotting analysis did not show differences in Akt-P among untreated and BK-treated cells, with or without LY294002 pretreatment. In addition to Akt, Rac has been indicated as a downstream effector of PI3K. Activated Rac induces membrane ruffles formation to facilitate cell motility which is according with our results. Since PI3 kinase is associated with G-coupled receptors, our results suggest that PI3K signaling downstream B2R could be implicated in the induction of collective migration which occurs during UB branching morphogenesis.

CB-C07
SUMO CONJUGATION TO SPLICEOSOMAL PROTEINS MODULATES SPLICEOSOME ASSEMBLY AND FUNCTION

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Since previous work from our laboratory has revealed the splicing factor SRSF1 as a regulator of the SUMO conjugation pathway, we started to explore a possible link between SUMO and the splicing process, focusing on the spliceosome, the multimegadalton ribonucleoprotein machine responsible for it. We found that the addition of recombinant SUMO-conjugating enzyme to an in vitro splicing reaction accelerates the appearance of mature mRNA while a SUMO protease retards it. By Mass Spec analysis of anti-SUMO immunoprecipitated proteins obtained from pre-mRNA-bound complexes at different steps of the splicing reaction, we identify several spliceosomal SUMO substrates, such as Prp3, Prp28 and Snu114, which we have validated in cultured cells. After identifying SUMO attachment sites in Prp3, we obtained a SUMOylation mutant (Prp3 K289,559R) that fails to increase splicing efficiency when overexpressed, and is unable to co-precipitate U2 and U5 snRNA as well as the spliceosomal proteins SF3a and Snu114, compared to the wt version. We are currently validating the hypothesis that the Prp3 SUMOylation mutant is unable to achieve similar splicing efficiency levels to the wt protein due to its diminished recruitment to active spliceosomes. We propose that SUMO conjugation to spliceosomal proteins could play a role in splicing dynamics by modulating protein-protein and/or protein-RNA interactions.

CB-C08
PROTEIN TRAFFICKING IN *Giardia lamblia*

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G. lamblia is an early diverging organism that parasitizes the gastrointestinal tract (GIT) of many mammals. Inside the host, the trophozoites attaches to the intestinal cells forming a barrier that impair nutrient uptake. However, when the parasites reach the lower parts of the GIT, the Encystation begins with trophozoite detachment and differentiation into cyst. During this process, the protein transport machinery adapts to sort and secret large amounts of Cyst Wall Material (CWM) to the surface throughout regulated Encystation Specific Vesicles (ESVs). Because no recognizable Golgi apparatus is observed in this parasite, our interest was focus on the functional characterization of COPII-COPI system, given its importance in the ER-Golgi protein sorting and delivery. For this, we expressed the Sec23/24 as component of the COPII coat and observed that the ESVs containing CWM are formed in specific COPII-enriched

zones of the ER. When we analyze the COPI-associated KDEL Receptor ortholog in *G. lamblia*, one of the component of COPI, we found that this receptor share its localization with COPII at the point of ESVs formation and unexpectedly mediates the *retention* of ER resident proteins during this process. Our findings showed for the first time a differential cellular rearrangement of the COPII/COPI machinery, acting in concert with the needs of fast and furious protein secretion.

CB-C09
DENGUE VIRUS HOST ADAPTATION IS ASSOCIATED TO A DYNAMIC PATTERN OF SUBGENOMIC RNA ACCUMULATION

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Dengue Virus (DENV) naturally cycles between Aedes mosquitoes and humans. Therefore, the virus must replicate in two different hosts, each with distinctive antiviral mechanisms. During DENV infection, the 5'–3' cellular exoribonuclease XRN1 degrades viral RNA stalling at highly structured regions of the 3' end of the genome. This results in the accumulation of subgenomic RNA fragments (sfRNA) corresponding to the viral 3'UTR, which have been involved in evasion of host antiviral responses. We have recently reported that when the virus adapts to mosquito or human cells distinct viral populations differing in their 3'UTR sequences emerge. Therefore, we hypothesize that genomic variations during host adaptation modulates sfRNA production. Using Northern blot techniques, we evaluated sfRNA accumulation during dengue virus replication in human or mosquito cells. Strikingly, we found different patterns of sfRNAs when the virus adapts to different hosts. To define the mechanism of sfRNA accumulation, we engineered viruses with specific mutations at the 3'UTR and identified an RNA structure responsible for the differential sfRNA synthesis. Disruption and reconstitution of this element was sufficient to mimic the pattern of sfRNAs obtained with mosquito or human adapted dengue virus populations. Our findings provide new information about viral strategies to evade host antiviral effects.

CB-C10
DENGUE VIRUS NS5 PROTEIN INTEGRATES THE SPLICEOSOME AND MODULATES CELLULAR SPLICING DURING INFECTION

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The plus strand dengue virus RNA genome is translated into a single polyprotein which is then cleaved into 10 different viral proteins. One of these proteins, NS5, contains an RNA–dependent RNA polymerase domain and is essential for viral replication. In order to identify cellular proteins that interact with NS5 during infection, we generated recombinant viruses with a purification tag fused to this viral protein and obtained replication competent viruses. Using an affinity purification and mass spectrometry strategy about 50 cellular proteins were identified as specific NS5 binders. Among these binders, spliceosome components were highly abundant with a clear enrichment of U5 snRNP components. Different studies including co–IP and RIP analysis confirmed the presence of the viral protein in active splicing complexes. Analysis of alternative splicing events from endogenous genes and transfected minigenes indicated that viral infection or NS5 overexpression alters cellular splicing patterns.To explore the relevance of NS5–spliceosome interaction on viral infection, different spliceosomal components were silenced. Interestingly, interfering with U5 components resulted in a significant increase in viral replication. These results support a model in which dengue virus reprograms cellular splicing during infection, creating a more favorable cell environment for viral replication.

CB-C11
ROLE OF N-GLYCOSYLATION IN THE CONFORMATIONAL MATURATION OF SECRETORY PATHWAY PROTEINS

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Chaperone and folding assisting enzymes have evolved to deal with proteins that adopt diverse conformations during their folding in vivo. Chaperone selection by a particular substrate depends on the structural features of its folding intermediates. Using Trypanosoma cruzi as a model, we investigated which chaperones and folding facilitating

enzymes assist to cruzipain (TcrCATL) folding, an abundant lysosomal protease. TcrCATL displays three N-glycosylation sites which are recognized by the folding sensor UDP-Glc: glycoprotein glucosyltransferase (UGGT), allowing its interaction with the lectin/chaperone calreticulin (CRT). We studied the in vivo folding pathway of TcrCATL lacking selected N-glycosylation sites in wt and in UGGT-/- parasites. We found that in wt cells TcrCATL associated sequentially with BiP and CRT. Early, extended conformations were bound to BiP, while more advanced and compact folding intermediates associated to CRT. In UGGT -/- cells the interaction between TcrCATL and CRT was impeded, while it associated with BiP only when displaying extended conformations. The absence of TcrCATL-CRT interactions in UGGT-/- parasites resulted in a drastic reduction of TcrCATL folding efficiency, which forms covalently bound aggregates that are retained in the endoplasmic reticulum.

CB-C12
Mycobacterium bovis REQUIRES P27 TO ARREST PHAGOSOME MATURATION AND SURVIVE IN BOVINE MACROPHAGES

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Mycobacterium bovis causes tuberculosis in mammals with strong tropism for cattle and eventually humans. P27 (LprG) is a secreted surface-expressed glycolipoprotein antigen and is involved in the mechanisms of virulence and persistence of *M. bovis* and *M. tuberculosis.* Here we have studied the role of P27 in the interaction with bovine macrophages (BMs), the natural host of M. bovis. BMs were infected with the mutant MbΔp27 or incubated with latex beads (LB) coupled to P27 and the phagosome maturation was determined by the acquisition of LAMP-3 and cathepsin D (late endo/lysosomal markers). Interestingly, the presence of P27 arrested the phagosome maturation in cells infected with *M. bovis* or in cells incubated with LB coupled to P27. We also found that phagocytosis of P27 coated LB was significantly increased in BMs, suggesting that P27 is involved in host-pathogen adhesion. Additionally, we evaluated the expression of cytokines and iNOS upon infection. The MbΔp27 strain elicited significantly lower transcript levels of iNOS than the wild type strain. Moreover, the expression of IL-1beta, IL-6 and IL-12p35 showed a reduction trend in macrophages infected with the mutant strain. Our results indicated that P27 modulates the intracellular phagosomal trafficking and the pro-inflammatory response in BMs, allowing the bacteria to replicate and remain in a suitable non acidic compartment.

CB-C13
CELL ADHESION MOLECULE AND SMALL GTPASE T3-REGULATED GENES IN *Xenopus laevis* GUT EPITHELIA

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Cadherins mediate cell-cell junctions forming dynamic adhesive complexes with α- and β-catenin. These recruit and integrate signals both from extra- and intracellular environment, playing an essential role in embryonic development and maintenance of epithelial architecture. The formation/maintenance of cell–cell adhesion contacts involves a relationship between these protein complexes, actin and its regulators, as well as the Rho-GTPase family. Triiodothyronine (T3) is a key regulator of amphibian gut development. T3 acts via nuclear receptors, which are T3-dependent transcription factors.To establish a molecular framework for understanding adherens junction dynamic and its importance in epithelial differentiation, the gut mRNA expression levels of adhesive complex molecules and GTPases were analyzed. *X. laevis* tadpoles were T3-treated by 0; 1 and 5-days and after gut extraction and processing, mRNAs were measured by sqRT-PCR. E-.cadherin, β- and α-catenin as well as Rac1 show significant increased mRNA levels at 24 h-treatment (0.7-1.2 times regarding no treated larvae) proving to be early-response genes. p120, RhoA, Rap1 and Cdc42 remain unchanged. After 5 days post-treatment, E-cadherin, β-catenin and Rap1 maintain increasing mRNA levels. In this report, we propose the T3-role over these molecules in the establishment of cell adhesion in the *X. laevis* gut epithelium

CB-C14
Staphylococcus aureus INDUCES A REORGANIZATION OF ENDOCYTIC MEMBRANES CAUSING THE FORMATION OF DYNAMIC TUBULES

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S. aureus is a pathogen that causes serious infectious diseases eventually leading to septic and toxic shock. One of the key features of *S. aureus* infection is the production of a series of virulence factors, including enzymes and toxins. After internalization *S. aureus* resides in a phagosome labeled by the autophagic protein LC3.We have shown that the pore-forming toxin α-hemolysin is the *S. aureus*–secreted factor responsible for the activation of this autophagic response. Recent results from our laboratory indicate that *S. aureus* at early times post-infection generates tubular dynamic structures marked with LC3. We determined that these structures correspond also to the late endocytic pathway, as they recruited Rab7. However, they are neither acidic nor degradative. Furthermore, we demonstrate that the formation of these filaments depends on the integrity of microtubules. In the endo-lysosomal system, several small GTPases of the Rab family facilitate transport by recruitment of motor proteins from the dynein and kinesin families. We have demonstrated that the protein Kinesin1 (Kif5B) and the Rab7-interacting lysosomal protein (RILP) are necessary for *S. aureus*-induced filaments elongation. When the formation of these tubular structures was inhibited a marked decrease in *S. aureus* replication was observed, suggesting these structures are necessary for bacterial replication.

CB-C15
ACYL-COA SYNTHETASE-4, A NEW POTENTIAL THERAPEUTIC TARGET IN HORMONE-RESISTANT BREAST CANCER

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Acyl-CoA synthetase 4 (ACSL4) expression, an enzyme working in arachidonic acid metabolism, has been associated with breast carcinoma. The triple-negative tumor (TN) is a subtype of breast cancer that exhibits poor prognosis and no effective therapy is readily available. Therefore, the identification of new therapeutic targets is critical to improve the management of a significant proportion of cancer patients. We show that knocking down ACSL4 expression in TN cell line, MDA-MB-231, induces estrogen receptor (ERα) expression. ACSL4 overexpression decreases the level of ERα. By means of the MCF-7 Tet-Off/ACSL4 model system, which involves a reduction in ERα levels, we used a pharmacological approach to inhibit cell proliferation through a combination of sub-maximal doses of tamoxifen and rosiglitazone, an ACSL4 inhibitor. Drugs alone did not produce a significant inhibition in cell proliferation. However, the combination of the two inhibitors was much more efficient, showing a remarkable synergistic effect. ERα and ribosomal p-S6 protein levels were monitored to confirm that rosiglitazone treatment indeed increased ERα expression and decreased the mTOR signal. The presence of ACSL4 could be a prognostic factor for hormone resistance in ERα-positive breast cancer tissues. A combined therapy could thus be very useful in actually preventing the appearance of hormone resistance.

CB-C16
CYTOPLASMIC FRA-1 AND C-FOS: POTENTIAL TARGETS FOR SPECIFIC BREAST CANCER THERAPY

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Breast cancer is the most common cancer in women worldwide. Most cases in less developed countries are diagnosed at late stages, so development of new therapies to eliminate established tumors is essential. Tumor cells require high rates of phospholipid (pl) synthesis to support membrane biogenesis necessary for their exacerbated growth. Fra1 and cFos activate pl synthesis to sustain proliferation and are highly expressed in breast tumors contrasting with their undetectable levels in the normal control. c-Fos activates particular enzymes of the pl synthesis pathway at the endoplasmic reticulum by physically interacting with them. As Fra1 is highly homologous to key domains of cFos, we propose a shared mechanism for this function. Here, we demonstrate by in vitro enzymatic reactions that Fra1, like cFos, activates CDP-DAG synthase (CDS) in MDA-MB231 cells. None of them affect phosphatidylinositol synthase activity. Similar experiments performed with deletion mutants show that CDS activation is mediated by the basic domain of Fra-1. FRET experiments revealed that Fra-1 binds to CDS through its N-terminal domain.

Preliminary results show that Fra1´s N-terminal domain acts as a negative dominant peptide to prevent breast tumor cell proliferation. These results highlight cytoplasmic Fra1 and cFos as potential targets for a novel breast cancer therapy by inhibition of pl synthesis

Enzymology

EN-C01
KINETIC AND DYNAMICAL CHARACTERIZATION OF AN ANTIOXIDANT SYSTEM FROM *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mt) is one of the most successful human pathogens. The molecular mechanisms of Mt pathogenesis are under active investigation, since they could provide the basis for a rationalized drug design. The decomposition of cytotoxic reactive oxygen species (ROS) formed upon phagocytosis, represents an important survival strategy. Among several enzymatic mechanisms of ROS detoxification, Mt expresses thiol-dependent peroxidases of the peroxiredoxin (Prxs) family, that plays essential roles in reducing hydrogen peroxide, peroxynitrite and organic hydroperoxides. Alkyl hydroperoxide reductase E (AhpE) represents a subgroup of Prxs, comprising MtAhpE and AhpE-like proteins. In this work, we present a combination of fast kinetics, binding experiments, and state-of-the-art hybrid quantum-classical simulations, in order to characterize the molecular basis of MtAhpE catalytic ability and substrate specificity. Our results show that the effects responsible for the fast H2O2 reduction are mainly due to an active-site arrangement, which creates a complex hydrogen bond network that activates the reactive species. In addition, we have confirmed the presence of a hydrophobic groove in the dimeric interface of the enzyme, capable to properly allocate fatty acid-derived hydroperoxides in a reactive conformation, explaining the enzyme´s preference for these kind of substrates.

EN-C02
A SINGLE MUTATION OF *Leptospira interrogans* HEME OXYGENASE GREATLY IMPAIRS ENZYME ACTIVITY

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Leptospira interrogans heme oxygenase (HO) is involved in the cleavage of heme releasing biliverdin, carbon monoxide and iron for subsequent use by the pathogen. Heme degradation is a complex process that requires O_2 and reducing equivalents. In this study we analyzed the role of phenylalanine-157, a residue located in a loop at 16.5 Å from the heme-binding site that is conserved in almost all HOs. It participates in an H-bond network that may deliver H^+ and propagates conformational changes to the active site. The wild type enzyme (HOwt) and a F157I mutant were characterized by optical absorption spectroscopy. We found that HOwt catalyzed the NADPH/ferredoxin-$NADP^+$ reductase dependent oxidation of heme to biliverdin, whereas the F157I reaction was arrested at verdoheme and did not proceed to biliverdin. There were no differences in the formation of the deoxyferrous complex under anaerobic conditions. However, exposing this intermediate to O_2 showed that the oxy-form of F157 was very susceptible to autoxidation in contrast to the stability observed in the HOwt. Furthermore, F157I has low efficiency to hydroxylate heme in the presence of H_2O_2. The present data point out the importance of F-157 in maintaining the appropriate environment for the HO reaction, the mutation probably produces steric effects that trigger a flexibility attenuation of the heme pocket affecting HO activity

Lipids

LI-C01
NUCLEAR LIPID METABOLISM IS DIFFERENTLY REGULATED BY POLYUNSATURATED FATTY ACIDS DURING AGING

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Former studies from our lab demonstrated an active nuclear lipid metabolism in central nervous system that is modified by aging. We detected several nuclear enzymatic activities related to glycerolipid metabolism, such as lipid phosphate phosphatase (LPP), diacylglycerol lipase (DAGL), monoacylglycerol lipase (MAGL), phospholipase A (PLA) and lysophosphate phosphatase (LPAPase). Interestingly, we also observed that they could be regulated by retinoic acid and polyunsaturated fatty acids (PUFA) through an unknown non-genomic mechanism in adult nuclei. Therefore, the aim of this work was to study the modulation of these enzymatic activities by arachidonic acid (AA) and docosahexaenoic acid (DHA) in nuclei from cerebellum of aged rats. To this end, rat cerebellums (28 mo) were homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Using the respective radiolabelled substrates co-incubated with these PUFA, we observed that AA and DHA promote a major DAG availability by increasing and decreasing LPPs and DAGL activity, respectively. A minor MAG availability was also observed due to a diminution on PLA and LPAPase activities. These results demonstrate a different PUFA-regulated lipid metabolism in aged nuclei with respect to adults which could be involved in signaling events related to the epigenetic changes during aging.

LI-C02
SK1 AS KEY G1-G0 TRANSITION MODULATOR IN RENAL EPITHELIAL CELL

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Sphingosine Kinase (SK) is a key enzyme involved in the synthesis of sphingosine-1-Phosphate (S1P), a lipid mediator that regulates several cellular processes. S1P has been characterized as a dual signaling molecule with the ability to activate different effectors. We demonstrated that S1P biosynthesis present a gradual decrease during kidney maturation and cell proliferation. In this report we evaluate the SK activity in renal epithelial cell cycle modulation and in the transit to cell differentiation For this, MDCK cells were cultured at low density to allow cell cycle progression and were treated with D,L-threo-dihydrosphingosine (tDHS), a SK1 inhibitor. SK inhibition induced a decrease in cell number after 24 h of incubation with no alteration in cell viability. Besides, treatment for 24 h with tDHS caused cell cycle arrest in G0/G1 phase with cyclin D1 accumulation. Cell cycle arrest was accompanied with hypophosphorilation of Rb protein. These results suggest that intracellular S1P was involved in cell cycle arrest. Moreover, SK inhibition induced an increase in the percentage of cell in G0 phase after tDHS treatment accompanied by cellular morphological changes. These suggest that S1P is not only involved in cell cycle arrest (with induction of cell quiescence), but also participates in cell differentiation.

LI-C03
CIRCADIAN REGULATION OF CLOCK GENE EXPRESSION AND PHOSPHOLIPID BIOSYNTHESIS IN GLIOBLASTOMA CELLS

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Circadian clocks present even in immortalized cell lines, temporarily regulated diverse physiological processes including cell proliferation and apoptosis while disruption of circadian rhythms can alter cell cycle to potentiate tumorigenesis. Here we analyzed whether the immortalized human glioblastoma T98G cells subject to proliferation (P) in the presence of serum, or maintained quiescent (Q) keep a functional clock, after synchronization, temporally regulating gene expression and phospholipid (PL) metabolism. We examined the expression of clock genes (Bmal1, Per1, Rev-Erbα) and PL synthesizing enzyme genes (choline kinase α: Chokα and CTP:phosphoethanolamine cytidylyltransferase 2:Pcyt-2), and the metabolic labeling of PLs. Cells grown in 10% FBS-DMEM for 3 days were synchronized with a 20 min shock of dexamethasone (100 nM) (time 0), maintained with (P) or without FBS-DMEM

(Q) for 48 h and collected at different times. Results showed that Bmal1, Per1, Rev-Erbα, Chokα and Pcyt-2 exhibited different temporal expression profiles, phases and amplitudes depending on the growth condition tested. Cell cultures also displayed a circadian oscillation in the labeling of 32P-PLs mainly when arrested. Overall, the temporal control of gene expression and metabolism persists in quiescent tumor cells and became more disorganized as proliferation progresses.

Microbiology

MI-C01
DEVELOPMENT OF A HIGH THROUGHPUT ASSAY FOR THE SCREENING OF ACYL-COA CARBOXYLASE INHIBITORS

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Acyl-CoA carboxylases (ACCases) commit acyl-CoAs to the biosynthesis of lipids in *Mycobacterium tuberculosis*. This pathogen has several genes coding for ACCase subunits in its genome: three α subunits (*accA1–3*), six β subunits (*accD1–6*) and one ε subunit (*accE5*). ACCase 5 complex is formed by the biotinylated α subunit AccA3, the carboxyltransferase β subunit AccD5 and the small ε subunit AccE5. To gain insight about the metabolic relevance of this enzyme in mycobacteria, we obtained *M. smegmatis* mutants in *accD5-accE5*, the two subunits specifically associated with ACCase 5. The analysis of this conditional mutant demonstrated that AccD5 and AccE5 are part of an essential ACCase involved in lipid biosynthesis, and proposed ACCase 5 as an attractive target for tuberculosis drug discovery. In this sense, we developed an enzyme-based assay to identify inhibitors of ACCase 5, and we optimized it for high throughput screening. We used this assay towards a library containing 11,000 compounds and found 34 candidates. We further analyzed these candidates by conventional methods and we found 6 compounds that inhibit ACCase 5 with different potency and now need to be characterized in more detail. These results validated the high throughput screening assay as a powerful tool for identifying novel enzyme inhibitors that could be developed as anti-tuberculosis drugs.

MI-C02
TREHALOSE-6-PHOSPHATE SYNTHESIS IN *Streptomyces* IS RELATED TO A NEW GDP-GLUCOSE PYROPHOSPHORYLASE

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The non-reducing disaccharide trehalose (α-D-glucopyranosyl-(1,1)-α-D-glucopyranoside, Tre) is a compatible solute stabilizing cell membranes and protein structures. It protects cells against different types of stresses and can serve as a carbon source or storage molecule. In some actinomycetes, Tre has a structural role and it is found as a cytoplasmic sugar in active turnover. The OtsAB route is the main Tre source in organisms synthesizing the disaccharide. Interestingly, in the group Actinobacteria, the key pathway enzyme Tre-6P synthase (EC 2.4.1.15; OtsA) exhibits particularities regarding nucleoside diphospho-glucose consumption, being highly specific for GDP-glucose in *Streptomyces*, as shown in this work. This scenario with the *Streptomyces*OtsA is supported by biochemical and genetic experiments. We further analyzed two putative pyrophosphorylases (PPases) from *S. venezuelae* to understand the glucose-1P routing towards Tre via GDP-Glc. Indeed, we found a specific GDP-GlcPPase (EC. 2.7.7.34), which had not been identified in prokaryotes to date. Thus, a pathway involving the intermediate GDP-Glc could be proposed, a feature never reported so far in any organism.

MI-C03
ELECTROCHEMISTRY OF EXTRACELULAR CYTOCHROME OMC FROM THE ELECTRO-ACTIVE BACTERIA *Geobacter sulfurreducens*

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Geobactersulfurreducens is an anaerobic gram (–) bacteria capable of using extracellular insoluble electron acceptor like Fe (III) (hydr)oxides during respiration as well as a polarized electrode thus producing an electric current. Elucidating the molecular mechanisms behind these modes of respiration is significant for the development of clean energy technologies. Soluble redox proteins like external cytochromes play a key role in the electron transport and reduction of insoluble electron acceptor in *Geobacter*. In this work we study external soluble cytochromes type c (Omc or Outter membrane cytochromes) of the extracellular matrix of *G. sulfurreducens* electro-active biofilms developed over polarized electrodes. For this we performed the purification and characterization of small cytochrome Omc12 involved in the current production mechanism. Our results showed that Omc12 is a small protein of 12 kDa with a redox behavior typical for mono-heme cytochromes type C and an electrochemical midpoint potential of -135mV. Its interaction with the electrode surface as well as its redox activity changed when electrode surface or physic-chemical conditions were modified. Characterization of this molecule allows us to better understand the Direct Electron Transfer mechanism in this bacteria and its possible application in biosensing as well as microbial fuel cell technologies.

MI–C04
PHOB MODULATION BY POLYPHOSPHATE LEVELS IN *Escherichia coli* AND ITS INVOLVEMENT IN BIOFILM FORMATION

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Polyphosphate (polyP) in *Escherichia coli* stationary phase is modulated by the media phosphate (Pi) concentration and its degradation induces biofilm formation via LuxS quorum sensing system. PhoB, the regulator of the two-component system phoBR, responds to media Pi limitation and inhibits biofilm formation. The aim of this work was to investigate if PhoB is implicated in the formerly studied biofilm formation regulated by fluctuations in polyP levels. Cells were statically grown at 30ºC in minimal medium varying Pi concentration (0.003, 2 and 40 mM). Alkaline phosphatase (AP) activity was measured as an indicator of PhoB activity. In stationary phase, a high AP activity was observed when polyP was accumulated, while it was similar to that of *phoB* mutant when polyP was degraded or absent. Consequently, when PhoB is repressed (polyP degradation) or absent (*phoB* related-mutants) cells form biofilm independently of the media Pi concentration, indicating that PhoB inhibits biofilm formation in a polyP accumulating condition. Data shows that PhoB activity is modulated in non-limiting Pi condition by fluctuation in polyP. PhoB seems to be a component in the signal cascade that regulates biofilm formation triggered by polyP levels in stationary phase.

MI-C05
ROLE OF INTERSPECIFIC INTERACTIONS IN THE EVOLUTION OF MUTATOR BACTERIA

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Mutator bacteria can reach high frequencies in natural bacterial populations and are a major cause of antibiotic-resistant infections. The causes and consequences of bacterial elevated mutation rates have been widely studied and results suggest that mutators might be selected because their higher probability of generating beneficial mutations. However, this evidence is based on pure test-tube studies that have largely ignored a crucial feature of the environment: the microbial community (MC). Here, we investigate for the first time the role of the MC in the selection of *Pseudomonas aeruginosa* mutators in a cystic fibrosis (CF) context. By competing *P. aeruginosa* non mutator and mutator strains in artificial CF communities (*B. cenopacea*, *S. aureus* and *A.baumanii*), we observed that mutator enrichment occurs only in the absence of the MC. Fitness determination of evolved strains suggest that the presence of the MC reduces the availability of beneficial mutations and might prevent mutator hitchhiking. Also, we conducted a survey to investigate whether there was a link between the MC diversity and *P. aeruginosa* mutation

frequency in natural CF communities. We found a negative correlation between *P. aeruginosa* mutation frequency and MC diversity. Our results demonstrate that interspecific interactions occurring in CF microbial communities act as a selective force for *P. aeruginosa* mutators.

MI-C06
HEPATITIS E VIRUS ORF3 PROTEIN IS A HUB PROTEIN WITH REGULATORY FUNCTIONS

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Hepatitis E Virus (HEV) is an emerging virus causing epidemic acute hepatitis in developing countries and with increasing importance in industrialized countries. HEV life cycle is still not well understood because of the lack of efficient cell cultures and small animal models. The objective of this study was to exhaustively examine all possible intraviral protein-protein interactions (PPIs) to get an insight into the function of HEV proteins using a systems biology approach. We used a systematic Yeast two-hybrid (Y2H) and LuMPIS (Luciferase detection MPB-Pull down Protein Interaction screen) to map the HEV genome-wide protein interaction map and provide a basis for studying the function of these proteins in the viral replication cycle. Key PPIs correlate with the already published HEV 3D structure. Thus, we report 20 novel PPIs including the homodimerization of the RNA dependent RNA polymerase (RdRp), the self-interaction of the papain like protease, and ORF3 interactions with the papain-like protease and putative replicase components: RdRp, methylase and helicase. We also present the Kd (dissociation constant) of ORF3 interactions with the viral helicase, papain-like protease and methylase, which suggest a regulatory function for ORF3 in orchestrating the formation of the replicase complex. Hence, these novel interactions may result in potential targets for new antivirals.

MI-C07
THE *Salmonella typhimurium* RcsCDB SYSTEM IS INVOLVED IN THE *std* OPERON REGULATION

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The genus *Salmonella* includes Gram-negative bacteria that cause foodborne diseases in human and animals. We are interested in the study of the RcsCDB system role in the control of *S*. Typhimurium virulence factors. This pathogen produces gastroenteritis in humans and typhoid fever in mice. The *rcsC11* allele activates constitutively the RcsCDB system. As we previously demonstrated that the *S*. Typhimurium *rcsC11* mutant shows reduced attachment to eukaryotic cells, we postulate that the activation of the RcsCDB system represses expression of fimbrial encoding genes. In order to study whether fimbrial expression depends on the RcsCDB system in *S*. Typhimurium, we selected the *std* operon because it was demonstrated that this operon encodes for a fimbriae required for the bacteria persistence in the distal region of the intestine. In this work, we demonstrated that the expression of the *std* operon is down-regulated by RcsB, in an HdfR-independent pathway. Finally, bioinformatic analysis and EMSA assay allowed us to conclude that the RcsB regulator binds to a specific *std* promoter region. The results obtained suggest that the reduced adhesion of the *rcsC11* mutant may be caused by repression of fimbrial genes like *std*.

MI-C08
Brucella MONOMERIC ADHESINS: VARIABILITY, FUNCTIONALITY AND POSSIBLE PSEUDOGENIZATION

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Brucella is an intracellular pathogen responsible for brucellosis. Adhesion to host surfaces is a critical step in the infection process. *Brucella* genomes are very similar among species; however, it seems that much of the variability is associated to surface proteins, suggesting they could contribute to host preference and tissue tropism. The genome of *Brucella suis* 1330 harbors three autotransporters from the monomeric family (BmaA, B and C). We aimed to explore the role of BmaA and BmaB in the interaction of *Brucella* with the host as well as the variability and functionality of their orthologs from different strains. A heterologous approach revealed that the *bmaA* and *bmaB* genes of *B. suis* confer adhesive properties to a non-adherent *E. coli* strain. Besides, mutants in *bma* genes of *B. suis* showed a

reduction in the adherence to HeLa, sinoviocytes, osteoblasts, and colorectal epithelial cells, suggesting that they may contribute to the interaction of *Brucella* with host cells before internalization. Bioinformatical analyses suggest that, in addition to variability in size and number of adhesion motifs among orthologs, BmaA and BmaB might represent pseudogenes in several strains of *B. melitensis* and *B. abortus*. These observations suggest that these adhesins could represent factors of bacterial surface variability between the different species, thus, contributing to host preference.

MI-C09
INSIGHTS INTO THE ROLE OF RAPA, A RHIZOBIAL EXTRACELLULAR LECTIN INVOLVED IN BIOFILM FORMATION

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Under nitrogen starvation, rhizobia establish symbiotic interactions with legumes inducing the formation of root nodules in which conversion of atmospheric nitrogen to ammonia takes place. In *Rhizobium leguminosarum*, it has recently been shown that attachment to roots hairs, increased biofilm formation and nodulation competitiveness are primarily due to enhanced expression of the Rap proteins (*Rhizobium* adhering-proteins). Among them, we have characterized RapA, a unipolar calcium-binding lectin. RapA is secreted by the type I PrsDE system and is composed of two Ra/CHDL (cadherin-like) domains that specifically recognize the acidic polysaccharides produced by *R. leguminosarum*. The exopolysaccharide (EPS) and capsular polysaccharide (CPS) are structurally and genetically related, and are important components of the biofilm matrix. In this work, we show that increased levels of RapA enhance in vitro biofilm formation but disrupt intimate cell-cell interactions in the biofilm. An examination of the polysaccharides produced by the *rapA* mutant and overexpressing strains revealed an alteration in the balance between CPS and EPS production. Interestingly, we also observed that the cleavage of EPS is altered by increased levels of RapA, giving rise to a particular distribution of EPS molecule sizes. We propose that the RapA lectin is important in the remodeling of the biofilm EPS-matrix.

MI-C10
IMPACT ON THE SOIL BACTERIAL COMMUNITIES BY THE HERBICIDES USED BY THE *Lotus tenuis* PROMOTION

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Promoting the forage *Lotus tenuis* is an important alternative for cattle-forage production in the Flooding Pampa, Argentina. This agricultural practice requires the application of herbicides to remove plant species competing. The use of them, in addition to the removal of native vegetation, may affect the diversity of bacterial communities. To evaluate this, we analyzed three different rangeland sites of the Flooding Pampa region. At each site, two paddocks were compared: one managed to promote de forage legume *L. tenuis*, and the second covered by natural grasses. To asses bacterial diversity we used 454-FLX pyrosequencing technology of the V4 region of the 16S rRNA gene, on genomic DNA isolated from soil samples. From the 18 soil samples studied, we obtained 135.918 sequences, representing 3187 Operational Taxonomic Units (OTUs). The main identified components of the bacterial community were *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Chloroflexi*. NMDS ordination in two dimensions based on Bray-Curtis distances and PERMANOVA test did not show differences in bacterial community composition between paddocks promoted or not with *L. tenuis*, although differences among sites were detected. Our results suggest that 5-6 years of land use with *L. tenuis* promotion using herbicides, does not produce enough impact on the microbial community structure in this ecosystem.

Plant Biochemistry and Molecular Biology

PL-C01
LIPID PROFILING OF PEACH CULTIVARS WITH DIFFERENT SUSCEPTIBILITY TO CHILLING INJURY

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Peaches ripen and deteriorate quickly at room temperature. Therefore, refrigeration is used to slow these processes and to extend fruit market life, however, several fruits can develop chilling injury (CI) during storage at low temperature. As the cell membranes are likely sites of primary effects of chilling, in the present work we analyzed the lipidome of six peach cultivars with different susceptibility to CI during ripening and after cold storage. By using ultra-performance liquid chromatography coupled to Fourier-transform mass spectrometry, we detected 59 lipid species, including diacyl and triacylglycerides. After 21 days of cold storage at 0 ºC, all the cultivars accumulated DGDG 36:4 and PC 38:2, and showed a decrease in the level of MGDG 36:5, with respect to harvest. In addition, levels of plastidic glycerolipids were also modified in fruits stored at 0 ºC for a short period, when compared with fruits of the same postharvest age under 20 °C ripening conditions (DGDG 36:3, DGDG 36:6 and MGDG 36:6 increase, MGDG 36:4 and MGDG 36:5 decrease). Finally, the relative abundance of some glycerolipids correlated with the susceptibility to CI, when compared woolly versus non-woolly fruits. Overall, the results allow the identification of lipids that are part of the common response of peach fruit to cold, and lipids that could be used as molecular markers of chilling susceptibility.

PL-C02
A MULTI-LEVEL REGULATION OF *Arabidopsis* FUMARASES REVEALS NOVEL EMERGENT PROPERTIES OF C4 METABOLISM

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Fumarase (FUM) is involved in the metabolism of C4 organic acids, catalyzing reversible hydration of fumarate to L-malate. *Arabidopsis thaliana* genome possesses two genes for this enzyme: *FUM1*, encoding for a mitochondrial isoform, and *FUM2* that could generate two cytosolic polypeptides (FUM2.1 and -2.2) differing in their carboxyl terminal region due to an alternative splicing process of the primary transcript. Here, FUM1, 2.1 and -2.2 were biochemically characterized by a recombinant approach. We could establish complex regulatory mechanisms of FUMs activity that occur at the mRNA and protein level, involving changes in mRNA maturation and abundance, switching the pH medium as well as the allosteric and redox regulation. Specifically, alkaline conditions and high asparagine or glutamine concentrations stimulated the fumarate generation by FUM2. Also, FUM1 and -2 activities are stimulated by the reduction of disulfide bridges between cysteine residues located on different subunits of the tetramer. This was observed either using reducing or oxidizing agents as well as components of redox systems of Arabidopsis leaves in the presence of NADPH. Overall, these results reveal a multi-level regulation of FUM activity in *A. thaliana* and new emergent properties for the control of C4 metabolism can be suggested.

PL-C03
LIGHT, CHROMATIN AND ALTERNATIVE SPLICING

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Light is not only a source of energy but also a key regulator of plant physiological adaptations. We have previously shown that light/dark conditions affect several alternative splicing events including that of the Ser-Arg-rich splicing factor RS31. This led us to investigate whether chromatin modifications play a role in the regulation of alternative splicing by light. Increasing concentrations of trichostatin A (TSA), a drug that inhibits histone deacetylase activity and therefore increases histone acetylation, mimic the effect of light on At-RS31 alternative splicing in a dose-dependent manner. Increasing concentrations of camptothecin, a drug that inhibits topoisomerase I, mimic the effect of darkness on At-RS31 alternative splicing. Using Arabidopsis mutants defective in different histone modifying enzymes we found that the effect of light to dark transition on alternative splicing is strongly reduced in mutants that show higher levels of histone acetylation, such as the histone deacetylase hd1 or the histone methyltransferase kyp6. In contrast, an Arabidopsis mutant defective in a histone acetyltransferase, taf1, shows a stronger light to dark

transition on alternative splicing compared to wild type plants. We are currently performing chromatin immunoprecipitation experiments to study changes in specific chromatin marks.

PL-C04
IS THE *Arabidopsis* ELECTRONIC SHUTTLE ADXR-ADX-P450 INVOLVED IN STEROID SYNTHESIS AND DEVELOPMENT?

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We have identified *Arabidopsis* gametophytic mutants with insertions in a nuclear gene encoding for a mitochondrial adrenodoxin reductase (ADXR). In mammals and insects mitochondria, adrenodoxin (ADX) mediates electron transfer from NADPH via ADXR to the terminal electron acceptor, a cytochrome P450 (P450), which constitutes an essential step for steroid biosynthesis. In this work, we study the occurrence of a similar steroid biosynthetic pathway in plants. Arabidopsis has an ADRX homologue and 2 ADXs located in mitochondria. However, no mitochondrial P450s have been described in *Arabidopsis*. We performed an Y2H screening to detect possible ADX redox partners. We found that the P450 CYP711A1 binds to ADX1, which was confirmed by BiFC assays. We also showed that CYP711A1 has a mitochondrial localization by means of GFP fusions and transient expression in *Nicotiana Benthamiana*. Using the same approach we studied the subcellular localization of different P450s with a predicted signal peptide targeting mitochondria. Two additional P450s were found localized to mitochondria and showed interaction with ADX trough BiFC assays. We collected ovules from wild type, *adxr/ADXR*, and *spl* (which lacks embryo sacs) mutants plants, to analyzed the female gametophyte steroid profile by UL-MS-MS. Additionally, insertional mutants for *CYP711A1* and different P450-encoding genes are being analyzed.

PL-C05
CONTRASTING THE BIOCHEMICAL AND PHYSIOLOGICAL RESPONSE OF *Lotus* ECOTYPES SUBJECTED TO COLD STRESS

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Lotus is an important genus of Leguminosae, used as forage due to their high nutritional value and adaptability to marginal conditions for agriculture. However, its dry matter production is drastically reduced in coolest season. To evaluate this situation, the acclimation response of two *L. japonicus* ecotypes (MG-1 and MG-20) was studied under cold stress. Plants of 3-weeks-old were analyzed at different times (first, fourth and seventh day) of stress (5-9 ± 1 °C) and control (21-25 ± 1 °C), showing that photoinhibition occurs differentially between ecotypes, being MG-1 more affected than MG-20. On the other hand, the Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) production were analyzed under stress, finding no differences between treatments or ecotypes. Nevertheless, NADPH levels were reduced in plants subjected to stress, and being more dramatically in MG-1. Moreover, antioxidant enzymes activities were measured. Only SOD isozymes showed a contrasting response between accessions and treatments, increasing in MG-20. Immunoblot of D1 and D2 proteins were done to analyze the effect in photosynthesis showing that while D1 protein contents remained constant, D2 turnover was evident. Our study provides a deeper insight into the response to cold stress in legumes.

Structural Biology

SB-C01
DETAILED ANALYSIS OF THE CATALYTIC MECHANISM OF HUMAN GLUTAMINE SYNTHETASE

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Glutamine synthetase (GS) catalyzes the formation of glutamine from glutamate and ammonia, using ATP as a cofactor. In plants and bacteria it is essential in nitrogen metabolism, making it a good target for herbicides and antimicrobial drug design. In human's brain it prevents glutamate dependent excitotoxicity and detoxifies ammonia.

The loss of GS activity as been related with neurodegenerative disorders, such as Alzheimer's disease. The human enzyme has 373 aminoacids (M.W. 44 kDa.) and exists as a decamer, formed by two stacked pentamers, with the active sites located at the interface between two subunits of the pentamer. Mainly from crystallographic results a reaction mechanism has been proposed, but since there is no information regarding the enzyme structure with its three natural substrates, a detailed study of the catalytic mechanism has still not been reported.
In the present work we used molecular dynamics simulations (MD), and combined quantum mechanics and molecular mechanics simulations (QM/MM), to examine human GS structural properties, as well as the reaction mechanism at an atomic level. The results provided an accurate study of human GS dynamics, and establishes the groundwork for the analysis of changes in GS activity due to post-translational modifications, as the inactivation of GS through nitration of tyrosine residues by action of peroxynitrite.

SB-C02
BA41, A NOVEL TPM-DOMAIN ATPASE FROM THE ANTARTIC FLAVOBACTERIUM *Bizionia argentinensis*

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The genome of the psychrophilic flavobacterium *Bizionia argentinensis* (Ba) was recently decoded. In order to tackle a structural coverage and accurate functional annotation of the Ba genome, we selected and expressed a set of domains with unknown function. BA41 is a member of the broadly conserved TPM domain family found across prokaryotes, plants and invertebrates. It is comprised by an N-terminal signal peptide, a single globular TPM domain followed by a transmembrane region and a C-terminal low complexity region. We´ve previously solved the crystal structure of the central TPM domain and found that it displays a Rossmann fold similar to an acid phosphatase from A. thaliana and other two uncharacterized prokaryotic domains. The structure revealed a Zn2+ atom in the putative active site, but this metal was also present in the crystallization conditions. We now present a new X-ray tridimensional structure obtained at near atomic resolution (1.4 Å) and show that this domain does not contains a structural metal. Functional assays showed that BA41 has a marginal reactivity against P-serine and cannot hydrolyze the general pNPP phosphatase substrate. Interestingly, the BA41 TPM domain displays high hydrolase activity against ATP and ADP and other triphosphate nucleotides, suggesting that this protein may represent a new class of the broad nucleotide specific ecto-NTPDases family.

SB-C03
CRYSTAL STRUCTURE OF THE NTRX RESPONSE REGULATOR AND ANALYSIS OF ITS DNA BINDING ACTIVITY

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Two-component systems are signaling pathways, formed by a histidine kinase (HK) and a response regulator (RR), that allow bacteria to sense environmental cues and generate a response. Upon reception of a signal, the HK autophosphorylates and then transfers the phosphate group to the RR, which is activated to perform an output.
Our group has been studying the two-component system NtrY-NtrX from Brucella abortus, which is involved in adaptation to low oxygen tension. The RR NtrX belongs to the NtrC family and comprises three domains: an N-terminal REC domain, a central AAA+ domain and a C-terminal DNA binding domain (DBD). Since NtrX is a poorly studied RR, we decided to undergo its structural characterization. Using X-ray crystallography, we were able to solve the structure of full-length NtrX. Overall, the protein crystallized as an asymmetric dimer in which the DBD adopts a conformation that is permissive for DNA binding. It is also noticeable that the presence of a long α5 helix in the REC domain sustains an interface that is different to that reported for this truncated domain. We confirmed the ability of inactive NtrX to bind DNA by EMSA using the promoter of its own operon, and we also established the sequence of the DNA binding site by footprinting. In conclusion, we provide the first crystal structure of NtrX and postulate a feedback mechanism regulating its own expression.

Signal Transduction

ST-C01
AKT POSTTRANSLATIONAL AND SUBCELLULAR LOCALIZATION PROFILING: TOWARDS AN AKT FINGERPRINT

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Cell-to-cell variability (CCV) is observed in normal and pathological conditions. Cancer is perhaps the most challenging situation in which to investigate how to use information present in CCV for therapy. Deregulation of Akt, a kinase that orchestrates many biological functions including cell proliferation and survival, has been widely linked to cancer. The information contained in the expression level, posttranslational modifications, activity and specific subcellular localization of each Akt isoform in each cell might explain differential Akt target specificity, CCV in cell fate choices as well as in anti-tumor drug sensitivity. We have developed immunofluorescence-based technics and designed fluorescent reporters to study CCV in the spatio-temporal dynamics of the Akt pathway. Here, we describe a protocol for automated imaging and quantitative measurement and analysis of the localization and phosphorylation profiles of Akt and its substrates. The combined dataset obtained defines what we call the "Akt fingerprint". We found novel subcellular compartments were Akt is recruited and we are now analyzing the molecular mechanisms that govern relocalization to these places. Analysis of Akt substrates by phosphorylation and localization profiles as well as classification of cancer cell lines by Akt fingerprint can lead us to understand complex cell and tumor behaviors.

ST-C02
CELL-TO-CELL VARIABILITY IN LIGAND-RECEPTOR BINDING DYNAMICS

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Biological systems are composed of physical constituents that constrain their performance leading to cell-to-cell variation, a feature often regulated by active mechanisms. Here we focus our attention on the cell-to-cell variability that arises from the dynamics of ligand-receptor interaction. We characterized in single cells the binding of fluorescently-labeled sexual pheromone (αF*, alpha factor) to its G-coupled protein receptor, Ste2, in Saccharomyces cerevisiae. By using competition experiments with unlabeled pheromone (αF) and strains lacking the receptor, we confirmed the specificity of the fluorescent analog association with the cell surface. Performing binding experiments, we measured a dissociation constant for αF*-Ste2 (KD) of 23nM that ranges in the order of αF KD, 5nM. Using quantitative fluorescence microscopy and image segmentation analysis, we determined binding dynamics for each cell and on/off rates, KD and total number of receptors were calculated. We observed a broad distribution of both KD values and total number of Ste2 between cells with coefficient of variation of 0.46 and 0.35, respectively. Finally, we studied the effect of mutations in the receptor and cell metabolic state on the control of cell-to-cell binding variability. Taking these data, we found that ligand-receptor binding dynamics is a new, previously unexplored, source of cellular signaling noise.

ST-C03
ROBUSTNESS TO VARIATION IN RECEPTOR ABUNDANCE IN A CANONICAL GPCR SIGNAL TRANSDUCTION SYSTEM

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G-Protein Coupled Receptors (GPCRs) are a important family of transmembrane receptors. They signal through the activation of heterotrimeric G proteins, which in turn activates downstream effectors. The pheromone response pathway in yeast is a prototypical and extensively studied GPCR signal transduction system. This system is notably robust to variations in receptor abundance, an observation that is hard to explain by the current model of G protein activation. We developed a detailed mathematical model of this system, which includes the activation of the heterotrimeric G protein and its coupling to the receptor. Analysis of the model suggested a novel working regime that could explain the observed robustness. This mechanism critically depends on the reported physical interaction between the RGS (an inhibitory component of the system) with the receptor. Consistent with our predictions, delocalization of the RGS activity resulted in loss of robustness to receptor abundance. Our model also explains many results from the literature that are difficult to explain by other mechanisms.

ST-C04
TETRAMERIC STRUCTURE AND INTERACTORS OF THE N-TERMINUS OF BCY1, THE YEAST PKA REGULATORY SUBUNIT

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PKA is classically a tetramer formed by a dimer of regulatory subunit (R2), which binds cAMP, and two catalytic subunits. In mammals the N-terminus of R2 (DD) is responsible for dimerization and for binding to AKAPs, through a hydrophobic surface. We have shown that Bcy1, the yeast PKA R subunit: 1) binds to specific interactors depending on its N-term 85 aa; 2) the interaction is dependent on charged residues; 3) a bacterial recombinant construct of Bcy1 (1-50) is a tetramer (dimer of dimers) both in crystal and solution. We now present characteristics of the tetrameric structure showing the importance of the orientation of Arg45 in the maintenance of the tetramer, in comparison with mammalian structures/sequences in a complete phylogenetic analysis. Two mutants that could affect the tetramerization are constructed and purified: Arg45Ala, and deltaQ43. In order to study whether Bcy1 (1-50) is sufficient to interact with specific proteins we overexpressed Tag.Bcy1(1-50) in a WT yeast strain; interactors were pulled-down with Ni-agarose; eluted with imidazol and analyzed by nanoHPLC-ESI-Orbitrap MSMS and compared to a control WT strain. Among the specific binders was endogenous Bcy1. This result indicates that Tag-Bcy1(1-50) and endogenous Bcy1 interact in vivo and raises the doubt on whether the specific interactors bind to the Tag-.DD domain directly or via endogenous Bcy1.

ST-C05
NOVEL STRESS GRANULE REGULATORS IDENTIFIED IN AN RNAI SCREEN IN *Drosophila*

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Stress granules (SGs) are cytoplasmic accretions that form transiently in all cell types undergoing acute stress. SGs contain polyadenylated mRNA, a number of translation factors and several RNA-binding proteins. Their significance to cell survival remains elusive. SG assembly and disassembly is a multi-step process that depends on I) Translation initiation blockage and destabilization of polysomes II) Retrograde transport by dynein and adaptor proteins III) Aggregation through specific proteins IV) Dissolution and dispersion mediated by stress-induced chaperones and kinesin. We performed an RNAi–based screen in Drosophila to identify signaling pathways that regulate SG dynamics. We identified 32 positive and 15 negative modulators of SG formation. As expected, eIF2α kinases facilitate SG assembly whereas the antagonistic phosphatase PP1α helps SG dissolution. Several hits are related to mRNA metabolism and translation, cytoskeleton organization and intracellular transport, and a number are linked to neurodegenerative diseases. We are currently investigating these novel SG regulatory mechanisms in the Drosophila brain. In addition, we are investigating how 2-Cys Peroxiredoxins, which are peroxidases and RNA binders with stress-regulated chaperone activity, regulate SG formation. We thank the DRSC, Harvard Medical School, and ANPCyT, CONICET and UBA, Argentina for funding.

ST-C06
CROSS-REGULATION BETWEEN *Bacillus subtilis* TWO COMPONENT SYSTEMS DESK-DESR AND YVFT-YVFU

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Two component systems (TCS) play a major role in adaptation to environmental changes in prokaryotes. In *Bacillus subtilis*, the DesKR TCS can detect changes in membrane fluidity upon a temperature downshift and induce the expression of a fatty acid desaturase, encoded by the des gene, allowing cell adaptation to cold shock. *B. subtilis* possess a DesKR homolog TCS, YvfTU, which regulates the expression of yvfRS genes, also in a temperature-dependent fashion. These genes encode a putative ABC transporter, but the transported solutes are unknown. In this work, we investigated whether the similarity of these TCS allows cross-talk between both pathways. First, we built a desR null mutant carrying a transcriptional fusion of PyvfR to lacZ wich allow us to evidence that DesR is essential for yvfRS expression. To know which is the phosphorylation state of DesR required for yvfRS expression, we made complementation tests with a DesR mutant in its phosphorylatable Asp54 residue. Also, by in vivo and in vitro assays we prove that DesK is able to phosphorylate YvfU, showing that DesK has an active role in yvfRS activation. On the

other hand, YvfTU TCS is not essential for des activation and YvfT cannot interact with DesR. These results suggest that together DesKR and YvfTU TCS regulate the transcription of yvfRS, evidencing one of the few examples of cross regulation known to date.

POSTERS

Biotechnology

BT-P01
ANTIMICROBIAL ACTIVITY OF A KAZAL-TYPE INHIBITOR FROM *Toxoplasma gondii* AGAINST PLANT PATHOGENS

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Plants are susceptible to be infected by several pathogens that secrete proteinases. A serine proteinase inhibitor of *Toxoplasma gondii* (TgPI) has shown to be a potent trypsin, chymotrypsin and elastase inhibitor. Since serine inhibitors have been established as an effective protective system for plants, we propose to evaluate the antimicrobial activity of the recombinant TgPI (rTgPI) against plant pathogens with the object to use this inhibitor as a novel strategy for improving plant pathogen resistance. Previously, we showed that rTgPI has bacteriostatic effect on *Pseudomonas'* growth. Following with the characterization of the inhibitory properties of rTgPI, the germination of *Botrytis cinerea* conidia in culture medium supplemented with rTgPI was analyzed. Germination rates of *B. cinerea* conidia in the medium containing rTgPI were significantly lower than control. To investigate whether rTgPI is able to inhibit the infection in planta, *A. thaliana* were co-inoculated with *B. cinerea* or *P. siryngae* and rTgPI. After *B. cinerea* infection, necrotic area was measured. Leaves co-inoculated showed a reduction of 70-80% of necrotic area respect to the controls. Similarly, bacterial counts were significantly reduced in leaves co-inoculated with *P. siryngae* and rTgPI respect to the controls. In conclusion, rTgPI would be able to inhibit the infectivity capacity of these plant pathogens.

BT-P02
CYTO- AND *IN VIVO* TOXICITY OF A BOVINE ALBUMIN NANOPARTICLE WITH ANTITUMORAL DRUG

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Serum albumin is one of the most abundant proteins in the body. Its main function is to be useful as a carrier for different substances. Our group managed to obtain a bovine serum albumin nanoparticle (BSA NP). The aim of this study is to characterise the BSA NP as a possible and efficient drug delivery system. Specifically the aim was to study the NP's toxicity in different study models. Human breast MCF-7 and prostate PC-3 carcinoma cells were tested for their viability while incubated with the BSA NP with and without drug. Cell viability was determined by crystal violet, MTT and LDH assays after 4, 24 and 48 hours. Moreover, toxicity studies in in vivo Zebrafish model were carried out. The effect of each treatment was tested by well activity, viability, cardiotoxicity and morphological malformations. Our results show that BSA NP is not toxic for tumoural cell lines whereas, BSA NP with an antitumoural drug diminishes considerably the cell viability (c.a. 55% viability). As regards Zebrafish toxicity, BSA NP and BSA NP with the antitumoural drug proved to be equally toxic. Both diminished the larvae activity and generated morphological changes in the larvae tested. While BSA NP proved to be a possible and efficient drug delivery system for tumoral cell lines. In the study model it to have toxic effect showing a preference for liver and brain causing toxicity.

BT-P03
CHARACTERIZATION OF FUNGAL STRAINS AS POTENTIAL BIOLOGICAL CONTROL FOR *Ilex paraguariensis* CROPS

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Yerba mate (*Ilex paraguariensis* St. Hill) crops are very important in the province of Misiones, Argentina. However these cultures are infected with diverse species of fungi causing disease and death of the plants. The present study aims to isolate and identify fungal microorganisms from diseased plants collected in Misiones, and to select *Trichoderma* native species as potential biological control agent using antagonism tests. 50 fungal microorganisms were isolated from leaves of diseased plants. Some fungi were morphologically and molecularly identified using ITS markers. The tests of antagonism were performed in PDA medium inoculating Trichoderma species and other microorganisms isolated. The mean radial growth rate was determined by measuring the diameter of the expanding colonies from the third day to the end of the experiment. The Inhibition Degree was calculated on the seventh day and the Antagonist Index was calculated on the tenth day. All the assays were repeated in triplicate. As a result, 77.78% of pathogenic strains was invaded and reduced by 75% or more by different *Trichoderma* species. Our results demonstrate the potential of *Trichoderma* species as biological control agents.

BT-P04
DEVELOPMENT OF VACCINE PLATFORM FOR MUCOSAL ADMINISTRATION USING *Lactobacillus* AS ANTIGEN CARRIER

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Lactobacillus (LB) is a bacterial genus that inhabits animal and human mucosal surfaces such as the gastrointestinal tract. On its membrane, *LB* displays a proteinaceous layer (S-Layer) that has an important role in bacterial growth and host-cell interaction, formed by the self-assembly of the protein SlpA. Here, we engineered the carboxy-terminus of SlpA (the membrane binding domain) from *L. acidophilus* to generate a GST-SlpA$^{240\text{-}378}$ (G-S) fusion protein that was recombinantly expressed and affinity purified. When coincubated with *LB casei* (that has no S-layer), G-S was bounded with high affinity to membrane. The amount of G-S bound to *L. casei* membranes was estimated by Quantitative Infrared Western Blots as 0.15pg/bacteria (about 2000 molecules/bacteria). The stability of the recombinant S-layer (r-S-Ly) was tested by exposing *LB* to conditions that mimics intestinal environment (pH, bile acid and proteases). Thus, r-S-ly was stable to different pHs and bile acids present within the intestinal tract and also the membrane-bound G-S protein was more resistant to protease degradation than free G-S. Here, we explore the use of *LB* as antigen carrier for vaccine development against the bacterial pathogen *Escherichia coli* O157:H7. Thus, we constructed a GST-EspA- SlpA$^{240\text{-}378}$ and GST-EspA-Intimin-Tir-SlpA$^{240\text{-}378}$ chimeric proteins for testing vaccine protection in a mouse model.

BT-P05
MARKER ASSISTED SELECTION FOR DURUM WHEAT ELITE LINES DEVELOPMENT TOLERANT TO FUSARIUM HEAD BLIGHT

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In Argentina durum wheat (*Triticum turgidum ssp. durum*) is the basis for dry pasta. Fusarium Head Blight (FHB) (*Fusarium spp.*) is a major global disease affecting this crop. The introgression line named as Langdon Dic-3A (LNDD3A), shows moderated tolerance to the disease, which is explained by a translocated fragment of chromosome 3A from *T.dicoccoides* (SSRs loci Xgwm2 and Xgwm674 linked). However, LNDD3A does not have good agronomic fitness. The aim of this study was to obtain a donor with FHB tolerance, emphasizing phenotypic selection in the field, assisted by SSRs. A single crossing was made between an elite line from a National Durum Wheat Breeding Program (NDWBP) and the LNDD3A. From F2 to F6, field selection was made following the pedigree method. In F4, F5 and F7 each selected genotype was characterized for the FHB linked loci. In F4 100% of individuals were heterozygous, while in F5 50% were heterozygous and 6% positive homozygous. In F6 the pressure selection for agronomic performance was stronger. Grains from these harvested plots (F7), were analyzed with SSRs and 50% showed positive homozygous for the loci, while the rest were negative. These positive lines will be

evaluated at a later stage, by direct infection, and those who show the best performance will be donors of a NDWBP. The procedure is presented as an alternative effortless to backcross method widely used.

BT-P06
ATARM-PROTEINS GENE EXPRESSION IN THE *Arabidopsis thaliana* POLLINATION PROCESS UNDER GIBBERELLINS STRESS

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The functional putative homologues of BnARC1, an armadillo repeat protein (ARM) of B. napus, known to regulate the pollination processes in this species, were studied in *A. thaliana*, by combining qRT-PCR gene expression, immunofluorescence and pollination bioassays. These studies were conducted both in normal conditions and under Gibberellin stress (GA), both in wild type (WT), gid1a and pub16 mutant plants. It was verified that AtPUB16 increases its expression 2.8 times in WT plants, 1.4 times in gid1a and does not exhibit changes in the pub16 mutant. AtPUB19 gene expression is increased by about 2.5 times in the three genotypes studied, the AtPUB5 expression is only significantly increased in the mutants gid1a and pub16, while AtPUB2 only exhibits significant changes in WT and pub16 plants (2 and 1.3 times respectively). By immunofluorescence was verified that, in stigmas of WT plants treated with GA, AtARM proteins significantly increase its expression, compared to controls, whereas in the gid1a and pub16 mutants decrease by 70% and 90% respectively. Also, in the pollination bioassay it was verified that GA causes increases in the pollen grains number attached on stigmas, only in WT plants, confirming our previous studies about the AtPUB16 key role, in GA mediate self-pollination mechanisms of *A. thaliana*.

BT-P07
CHITOSAN HYDROGELS: AN ALTERNATIVE FOR A BIODEGRADABLE ORAL DELIVERY DEVICE

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Severe periodontitis represents a priority in oral care. It´s the sixth most prevalent condition worldwide, which affects 10% of the population. This advanced stage of periodontal disease characterized for the formation of dental pockets and gingival tissue recession could lead to tooth loss. Thymol chitosan hydrogels exemplifies a possible alternative for a biodegradable oral delivery device for severe periodontitis treatment which would also serve as a scaffold for periodontal tissue regeneration. Oxidative stress is known to be involved in periodontitis pathogenesis. Thymol release, a phenol compound with antioxidant properties, could help as a supplementary therapy agent. Antioxidant agents have already been added in many dental products, such as oral rinses and toothpastes. Thymol chitosan hydrogels showed *in vitro* antioxidant activity after 24hs, carried out through the DPPH assay, which analyses free radical scavenging activity. Thymol release was determinated by the Folin-Ciocalteu method using as release medium modified Fuyasama-Meyer artificial saliva. Chitosan hydrogels were characterized by scanning electron microscopy. It was observed that thymol *in vitro* release from chitosan hydrogels in artificial saliva preserved its antioxidant characteristics. Thymol antioxidant therapy could decrease periodontal inflammation through its free radical scavenging activity.

BT-P08
IN VIVO BIOCOMPATIBILITY EVALUATION OF GENTAMICIN SILICA-COLLAGEN NANOCOMPOSITES

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Nanocomposites are composites in which at least one of the phases shows nanometric dimensions. In this work we evaluated in vivo biocompatibility of Silica nanoparticle-collagen I hydrogels composites as gentamicin releasing systems for medical applications, that showed prolonged antibacterial activity in a previous work. One step loaded 500nm silica nanoparticles (SiNPs) were synthesized according to the Stöber method. For this purpose Tetraethyl orthosilicate (TEOS) and ammonium hydroxide were added to a water-ethanol mixture with or without (blank) gentamicin sulphate. Monodisperse drug free and gentamicin loaded SiNPs were observed by scanning electron microscopy (SEM). Collagen type I was purified from rat tails and nanocomposites were prepared by mixing 30mg

SiNPs with 5mg/mL collagen suspension (0,5M Si). Antibacterial activity of gentamicin SiNPs (338 IU per g NPs) were evaluated by the disk diffusion method as described in the US Pharmacopeia. *Pseudomonas aeruginosa* was used as sensitive microorganism and LB agar as growth medium. Collagen hydrogel and nanocomposites morphology were also observed by SEM. Biocompatibility was evaluated in vivo by implanting subcutaneously 300uL hydrogel or composite on Wistar rats (250g). No clear toxicity was observed by histology and immunohistological studies after 15 days.

BT-P09
NEGATIVE EFFECTS OF A NON-CONSENSUS CYS RESIDUE OF A SINGLE CHAIN ANTIBODY FRAGMENT (SCFV)

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scFv(s) have the potential to replace mAbs in several applications. The aim of this work was to obtain a scFv anti-recombinant human follicle stimulating hormone (rhFSH). Besides, given the attained sequence, the effect of two contiguous Cys residues on the antigen interaction was analyzed. A pool of heavy and light variable fragments (VH-VL) was constructed from an anti-rhFSH hybridoma-extracted RNA. VH and VL fragments were identified by sequencing and bioinformatics. Then, the scFv was constructed by splicing of VH and VL nucleotide sequences through a linker. The scFv was expressed in the periplasmic space of *E. coli BL21(DE3) Rosetta*™ cells and it was extracted by osmotic shock. scFv binding activity to rhFSH was determined by ELISA assays. The scFv sequencing revealed the presence of two contiguous Cys residues in VL fragment. In order to study the impact of these residues, Cys-by-Ser mutations were carried out considering their similar physicochemical properties and the absence of the sulfhydryl group in Ser. Thus, C87S and C88S mutants were constructed by site-directed mutagenesis. The mutation of Cys-VL88 confers a non scFv binding to rhFSH and the mutation of Cys-VL87 produced an increase of the scFv anti-rhFSH affinity. These results led to the conclusion that Cys VL87 would cause the structural destabilization of the scFv to bind to the hormone.

BT-P10
DEVELOPMENT OF BACTERIAL PLATFORM FOR BIOPLASTICS MANAGEMENT

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The development of environmentally friendly alternatives to conventional petroleum derived plastics, such as bioplastics, is urgently desired due to depletion of petroleum resources, global warming, and environmental damage. Among bioplastics, PHAs represent a good alternative since they are produced biologically directly from renewable resources. More than 150 different monomers can be combined within PHA family to give materials with extremely different properties. However, the use of PHA in a wild range of applications has been hampered mainly by their high production cost. Furthermore, the difficulty in controlling their biodegradability and their efficient recycling has become increasingly important. Until now, recycling has been carried out by chemical degradation. A biodegradation system needs a high variety of specific degrading enzymes sufficiently active and stable in different reaction conditions, which has been poorly reported. We performed a data mining analysis looking for high temperatures stable depolymerases. We selected enzymes able to degrade different types of plastics including copolymers. An *E. coli* recombinant clone, carrying and simultaneously expressing three depolymerases would derive in a best managemet of discarded bioplastics. This is a first step towards a total recycle system of bioplastics.

BT-P11
BIOLOGICAL ROLE OF NON-CODING REGIONS IN THE REPLICATION OF *Baculovirus* GENOMES

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Baculoviruses are insect pathogens carrying large circular dsDNA genomes (80-180 kbp). There are hundreds of species reported worldwide and are classified in four genera: *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus*. The biotechnological interest on these viruses is demonstrated by the existence of numerous applications that assist in pest biological control, recombinant protein expression or gene delivery in mammals,

among others. Despite the success of baculovirus-based technologies, some viral molecular mechanisms are still poorly understood such as replication. Particularly, the baculovirus genome contains 90-180 genes according species and very few non-coding regions. Among them, tandem repeats recognized as homologous regions (*hr*s) and direct repetitions (*dr*s) were associated with DNA synthesis. Besides, the mechanisms involved in genome replication are still discussed and the evidences suggest that *Theta*, rolling circle or recombination-dependent processes may occur. Considering the above, in this work the dynamics of DNA synthesis and the role of *hr*s and *dr*s from the nucleopolyhedrovirus of *Anticarsia gemmatalis* (AgMNPV, alphabaculovirus) is analyzed and discussed using the UFL-Ag-286 cell line as host, the virus isolate 2D and infection-dependent replication assays of plasmids carrying viral regions as the main experimental strategy.

BT-P12
RECOMBINANT EXPRESSION OF PIGGYBAC TRANSPOSASE FOR USE IN dsDNA VIRUS GENOME MUTAGENESIS

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Functional genomics is a field of life sciences which aims include the study of gene function in the context of an organism. Generally, these analyses require the introduction of mutations such as gene knock-outs with the goal to collaborate in the understanding of the role played by proteins and non-coding regions. For this purpose, the use of insertional transgenesis based on engineering transposons has been reported as a good molecular tool. PiggyBac, a class II mobile element isolated from insects, is one of the most employed transposons in functional genomics having successful references in organisms from different lineages including bacteria and mammals. Baculoviruses are insect pathogens carrying large circular dsDNA genomes which are infective *per se*. They are very exploited in different technologies including protein expression, bioinsecticide uses and gene delivery in mammals, among others. Considering that the biological role of baculoviral gene content is not fully understood, we decided to develop an *in vitro* mutagenesis approach based on a binary system containing the recombinant PiggyBac transposase and an insertional cassette which confers a bacterial ORI to transform virus genomes in plasmids. In this work the heterologous expression of transposase in bacteria, *Pichia pastoris* and insect cells is reported and the preliminary activity studies are discussed.

BT-P13
HIGH LEVEL PRODUCTION OF FUNGAL RECOMBINANT INULINASE IN HIGH-CELL-DENSITY CULTURES OF *Pichia pastoris*

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Inulinases comprise an important group of enzymes industrially used for production of fructose syrups and fructooligosaccharides, valuable compounds extensively used as sweeteners and functional food additives. *Aspergillus kawachii* (IFO 4033) produces an acid active inulinase (INU) with potential industrial applications. Due to the low expression levels, INU was cloned and over expressed in order to increment enzyme production. A. kawachii INU ORF was cloned into *P. pastoris* expression vector pPICZαA and expressed in X-33 *P. pastoris* cells. A high expressing clone was selected and cultivated under suitable culture conditions to achieve substantially high levels of INU activity. Fed-batch cultures, limited by methanol concentration and regulated by oxygen consumption, were carried out in a 5-L bioreactor. After 72 h of induction, recombinant INU activity reached 840 U/mL, value which is 105 times higher than that obtained using the wild type *A. kawachii*. The enzyme was purified to homogeneity using chromatographic techniques, and the biochemical characterization was performed. By the strategy proposed, it was possible to obtain substantial amounts of a stable and robust biocatalyst to be applied in industrial processes for the production of high fructose syrup.

BT-P14
PROTEIN ENGINEERING OF BLS TO GENERATE A MULTIVALENT BIFUNCTIONAL PLATFORM

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The Brucella Lumazine Synthase (BLS) is a homodecameric protein formed by a head-to-head dimerization of homopentamers. We have previously demonstrated its high quaternary stability and immunogenicity, allowing it to

be used as antigen carrier. In this work we decided to mutate the homodimer interface in order to interrupt the association between homopentamers and simultaneously promote the association between mutant heteropentamers. In this way, we aim to produce two different BLS fusion proteins, five copies of each one on a different pentamer and therefore to bifunctionalize BLS in a multivalent manner. The mutations have been rationally designed based on its crystallographic structure using the bioinformatic softwares FoldX and Pymol. The BLS mutants have been named as BLSa and BLSb and possess the introduction of negatively and positively charged aminoacids in the pentameric interface. These mutations disrupt in cis interactions between pentamers and complement in trans. Both mutant variants have been constructed and purified to homogeneity. Structural analyses demonstrate that BLSa and BLSb form pentamers in solution and when incubated together they are able to heterodimerize (BLSab). In addition, we have created fusion fluorescent proteins (YFP and CFP) to BLSa and BLSb and demonstrated that this system allows the building of a multivalent bifunctional BLSab.

BT-P15
PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANT WITH APPLICATION POTENTIALS

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AIM: Producing and characterizing a surfactant from a not pathogenic bacterial strain. MATERIALS AND METHODS: The *Pseudomonas sp.* strain was cultivated in controlled conditions. The production of biosurfactant was corroborated by measures of surface tension (γ). The culture was concentrated, sterilized and lyophilized. The analytes of interest were isolated through of an absorption chromatography. They were analyzed by UPLC-MS, Spectral Scanning, HPLC-UV and NMR, and the critical micellar concentration (CMC) was determined. RESULTS: The supernatant of culture gave a value of 31 Dyn/cm for a dilution 1/3000 in the optimized conditions. Was isolated a fraction by chromatography, it showed a CMC of 160 ppm and γCMC = 29 Dyn/cm. Masses, that vary 1200-1250 Da, were found by UPLC-MS. A pick to 210 nm was observed by HPLC-UV. Furthermore, this had a soluble (S) and insoluble (I) portion in water. This allowed to obtain signals characteristic of aliphatic group and amides by NMR, that could correspond to cyclic lipopeptides family. CONCLUSION: It was possible to obtain biosurfactant from a not pathogenic strain with excellent values of (γ). We are currently researching the chemistry structure of lipopeptides found, as well as, its potentials application in different areas, such as medicine and cosmetic.

BT-P16
MUTATIONAL ANALYSIS OF ACYLTRANSFERASE PAPA5 IN ORDER TO MODIFY ITS ALCOHOL SPECIFICITY

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Wax esters are high-value neutral lipid compounds that serve a variety of functions in biological systems and have specific industrial uses as cosmetics, high-grade lubricants, and food additives. In our laboratory, we have developed a biosynthetic pathway that is based on an iterative PKS system in order to produce multi-methyl branched fatty acids (MBFA) and wax ester derivatives (MBE). This system consists of a PKS called mycocerosic acid synthase Mas, an acyl-AMP ligase Faal28, and an acyltransferase called PapA5 from *Mycobacterium tuberculosis*. The PapA5 protein can directly transfer the MBFA from Mas protein to alcohols. Structural analysis of PapA5 reveals a two-domain protein that shares unexpected similarity to structures of other unrelated acyltransferases. Binding pockets of PapA5 substrates were defined based on the modeling of the ligands of these homologous acyltransferases. It was shown that *in vitro* PapA5 can use a wide range of alcohols as substrate with more efficiency for medium chain and long chain alcohols, mainly 1-octanol. From the crystal structure of PapA5 we rationally designed and constructed mutants in residues predicted to have relevance in substrate specificity in order to improve short chain-alcohol affinity that finally generate more efficiently MBFA esters with different chemical structures and hence with different physicochemical properties.

BT-P17
FED-BATCH FERMENTATION OF *Escherichia coli* FOR A PKS-DERIVED METHYL-BRANCHED ESTER PRODUCTION

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Microbial fatty acid (FA)-derived molecules have emerged as promising alternatives to petroleum-based chemicals for reducing dependence on fossil hydrocarbons. In this sense, we have successfully engineered into *Escherichia coli* a mycocerosic polyketide synthase-based biosynthetic pathway from *Mycobacterium tuberculosis* and redefined its biological role towards the production of multi-methyl-branched-esters (MBE) with novel chemical structures. In this work we aimed to develop a fed-batch fermentation process in order to increase MBE production yields. To achieve this goal we cultured the *E. coli* MBE producer strain in 1 L fermentors, using a glucose-based minimal media. After optimization of medium composition and growth parameters for this particular strain, we focused our study on determining the optimal protein induction timing and inducer concentration, in order to maximize the volumetric production of the lipid product. After all of these steps, we scaled up the process to a 75 L fermentation. Finally, we developed a protocol for MBE purification from total lipid extracts, based on the differential silica adsorption affinity of the MBE esters in comparison to the phospholipid and fatty acid fractions of the extract. After that, a silica column based purification of MBE led us to obtain the pure product, suitable for physicochemical properties measurements.

BT-P18
NOS OVEREXPRESSION CONFERS ADAPTIVE ADVANTAGES TO BACTERIA AND PLANTS DURING NITROGEN DEFICIENCY

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Nitric oxide (NO) is a free radical that acts as a signal molecule in diverse biological processes. In plants, NO acts as a promoter of growth and developmental processes and participates in defense responses to biotic and abiotic stresses. The enzyme nitric oxide synthase (NOS) catalyzes the biosynthesis of NO. Our laboratory characterized the first NOS enzyme from the plant kingdom belonging to the green alga *Ostreococcus tauri* (OtNOS). Later, through bioinformatics studies, a new NOS sequence was found in the cyanobacteria *Synechococcus PCC7335* (SyNOS), with a yet undescribed function for the N-terminal globin domain. The objective of this work is to analyze the impact of the expression of NOS from photosynthetic organisms on nitrogen (N) metabolism in plants and bacteria. Results indicate that *E. coli* cultures expressing either OtNOS or SyNOS increase their growth rate compared to bacteria expressing the empty vector when growing in N-deficient media. We have transformed Arabidopsis with *SyNOS* under the control of *CaMV 35S* promoter. We are currently conducting analysis to detect SyNOS transcript and protein levels and phenotyping the transgenic lines growing under optimal and suboptimal N conditions. The finding that NO improves the N assimilation in plants under suboptimal N availability would represent a new and valuable biotechnological tool to increase yields in crops.

BT-P19
DELIVERY OF THE C-TERMINAL REGION OF THE ROTAVIRUS VP6 PROTEIN BY JUNÍN VIRUS Z VLPS

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The ability to boost the viral particle budding of viral matrix proteins has been studied in several RNA viruses. For arenaviruses, the only expression of the Z protein in mammalian cells allows the generation of virus-like particles (VLPs) containing this protein inside. In previous studies, we have shown that the Junín Z protein, belonging to the Arenaviridae family, is able to maintain its activity even when fused to a heterologous sequence at its C-terminal region. We have also demonstrated the *in vivo* immunogenicity of these chimeric virus-like particles using GFP and pther viral antigens. The aim of this study is to evaluate this antigen presentation system using the C-terminal region of the Rotavirus VP6 protein. We have cloned the C-terminal region of the VP6 protein (332-397) in an eukaryotic expression vector fused to the Z protein, pZ-CT-VP6. We have also made a reporter construction using GFP, which allow cellular localization studies in mammalian cells (pZ-CT-VP6-GFP). In all cases the expression of the cloned proteins was confirmed, and the formation of VLPs was analyzed. Before testing the immunogenicity of the CT-VP6 VLPs, performed an immunization schedule using the Z-EGFP chimera. And from these results, we will select the best condition for the stimulation of the immune response against CT-VP6.

BT-P20
DEVELOPMENT OF A MULTIEPITOPE RECOMBINANT PROTEIN PRODUCTION STRATEGY FOR VIRAL DIAGNOSIS

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The goal of this project is the production of recombinant antigens to be used in the serological diagnosis of different Flavivirus and Alphavirus, with the overall aim of improving its diagnosis and epidemiological surveillance. Currently, the diagnostic of these viruses rely on the use of whole virus antigens and are consequently associated with false positives due to serologic cross-reactivity, high cost of antigen production, and biohazard risk. This has prompted the need to develop an alternate antigen to replace the whole virus in diagnostic. We have designed and expressed a novel recombinant protein antigen by assembling linear IgG-specific SLEV or VEEV epitopes, chosen on the basis of computer predictions and bibliographic data of related viral species. The recombinant virus multiepitope protein was expressed in *Escherichia coli* and fused to a his-tag tail as a purification method. Also it will be expressed as a fusion protein using GST, maltose or tioredoxin. Finally we planed develop an in-house ELISA to detect anti-SLEV or anti-VEEV antibodies in a panel of positive and negative sera using the purified recombinant multiepitope protein as the capture antigen. The selection of epitopes and the use of *E. coli* as expression system, along with simple purification, have the potential to lead to the development of an inexpensive, highly specific and sensitive diagnostic test.

BT-P21
VEHICULIZATION OF THE NS1 PROTEIN FROM SLEV USING A GENERIC SYSTEM FOR ANTIGEN PRESENTATION

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Arenaviruses' Z protein directs the viral budding process at the end of the infection cycle, associated to the inner face of the plasma membrane in host cells. In previous studies in our laboratory it has been shown that the Z protein from Junin virus (JUNV) fused to the Green Fluorescent Protein (GFP) retains its ability to sprout autonomously in eukaryotic expression systems (mammals and insects) and generates virus-like particles (VLPs). Also, these VLPs were able to stimulate an immune response anti-GFP in inoculated mice. In this work, we present a plasmid vector (pResAg) designed in our laboratory from JUNV's genomic elements in order to optimize the generation of VLPs. To assess the ability of this system as a vehicle for surface antigens, we selected the nonstructural protein NS1 protein from the Saint Louis Encephalitis Virus (SLEV), an emerging flavivirus in Argentina, due to its immunogenic characteristics. As first approach, we were able to detect the expression of NS1 in the monolayer and culture supernatant from transfected COS-7 cells using immunodetection techniques with specific antibodies. These results, along with protease protection assays, electron microscopy, and inoculation assays, would demonstrate the expression of VLPs containing NS1 as a viral antigen in pResAg and the capability to stimulate a noticeable immune response in mice.

BT-P22
HETEROLOGOUS EXPRESSION OF XYLANASE AND α-AMILASE GENES IN LACTIC ACID BACTERIA

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The enzymes of the glycoside hydrolase family are used industrially as dietary additive in animal feed. This innovative practice helps improve weight gain of the animals when they are fed mainly with fibrous nutritious low quality forages. In dietary supplements, the additions of enzymes (cellulase, xylanase or amylase) in which degrade polysaccharides (cellulose, hemicellulose or starch) to simple sugars, improves digestibility of fiber in foods like corn, barley, soybeans, sorghum or poor pastures, allows the reduction of diarrhea, increased weight of the animals (such as chickens and pigs monogastric or ruminant) and improves overall health thereof. The use of BAL for heterologous expression is interesting since the most of lactic acid bacteria (LAB) species are safe for animal consumption and in many cases beneficial, besides contributing to the biological process of fermentation in silage fodder. In this work, the α-amylase enzyme from *Bacillus licheniformis* was expressed constitutively both in *Escherichia coli* as in *Enterococcus faecalis*, using pGAL9 plasmid. In addition, the xylanase enzyme from B. subtilis was expressed an inducible way both in *E. coli* (pET28 system) as in *Lactococcus lactis* (pNZ system).

Enzymes were exported using different signal peptides and the corresponding activities were detected in the culture supernatants

BT-P23
EFFECT OF GLUTATHIONE ADDITION ON *Oenococcus oeni* TOLERANCE TO STRESSFUL WINE-LIKE CONDITIONS

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During wine-making process, the malolactic fermentation (MLF) is a critical step to obtain high quality wines being *Oenococcus oeni* the most important species involved in this fermentation. *O. oeni* viability in wine depends on its resistance to several stress factors such as low pH, ethanol and other inhibitory metabolites produced by yeasts. Glutathione is a nonproteic tripeptide that in its reduced form acts as an antioxidant through the thiol group of cysteine. The aim of this work was to evaluate if glutathione addition could increase tolerance of *O. oeni* to stressful conditions. *O. oeni* X_2L was cultured in MRS medium with and without glutathione (5 mM). The strain showed better growth parameters in presence of glutathione. This positive effect was more remarkable when the bacterium grew at low pH (4 and 3.4) and/or in presence of ethanol. Moreover, expression of *gsh* gene encoding glutathione reductase increased 3-fold by glutathione addition at different culture conditions while expression levels of *cfa* gene encoding cyclopropane fatty acid synthase increased 2-fold only in presence of glutathione and ethanol. Also, glutathione addition produced modifications in the fatty acid composition of bacterial membrane. These results showed the potential protective role of glutathione on *O. oeni* which could improve its stress response as well as MLF performance during wine elaboration.

BT-P24
DIFFERENTIAL PROTEIN EXPRESSION BY *L. sakei* CRL1862 DURING BIOFILM FORMATIONAND PLANKTONIC CULTURE

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Bacteria are able to grow in planktonic (free) or biofilm form. Biofilms are cells bound together by extracellular polymeric substances attached to a surface. In order to characterize the biofilmogenic *Lactobacillus sakei* CRL1862, was studied the differential pattern of proteins expressed in planktonic and biofilm growth using two-dimensional electrophoresis. Biofilm proteome was obtained by incubation in MRS at 10 °C during 6 days on stainless steel chips and were compared with planktonic proteome obtained under the same conditions.The cells were harvested and 340 µg of proteins were seeded in isoelectrofocusing strips (pH 4-7).Twenty-nine proteins were identified by MALDITOF MS/MS. Tenof them were overexpressed during growth in biofilm while 19 proteins corresponded to planktonic conditions. Proteins involved in the hydrolysis of peptide bonds (acquisition of nutrients), purine synthesis (extracellular matrix compounds), glutamate (peptidoglycan synthesis) and pentoses (present in sessile cells) were overexpressed under biofilm growth. Whereas, in planktonic conditions, enzymes related to energetic pathways were mostly expressed. The proteomic approach here applied allowed understand some of the mechanisms and molecular basis used by *L. sakei* CRL1862 for its adaption to biofilm growth conditions.

BT-P25
Helianthus petiolaris: A NEW SPECIES TOLERANT TO HEAVY METALS

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The environmentally neglected exploitation of mineral resources pollutes soils and sediments with heavy metals, which accumulate in the environment affecting human health and biodiversity. Conventional remediation methods are expensive and usually spoil soil properties. Hence, phytoremediation rises as a “green” alternative to stabilize, remove or degrade pollutants through a low cost and environmentally compatible biological process. In this context, *Helianthus petiolaris* grows in the arid environments and sandy soils of the central region of Argentina. These characteristics make it able to grow on degraded and poor soils, as those produced by the mining activity. In addition, this species has potential for technology spin off, since its essential oils can be used for pest control in stored grain. The aim of this work was to investigate the competence of *H. petiolaris* as a phytoremediating species by assessing its tolerance to heavy metals. Tolerance was assessed by sterile seeding each agarized medium containing Cd 10 ppm, Pb 100 ppm, Zn 400 ppm respectively. *H. petiolaris* was able to germinate at the following rates: 87 % Cd, 84 % Pb,

76 % Zn. However, only the seedling germinated in the presence of Cd and Pb reached the "true leaves" stage. After 32 days, tolerant seedlings were successfully transplanted to sterile soil to complete their growth.

BT-P26
ISOLATION AND CHARACTERIZATION OF GLYPHOSATE DEGRADING BACTERIA FOR RHIZOREMEDIATION STRATEGIES

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The Humid Pampa is the main agricultural region in Argentina and one of the most important crop fields in South America, covering about 52 million ha. As part of the modern agriculture practices, more than 300,000 tons of pesticides are applied every year, were about 65% are formulations of the herbicide glyphosate (N–phosphonomethylglicine). To develop an efficient, eco–friendly, solar driven system to mitigate the environmental impact of glyphosate use in the agricultural exploitation context is a priority strategy. To achieve this goal, the aim of this work is to find and characterize glyphosate degrading bacteria associated to *Lotus spp.* roots. This plant–bacteria system will be use to enhance the glyphosate degradation in the rhizosphere influence zone and reduce its residues in soils and running off waters. To carry out this objective, bulk and Lotus rhizospheric soil samples were collected from both, glyphosate treated and control pasture plots. Batch liquid cultures were performed using basal salt media enrichment with glyphosate as sole carbon or phosphorus source respectively. Among isolates, two glyphosate–degrading strains were selected and culturally/genotipically characterized. Glyphosate degradation in culture media was determined by FMOC–Cl derivatization followed by UPLC. In addition, plant growth promotion capabilities were also determined for both strains.

BT-P27
ESTIMATION OF BIOSURFACTANT PRODUCTION BY *Pseudomonas* sp. GROWING ON THREE DIFFERENT SUBSTRATES

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The microorganism used in this study was isolated from a chronically hydrocarbon–contaminated area in Campana (Bs.As.). Experiments were carried out in Erlenmeyer flasks and were divided in two stages: initially, the strain was cultured for 48h in a minimal saline medium (MSM) supplemented with glucose (Glu, 20g/l) or glycerol (Gly, 3%v/v) as sole carbon and energy source. Next, biomass produced with each substrate was transferred to a fresh MSM supplemented with a mixture of three commercial hydrocarbons (HC), and the process was monitored for 72h. Biomass and biosurfactant production throughout the experiment were estimated by cell dry weight and direct measurement of surface tension (ST) methods. Results showed that bacteria was able to grow using all the carbon sources tested, reaching concentrations of 1,29g/l and 0,92g/l in Glu and Gly respectively, and 0,24g/l and 0,32g/l in HC respectively. Regarding ST measurement, culture supernatant showed a marked decrease in ST value when the bacteria grew on Glu and Gly (46,30% and 54,72%). No decline in ST value was observed when HC was used. Results suggest that the isolated microorganism might be used to perform a biosurfactant production using renewable carbon sources (like Gly), with the aim of designing a bioremediation strategy of hydrocarbon contaminated sites with native strains

BT-P28
FATTY ACID 2-HIDROXYLASE GENE IDENTIFICATION IN THE CILIATE *Tetrahymena thermophila*

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Sphingolipids are lipids of great importance not only because of their structural function on cellular membranes, but also they are bioactive molecules, since them participate in several process as cellular signaling, vesicular traffic and apoptosis. Ciliates, as *Tetrahymena thermophila*, are promising organism to apply to the field of sphingolipid research, since they have many advantages in their use, including complex biological processes, ease of cultivation and various molecular tools. However, although it has been reported the presence of these lipids in *Tetrahymena*, neither their biological role, nor the genes involved in metabolism, are known. Addressing these issues, it has been

built by bioinformatics analysis a putative metabolic pathway in the ciliated, identifying possible paralogs for most of the synthetic steps involved. According to this, we proceeded to knock the only candidate of a fatty acid hydroxylase gene, transforming the macronucleus by biolistic bombardment. Results showed the loss of hydroxylase activity by gas chromatography without observing other significant phenotypic changes from the wild strain. In conclusion, the fatty acid 2-hydroxylase thus becomes the first gene of sphingolipid metabolism to be identified, not only in *T. thermophila*, but also in ciliates.

BT-P29
HETEROLOGOUS EXPRESSION OF BABESIA BOVIS VACCINE CANDIDATE MSA-2C IN *Tetrahymena thermophila*

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Tetrahymena thermophila is a non-pathogenic protozoa used for the expression of recombinant proteins. Several glycosylphosphatidylinositol (GPI) anchors proteins have been expressed successfully in the surface of this ciliate. Some examples are the circumsporozoite protein (CSP) of *Plasmodium falciparum* and the immobilization antigen of *Ichthyophthirius multifiliis*, which are used as vaccine candidates for malaria and white dot disease respectively. *Babesia bovis* is a tick-transmitted hemoprotozooan that causes infection diseases in vertebrate hosts. It is responsible for the mortality of cattles, leading to significant economic losses. Up to date, no effective recombinant vaccines are available. The GPI protein MSA2c of *B. bovis* represents a prominent vaccine candidate that was chosen to be expressed in *T. thermophila* for the preparation of subunit vaccines. We could achieve episomal expression of MSA2c in whole cell of *T. thermophila* and also in enrich GPI fraction. Preliminary analysis, based on a detergent phase-partitioning procedure followed by HPLC-MS, has confirmed the expression of MSA2c as an endogenous GPI protein of 35 kda member of the SerH family. This result demonstrated that *T. thermophila* expresses successfully heterologous GPI proteins and supported the idea that it can be used as a platform to produce recombinant surface antigens.

BT-P30
EXPRESSION OF HBSAG VARIANTS TO IMPROVE THE EFFICACY OF THE CURRENT ANTI-HEPATITIS B VACCINE.

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Introduction: Cocirculation of HBsAg and neutralizing anti-HBs antibodies in patients with adequate active/passive immunization or during the natural course of infection is a problem of health concern. The most widely reported S-escape mutants are D144A, G145R and those at cysteines. All anti-HBV vaccines available are only composed of wild-type (wt) HBsAg, the S-escape mutants described to date in the world are not included. Some of them may also escape immune detection by some commercial kits used in the diagnosis of HBV infection and in the screenings of blood donors. Aims: To analyze the effect of S mutants at various positions in relation to the interaction with anti-HBs antibodies from wt strains. Methods: Wt and mutant S genes were cloned into the expression vector pPICZa and transformed into *Pichia pastoris* X-33. For detection of HBsAg, commercial ELISA assays were performed. VLPs were characterized and reactivity of wt and mutant HBsAg was assayed in vitro (TEM and DLS) and in silico. Results: High expression levels were obtained, and the resulting VLPs had a size of about 20 nm. Reactivity with anti-HBs antibodies was variable. Conclusions: This may lead to the development of a new anti-HBV vaccine covering such escape mutants, as well as for the development of diagnostic tools needed - and still not available, for their detection, as some of them may escape their detection.

BT-P31
EXPRESSION OF HBSAG FROM DIFFERENT GENOTYPES TO IMPROVE THE EFFICACY OF THE CURRENT ANTI-HEPATITIS B

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Introduction: Phylogenetic analysis based on the comparison of complete HBV genomes has defined at least 8 genotypes (A-H) and several subgenotypes that display a characteristic geographic distribution and show differences in biological properties. Genotypes E and F are the most divergent among all genotypes within the a determinant (aminoacids 107-147 of HBsAg) and within the S gene, respectively. These features, together with the emergence of S-escape mutants, have raised concerns about the efficacy of the current genotype A-based vaccines on the African and American continent, where genotypes E and F are prevalent, respectively. Aims: To analyze the effect of the most divergent genotypes (E, F1b and F4) and their interaction with anti-HBs antibodies derived from vaccination with genotype A2. Methods: Genes for each genotype were cloned into the expression vector pPICZa and transformed into *Pichia pastoris* X-33. For detection of HBsAg, commercial ELISA assays were performed. VLPs were characterized and reactivity was assayed in vitro (TEM and DLS) and in silico. Results: High expression levels were obtained, and the resulting VLPs had a size of about 20 nm. Reactivity with anti-HBs antibodies was variable. Conclusion: Our results point out the pressing need to assess the incorporation of genotypes E/F-derived HBsAg vaccine in those endemic areas where said genotypes are prevalent.

BT-P32
IDENTIFICATION OF Δ12 FATTY ACID DESATURASE GENE BY SOMATIC KNOCKOUT IN *Tetrahymena thermophila*

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Polyunsaturated fatty acids (PUFAs) play important roles as structural components of membrane phospholipids and as precursors of eicosanoids. Mammals synthesize eicosanoids, which are involved in inflammatory and immune responses, reproduction, and blood pressure regulation. However, they are unable to add double bonds at Δ12 or Δ15 positions, for this reason PUFAs linoleate (18:2Δ9,12) and linolenate (18:3Δ9,12,15) must be obtained from the diet. The study of PUFAs metabolism in microorganisms can contribute to develop better biotechnological strategies for their production. In this regard, the ciliated *Tetrahymena thermophila* seems to be a good model for this purpose. We have undertaken a genome-wide survey of putative genes involved in PUFA metabolism in *T. thermophila*. A number of candidates were selected as possible fatty acid desaturases (FADs). Gene deletion by somatic knockout of the only Δ12 FAD ortholog present in *T. thermophila* (TTHERM_00535680) unables to synthesize a double bond at position 12 of lipid´s fatty acid (FA) chain. Gas chromatography-Mass spectrometry analysis of purified FAs showed that the main PUFA (18:3Δ6,9,12) present in the wild type couldn´t be detected in the KO strain. Further studies of FADs metabolism in ciliates could contribute to the understanding of lipid biosynthesis pathways and to generate recombinant strains with biotechnological value.

BT-P33
GP60 FROM *Cryptosporidium parvum*. HETEROLOGOUS EXPRESSION IN *Tetrahymena. thermophila* AS VACCINE CANDIDATE.

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Cryptosporidium parvum is a protozoan parasite of the phylum Apicomplexa which is responsible of cryptosporidiosis, a disease that generates significant economic deficits in livestock. One of the main determinants of the different subtypes of *Cryptosporidium* is the surface protein GP60. This protein like most phylum surface proteins is attached to the membrane by a glycophosphatidylinositol anchor-type (GPI) and often involved in recognition processes, adhesion and invasion of host cells. These characteristics make the GPI proteins essential for the survival of protozoa and also turn into vaccine candidates in high biotechnological value. In this work the heterologous expression of GP60 antigen from *C. parvum* was held in the ciliate *Tetrahymena thermophila* using ribosomal DNA vectors. The presence of GP60 was evaluated by western blot with specific antibodies. The protein is expressed in a 40 kDa variant and is localized in an insoluble fraction after Triton X-114 phase-partitioning procedure. It was also detected by Coomasie Brilliant Blue staining and confirmed by HPLC-MS indicating that this ciliated GPI is not only

able to express but also to perform the post-translational modifications necessary for obtaining GP60 antigen from *C. parvum*.

BT-P34
NANOIMMUNOLIPOSOMES TARGETED TO CANCER CELLS WITH ANTI-EGFR ANTIBODIES.

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The aim of this study was to develop a nanoimmunovehicle to deliver a genetic medicine to EGFR (epidermal growth factor receptor) overexpressing cancer cells. The final goal is to deliver a chimeric ROS-responsive DNA element (ROS-RE) driving the expression of therapeutic genes. We compared several methods for the efficient encapsulation of the DNA in stealth liposomes. Subsequently, both a humanized anti-EGFR Fab' fragment as well as a camelid VHH nanobody fragment was conjugated to the nanovehicle. As a proof of concept we encapsulated a plasmid where luciferase expression was driven by the ROS-RE. The internalization of the nanovehicle was followed by confocal microscopy. Initial studies showed poor gene expression indicating that the DNA was unable to reach the nucleus. In subsequent studies the cationic polymer PEI was used to improve immunoliposomes exit from the lysosomes. Complexes of PEI/DNA performed at different nitrogen to phosphate ratio (N/P=3, 5 y 10) were encapsulated in liposomes and luciferase activity was evaluated in cell cultures. Luciferase expression of liposomes containing complexes at N/P 10 was 1300 times higher than liposomes with complexes at N/P 3 ratio. In addition, liposomes containing PEI/DNA complexes did not shown toxicity over treated cells (95-98% cell survival). In future studies, we will evaluate the therapeutic efficacy in *in vivo* models.

BT-P35
DESIGN AND DEVELOPMENT OF MAGNETIC NANOCARRIERS FOR THE OPTIMIZATION OF siRNA DELIVERY

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Nonviral gene vectors, such as liposomes, are widely used for nucleic acid transfer due to their low immunogenicity and biocompatibility. On the other hand, it has been proved that complexes performed by magnetic nanoparticles, polyethyleneimine polymer and nucleic acid (MPNA) enhance nucleic acid transfection efficiency in vitro and in vivo, with low toxicity. The aim of this work was to obtain optimized magnetoliposomes (MLs) that can be magnetically directed to cell surfaces to increase nucleic acid transfer. SO-Mag5 magnetic nanoparticles (MNP) were used to perform MPNA complexes with different Fe/DNA ratios (0,2:1, 0,4:1, 0,75:1, 1:1, 3:1) using a fixed nitrogen to phosphate ratio of 10. We have determined MPNA hydrodynamic diameter (200-230nm) and polydispersity index (0,2) and also were visualized its stability in agarose gel. We chose Fe/DNA 3:1 ratio to encapsulate the MPNA complexes in negatively charged lipid films. We tested different separation methods to obtain pure MLs, that were visualized by Transmission Electron Microscopy. In addition, we evaluated MLs activity in colorectal cancer cell lines, HCT116 and Caco-2. We obtained high levels of gene activity after expose cells to the magnetic nanovehicles combined to magnetic external fields. We think that the obtained MLs are promising nanovehicles to be used in siRNA delivery for anticancer therapies application.

BT-P36
CLONING, EXPRESSION AND CHARACTERIZATION OF A BACTERIAL BAEYER-VILLIGER MONOOXYGENASE

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Baeyer-Villiger monooxygenases (BVMOs) are FAD- or FMN-dependent oxidoreductases that catalyze the insertion of an oxygen atom from molecular oxygen next to a carbonyl group at the expense of NAD(P)H and the other oxygen atom is reduced to water. These enzymes are useful biocatalysts for the production of lactones or esters using cyclic or lineal ketones as substrates, respectively. The use of BVMOs to gain access to different compounds is a convenient process due to the high selectivity and efficiency of these enzymes and the mild conditions required for the enzymatic Baeyer-Villiger oxidation. The aim of our work is to expand the number of BVMO biocatalysts available for the

production of esters or lactones that are valuable precursors for chemical applications. In this study, we cloned a gene from *Bradyrhizobium japonicum* that encodes a protein with the characteristic consensus sequences and dinucleotide-binding motifs of Type I BVMOs. This protein also contains an amino terminal extension, a feature observed in few BVMOs. We expressed this gene in *Escherichia coli* BL21(DE3) and evaluated the substrate preferences of this novel BVMO from *B. japonicum* by whole-cell biotransformations. Different lineal aliphatic, monocyclic, bicyclic and aromatic ketones were tested as substrates. Acknowledgments: ANPCyT, CONICET, UNR.

BT-P37
EXPLORING EXPRESSION PLATFORMS FOR DENGUE VIRUS ENVELOPE PROTEIN DOMAIN III IN *Escherichia coli*

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Domain III of dengue virus envelope protein (DomIII) presents multiple epitopes that induce serotype specific antibodies and is an interesting antigen for being used in diagnostic kit for dengue virus. The aim of this work was to express the DomIII fused to two different tags that confers different purification strategies in *Escherichia coli*, with the final purpose of develop a diagnostic kit. On one hand, the protein was fused to hidrofobin (DomIIIHFB) for a later purification by surfactant-based aqueous two-phase systems, on the other, fused to chitin-binding domain (DomIIICBD) for a later purification by affinity for chitin beads. We expressed DomIIIHFB, using pET32a vector in three different strains Origami(DE3), Origami(DE3)B, and Origami(DE3)pLysS. There were no significant differences in the expression level between the strains and in all cases the protein was expressed in inclusion bodies. DomIIICBD was expressed using pTWIN1 vector in Rosetta(DE3)pLacI strain. In all cases the fused proteins were analyzed by SDS-Page and Western Blot, they presented the expected molecular weight and were recognized by rabbit polyclonal anti-dengue serum. In conclusion, DomIIIHFB and DomIIICBD were successfully expressed in *Escherichia coli*.

BT-P38
MOLECULAR ANALYSIS OF BACTERIOCIN EXPRESSION IN SEMI-HARD CHEESES

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Many lactic acid bacteria are able to secrete ribosomally-synthesized peptides that have antimicrobial activity, known as bacteriocins. *E. faecium* CRL1879 is a native strain isolated from an artisanal cheese in the northwestern of Argentina. This strain displays a remarkable antibacterial activity against *L. monocytogenes* and has six class II bacteriocin clusters in its genome. The aim of the present work was to evaluate the expression of enterocin CRL1879 genes (*ent*A, *ent*B, *ent*SE-K4, *ent*P, *ent*Xαβ, *ent*CRL1879αβ) during the manufacture and storage of a semi-hard cheese by RT-PCR. In addition, this bacteriocin-producing strain was tested with regard to controlling *L. monocytogenes* growth during the manufacture and storage of semi-hard cheeses. Pilot-scale semi-hard cheeses namely A (starter culture), B (starter culture + CRL1879), C (starter culture + *L.monocytogenes*), D (starter culture + CRL1879 + *L.monocytogenes*) were manufactured in duplicate in the pilot plant according to CERELA protocol. Results demonstrated that *E. faecium* CRL1879 express all enterocin genes except for enterocin A and SE-K4 during cheese ripening. Consequently, bacteriocin titration was detectable at 1600 UA/ml during the whole process and completely inhibits *L. monocytogenes* growth during cheese ripening (30 days) at 8°C. *E. faecium* CRL 1879 represents a promising tool for biopresevartion.

BT-P39
Lactococcus lactis AS A PLATFORM FOR ROTAVIRUS ANTIGEN EXPRESSION

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Group A rotaviruses are the major etiologic agents of acute gastroenteritis in children. Mortality rates have been reduced since the implementation of attenuated vaccines. However, research on alternative vaccination strategies continues. Among rotavirus structural proteins, VP6 is the most immunogenic. The nisin-controlled expression system was used to display the recombinant protein at the cell surface of *Lactococcus lactis*. Different genetic constructs were made by cloning VP6 sequence (398 aa), an optimized *L. lactis* codon usage VP6, and three different

optimized VP6 fragments: AB, BC, and CD, fused to a signal peptide (SP) and a cell wall anchor (CWA) fragment. Moreover, an optimized *L. lactis* codon usage version of SP-VP6-CWA was also constructed. Protein expression was analyzed in cytoplasmic and cell-wall fractions by SDS-PAGE, Western blot, and ELISA. Results showed that VP6 fragments were efficiently expressed presenting higher protein expression compared to that of complete VP6. Although AB fragment was efficiently expressed and localized in the cell wall of the recombinant *L. lactis*, it failed to induce a specific humoral immune response when administered intranasally to mice. Immunogenicity and protection capacity elicited by mucosal inoculation of *L. lactis* expressing BC and CD VP6 fragments as well as total cell lysates are being evaluated.

BT-P40
SELECTIVE ACYLATION OF ANTITUMORAL COMPOUNDS BY NOVEL BACTERIAL LIPASES

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In the last times, there has been a breakthrough in the antitumor therapies. In this sense, it has proven the effectiveness of different nucleoside analogues as 5-fluorouracil-2'-desoxiribósido derivative, known as floxuridine, which is associated with cancers treatment of the digestive system. In addition, this compound is used as prodrug for the synthesis of acylated compounds with improved bioavailability and low toxicity. In general, these compounds must be obtained through costly chemical synthesis processes. Alternatively, they may be biosynthesized using biological catalysts, thereby allowing the use of renewable raw materials. In this work, two bacterial strains with lipase activity have been identified from a screening from more than 30 microorganisms. The extracts which showed activity were concentrated by precipitation techniques and purified by adsorptive immobilization, achieving a specific activity 3-fold higher than basal. Besides, the lyophilization process allowed to obtain nucleosides selectively acetylated with an enantiomeric excesses (e.e) of 88%. Additionally, using *C. antarctica* lipase reaction parameters as acyl donors, solvents, and temperatures were optimized. Using acyl donors from 2-8 carbons, e.e about 99% in less than 6 h were achieved. Therefore, antitumor compounds with enhanced capabilities could be obtained through an eco-efficient methodology.

BT-P41
DEVELOPMENT OF AN IMMUNOASSAY FOR THE SEROLOGICAL DIAGNOSIS OF DENGUE VIRUS INFECTION

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Dengue is a mosquito borne viral infection that causes a febrile illness in infants, young children and adults. Nearly a hundred million new infections occur annually worldwide. Because four dengue virus (DENV) serotypes exist, humans can be exposed to DENV infections several times. While dengue fever is usually associated with a rather low mortality, development into a hemorrhagic fever may give rise to lethal complications and it has been shown that this is frequently due to secondary infections. There is no specific treatment for dengue, but early detection and access to medical care diminishes mortality below 1%. Confirmation of a case of dengue should be done either by direct virus detection during the acute phase or detection of specific antibodies. The immune response to DENV infection is mainly directed against the envelope protein, and the most widely used techniques for serological diagnosis are ELISAs that detect specific IgM and IgG antibodies against the virus particle. There is a need for diagnostic tests that can be used as point of care for clinical management and early detection of outbreaks. Our aim is to develop a DENV diagnostic assay suitable for use under minimal infrastructure. For this purpose we are developing a serological test based on the use of the structural domain III of the envelope protein as an antigen for the detection of specific IgM/G antibodies.

BT-P42
INDUCTION OF SYSTEMIC RESISTANCE IN SOYBEAN PLANTS AGAINST *Corynespora cassiicola* BY *Pseudomonas* spp.

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The ring spot disease of soya caused by *Corynespora cassiicola*, is commonly found in the north Argentinean soybean-growing zones. The inadequate use of agrochemical products to treatment this disease is dangerous for the environment, so is interesting to search effective management alternatives that do not select resistance in the pathogen and that can be produced without petrochemical industry energy use. One alternative is the use of biological control agents. Previously, our laboratory made a screening for rhizosphere bacteria that are able to promote plant growth or to induce systemic resistance in the soybean plant. In this work, we evaluated the ring spot disease control using a *Pseudomonas* isolated from rhizosphere of soybean. To this end, the soybean plants were inoculated by foliar application with a bacterial suspension three and seven days before infection with the pathogen *C. cassiicola* and kept in controlled conditions to favor the disease development. After the evaluation for ten days, a protective effect against the disease was observed. In addition, evaluation of biochemical markers such as oxidative burst, the accumulation of phenolic compounds and callose was performed. Our results indicate that this *Pseudomonas* isolated induce plant resistance and could be used as a "bioinoculant" for disease control, contributing to the development of sustainable agriculture.

BT-P43
REDUCTION OF CAPACITY FORM BIOFILMS OF *Pseudomonas aeruginosa* USING CONDUCTIVE POLYMERS

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A major cause of cases of nosocomial infections that occur each year are associated with implantation of medical devices. One of the main causes of failure of devices and materials (catheters, implantable orthopedic implants) is the establishment of infection by bacteria due to the ability to produce biofilm. The aim of this study is to evaluate the interaction between *Pseudomonas aeruginosa* (*P. aeruginosa*) and modified surfaces with conductive polymers. *P. aeruginosa* is a Gram negative bacteria that has the ability to form biofilms, requirement for chronic colonization of human tissue and persistence in implanted medical devices. This works supports polyamide polymers, which were surface-modified polyaniline and micro-structured surfaces are used. The polymer films were characterized by UV-visible spectroscopy and Infrared. Subsequently, the effect of surfaces on bacterial adhesion was evaluated. From these experiments were observed that these bacteria when placed against a surface of PANI exhibit a marked reduction in the ability to form biofilms compared to unmodified polyamide film. While on the other hand, a decrease in biofilm formation was observed although not as marked between the micro-structured surfaces PANI and PANI surface. Therefore, depending on chemistry and topography of the surfaces these bacteria have different behaviors as regards the ability to form biofilms.

BT-P44
MOLECULAR PHYLOGENY OF *Beauveria bassiana* NATIVE FROM MISIONES USING THE ELONGATION FACTOR 1-α

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Beauveria is an entomopathogenic fungus of interest for biocontrol of pest insects. Species recognition and diversity studies in this genus are difficult due to a lack of taxonomically informative morphology. A strain of *B. bassiana* was isolated from an insect (Hemipthera) from the province of Misiones. A molecular approach using elongation factor 1-alpha (EF1-α) was used to investigate the molecular phylogenetic diversity of this genus. Analyses were based on EF1α sequences from twenty-seven isolates from diverse geographic origins and insect hosts. Phylogenetic trees were calculated using MP, ML and NJ methods. All sequences evaluated resolved three main *Beauveria*'s genus clades. Also four well supported subclades within *B. bassiana* species were attained in the EF1-α phylogeny. Related to geographic origins all *B. bassiana* isolates from South America were included in a sole subclade but no other clear trend was observed. The subclade was supported as a monophyletic group by the three methods. Insect-fungal associations were heterogeneous in all clades and subclades, and no clear trend was observed. The clades molecularly resolved correspond closely to clades morphologically defined within *Beauveria* genus. The development of methods to differentiate among sympatric lineages will be an essential step toward the elucidation of the community structure of *B. bassiana*.

BT-P45
IDENTIFICATION OF A GENE SEQUENCE ENCODING AN ENDOGLUCANASE FROM *Irpex lacteus* (BASIDIOMYCOTA)

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Endo-β-1,4-glucanases are enzymes that hydrolyze beta-1,4 bonds between two glucose units in the cellulose chain, having key role in several biotechnological processes such as cellulosic bioethanol production. The aim of this study was to identify DNA sequences encoding enzymes with endoglucanase activity from the fungus *Irpex lacteus* (Basidiomycota) LBM 034, isolated from the subtropical rain-forest of the province of Misiones, by PCR amplification using a pair of degenerate primers. The primers were designed based on the endoglucanase sequences from other fungi belonging to Phylum Basidiomycota. A fragment of ~2000 pb was found in the genomic DNA of *I. lacteus* LBM 034 when the PCR amplification was performed using the degenerate primers. The putative endoglucanase ORF identified, excluding signal peptide, encoded a fragment of 384 amino acid residues, interrupted by 14 introns. This deduced amino acid sequence revealed a multidomain structure composed of a Carbohydrate-Binding Module belonging to the Family 1, a Ser-/Thr-rich linker and a catalytic domain from the N-terminus. This putative endoglucanase showed high similitude compared to those belonging to family 5 of glycosyl hydrolases. Future studies will involve cloning and expression of this endoglucanase gene from *I. lacteus* LBM 034 as promising target in the uncovering of new recombinant enzymes of industrial application.

Cell Biology

CB-P01
IMPACT OF PROTEIN FATTY ACYLATION IN *Toxoplasma gondii*

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In higher eukaryotes protein fatty acylation plays key roles in cellular signaling pathways, in mediating subcellular targeting and in protein-protein interactions required for activity. However little is known of this modification in apicomplexan parasites. With the aim of unveiling the role of protein fatty acylation in *T. gondii,* we analyzed the impact of protein palmitoylation and myristoylation in this parasite. The use of the biotin-exchange method allowed us to obtain the *T. gondii* palmitoylome, which shows that these proteins are found in multiple cellular compartments, with a wide range of functions that vary from metabolic processes, gliding and host-cell invasion to even regulation of transcription and translation. Furthermore, it is evident that this modification affects the majority of cellular functions since we were able to identify proteins involved in signaling, gliding motility, transcription and translation. In parallel, an in silico analysis shows that *T. gondii* expresses one N-myristoyl transferase and that myristoylated proteins are predicted to be involved in important events for this parasite such as calcium homeostasis and kinase activity regulation which are critical for invasion and gliding. Therefore, our data indicates that protein fatty acylation is a general mechanism of protein regulation that affects vital processes of this parasite.

CB-P02
STUDY OF THE ROLE OF EISOSOMAL MEMBRANE DOMAINS IN AGING

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Saccharomyces cerevisiae plasma membrane (PM) organization is partially dependent on eisosomes assembly, large protein complexes that delimit nanoscale PM invaginations. Eisosomes are composed of more than 25 proteins including transporters, signaling molecules and proteins of still unknown molecular function. Some of these proteins have been reported to be involved in the cellular aging process. We are interested in understanding eisosomes´ role in aging using *S. cerevisiae*, an experimental model that has contributed to the identification of many genes involved in aging in mammals, flies and worms. In knockout strains for *PIL1*, a gene encoding a major eisosomal structural protein, the PM topography is abnormal with large invaginations and eisosomes´ organization is lost. Performing both replicative and chronological yeast aging assays we have found that *PIL1* deletion leads to significantly enhanced

longevity in different *S. cerevisiae* genetic backgrounds, strongly suggesting that eisosome structure plays a key role in the yeast aging process. In order to characterize the observed pro-longevity effect, we analyzed the interaction between *PIL1* and key genes involved in aging pathways. Describing eisosomes´ role in aging will likely contribute to further understanding of PM domains' functions as well as the complex aging signaling network.

CB-P03
CHARACTERIZATION OF MC540 PHOTO OXIDATED PRODUCTS AND CYTOTOXICITY IN HUMAN CARCINOMA CELL LINES

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Merocyanine 540 (MC540) is a photosensitive dye that in the presence of light and oxygen showed antitumor activity against leukemia cell lines. The aim of this study is to characterize MC540 photoproducts (pMC540) and evaluate their cytotoxicity in vitro in human carcinoma cell lines. MC540 solutions were irradiated with fluorescent lamps during 6 hs at 25°C. MC540 and pMC540 were characterized by UV-visible, fluorescence, FT-IR and mass spectrometry. Human breast MCF-7, prostate PC-3 and colorectal Caco-2 carcinoma cells were plated in 96 well plates and treated with different doses of pMC540 or MC540. Cell viability was determined by crystal violet, MTT and LDH assays after 4, 24 and 48 hs. Our results show that light irradiation of MC540 produces a mixture of at least two major pMC540 that resulted to be dose-dependent cytotoxic ($p<0.05$) for PC-3 cells (c.a. 65% cell death) and MCF-7 cells (c.a. 85% cell death) at 120 ug/ml after 48 hs incubation. Caco-2 cells was not susceptible to pMC540 ($p>0.05$). None of the cell lines were susceptible to MC540 ($p>0.05$). We conclude that photooxidation breaks down MC540 generating at least two biologically active pMC540 which cytotoxicity is dependent on the cell line type. Our hypothesis is that pMC540 interacts differentially depending on specific cell membrane constituents. Further assays and analysis are ongoing to strengthen this hypothesis

CB-P04
REGULATION OF THE SECRETORY PATHWAY IN A NEURONAL DIFFERENTIATION MODEL

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The secretory pathway is ubiquitious, however, its components can adapt to different physiological situations such as increased secretory demand or differentiation. One example of cell differentiation are developing neurons, where the secretory pathway must adapt to cope with size increasement and acquirement of plasma membrane proteins like neurotransmitter receptors. The mechanisms underlying this process of adaptation are still poorly understood. Our aim is to describe biochemical and morphological changes involving the secretory pathway during neuronal differentiation and to understand how they are regulated. We analyzed this process using PC12 cells, which are rat pheochromocytoma cells that can differentiate into neuron-like cells in response to Nerve Growth Factor (NGF). We have found that, after addition of NGF there is a time-dependent increasement in the levels of different components of the secretory pathway, such as Calreticulin and Calnexin (markers of the ER); proteins of the COPII Complex (ER Exit Sites); GM130 and GalNAc-T2 (Golgi Complex). We propose that, during PC12 cells differentiation, the secretory pathway undergoes a big adaptation process in order to respond to the high demand of its function. Our future goals are to elucidate the mechanisms responsible for this adaptation and to extend our studies to other neuronal cell models.

CB-P05
PTP1B TRIGGERS INTEGRIN-MEDIATED REPRESSION OF MYOSIN ACTIVITY AND MODULATES CELL CONTRACTILITY

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Modulation of cell contractility and migration by integrins depends on the regulation of protein tyrosine kinase and Rho-family GTPase activities in specific spatiotemporal patterns. Here we show that protein tyrosine phosphatase PTP1B is critical for this regulation. Using PTP1B null (KO) cells and PTP1B reconstituted (WT) cells, we determined that lamellipodium assembly and aggregation of beta-3 integrins, adaptor proteins, and activation of Src/FAK-dependent signaling, triggered by cell adhesion to fibronectin and vitronectin, occurred robustly in WT cells but were significantly impaired in KO cells. Inhibition of Src/FAK function and hyperactivity of RhoA in WT cells

induced a KO cell phenotype. Conversely, hyperactivity of Src and myosin inhibition in KO cells restored the WT phenotype. Analyzing WT and KO cells expressing a PQ-FRET sensor based on filamin A, and the capacity of these cells to remodel 3D collagen gels, we established that cell contractility was highly enhanced at the periphery of KO cells compared to WT cells. We propose that PTP1B cooperates with beta-3 integrin to activate the Src/FAK signalling pathway and to repress RhoA-myosin-dependent contractility at the cell periphery. These events stimulate permissive conditions for adhesion and lamellipodium assembly at the protruding edge during cell spreading and migration. Supported by CONICET and ANPCyT.

CB-P06
CHLOROGENIC ACID BIOAVAILABILITY IN *Caenorhabditis elegans*

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Previously our workgroup has demonstrated that tomato extracts confer thermal stress resistance in *C. elegans*, where chlorogenic acid (CGA) was their main bioactive compound. The CGA activity might be explained by the effect of intact CGA or/and their biotransformed metabolites. In fact, we have identified four CGA metabolites by HPLC-MS in worms exposed to CGA active doses for 24h. Here, two metabolites (caffeic acid and ferulic acid), and the intact CGA, was evaluated in *C. elegans*. Adult worms were incubated with each compound for 18 h and then submitted to thermal stress conditions. Nematodes were fed with living and gentamicin-treated E. coli. Both metabolites showed the ability to rescue the worms from death by thermal stress; this activity was independent of the feeding conditions (live or dead bacteria). However, CGA was bioactive only in worms fed with live bacteria. To confirm these results, liquid bacteria (E. coli OP50) culture were fed with CGA. Dead bacteria fed with CGA and living bacteria culture were used as controls. The culture supernatants were used to supplement the NGM agar plates and nematodes were exposed to thermal stress. Only worms exposed to supernatant from living bacteria fed with CGA were rescued. These results evidence the relevance of the nematode's gut microflora, and reveals that thermotolerance exerted by CGA is dependent on its biotransformation

CB-P07
HIV-1 TAT PROTEIN IMPAIRS HUMAN SPERM ACROSOME EXOCYTOSIS

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Human immunodeficiency virus type 1 (HIV-1) transcription relies on its transactivating Tat protein. Although devoid of a signal sequence, Tat is released by infected cells. Secreted Tat can affect uninfected cells, thereby contributing to HIV-1 pathogenesis. Tat inhibits neurosecretion in PC12 cells and these cells actively internalize Tat by endocytosis. In this work, we focused on the role of Tat in human sperm acrosome secretion, a regulated calcium-dependent exocytosis necessary for fertilization. Given that the spermatozoon is a non-endocytic cell, we first tested if the protein was able to penetrate intact cell membranes. We incubated sperm with recombinant WT Tat (using the physiological concentration present in HIV patients' serum) and combined biochemical, functional, and microscopy-based methods to show that WT Tat is capable to permeate intact sperm membranes. Further, by using exocytosis assays, we demonstrated that WT Tat inhibited progesterone-induced acrosome reaction. Additionally, we evaluated a variety of Tat mutants to elucidate the mechanisms involved in protein internalization and exocytosis inhibition. So far, the results obtained indicate that Tat is able to permeate through the sperm plasma membrane impairing the acrosomal exocytosis. Our data provide insights compelling the physiological role of Tat present in semen of HIV patients.

CB-P08
ROCK DOWNSTREAM EFFECTORS PARTICIPATE IN THE INTERNALIZATION PROCESS OF *Coxiella burnetii*

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Phagocytosis is an important host defense mechanism against pathogens. In this process the actin and actin-interacting proteins play important roles. *Coxiella burnetii* (*Cb*) is an intracellular pathogen that enters into host cells

by a mechanism poorly characterized. Our goal was to study the role of ROCK downstream effectors ezrin, LIMK 2 and non-muscle myosin II (NM II) in the phagocytosis of *Cb*. HeLa cells were transfected with siRNA targets to Ezrin, or with pEGFP-Ezrin WT, -Ezrin T567D, pHA-LIMK 2 WT, -LIMK 2 TA or -LIMK 2 TEE and then infected with *Cb*. After 6h, cells were processed for indirect immunofluorescence and analyzed by confocal microscopy. We observed a decrease in the uptake of *Cb* when ezrin was depleted by the specific siRNA. Treatment of HeLa cells with the ROCK inhibitor Y27632 before *Cb* infection, inhibited uptake nevertheless overexpression of the ezrin phosphomimetic (T567D) mutant partially recovered the conditions without the inhibitor. In cells overexpressing LIMK 2 WT or their mutants the entry of *Cb* was not affected. Finally, we investigate the possible participation of NM II in the internalization of *Cb* by using a NM II specific inhibitor blebbistatin. This inhibitor diminished the uptake of the bacteria. These results suggest that ROCK and downstream effectors ezrin and NM II participate in *Cb* internalization into non-phagocytic cells.

CB-P09
ROLE OF ORP1L AND NPC1 PROTEINS IN THE BIOGENESIS OF THE *Coxiella burnetii* PARASITOPHOROUS VACUOLE

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Rab7, RILP (Rab7-interacting lysosomal protein), ORP1L (OBP-related protein 1L) and Dynein form a motor complex that plays role in endocytic trafficking. ORP1L senses cholesterol in membranes of endo-lysosomes and NPC1 (Niemman-Pick protein C1) is an endo-lysosome protein that transport cholesterol to other membranous compartment. Motor complex transports late endosomes containing high cholesterol concentration toward microtubule minus end. *C burnetii* (*Cb*) is an intracellular pathogen that transits the phagocytic pathway of host cell to form a parasitophorous vacuole (PV) with endo-lysosome characteristics. To determine the role of ORP1L in the PV biogenesis, HeLa cells were co-transfected with pGFP-ORP1L or its mutant -ORP1L$^{\Delta ORD}$ and pRFP-p150^{Glued} or -RILP, and then infected. To study the NPC1 in PV formation, HeLa cells were infected followed by treatment with NPC1 inhibitor U188666A. Cells were processed for Indirect Immunofluorescence. *Cb* intracellular multiplication, number and size of PVs were estimated. The results showed that wild type forms of overexpressed proteins did not affect the determined parameters while the mutants decreased the size and increased the number of PV and diminished *Cb* replication. The U188666A produced similar effects to the mutant. Our results suggest an important role of proteins that regulate membrane cholesterol homeostasis in the PV biogenesis.

CB-P10
INTERLEUKINE 6 CONTRIBUTION TO CELLULAR PROLIFERATION AND SENESCENCE IN PITUITARY TUMOURS

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Pituitary tumors are mostly benign, suggesting the participation of intrinsic mechanisms as cellular senescence, a program with anti-tumorigenic potential. In previous studies we showed evidence of cellular senescence in experimental pituitary tumors development. Inflammatory secretome, in particular IL-6 cytokine, regulates cellular senescence. In this study we evaluated IL-6 contribution in cellular proliferation and senescence of normal and tumoral pituitaries. Primary cultures from normal or 40 days-estrogenized Wistar male rats were treated with IL-6 or with LPS alone or after BFA (secretion inhibitor) or BAY (NF-kB blocker) incubation. The proportion of SA-b-gal (senescence) or Ki67 (proliferation) positive cells was evaluated. Exogenous IL-6 decreased normal and tumoral cell proliferation while increased senescent cells number only under tumoral setting. Also, LPS, exclusively through its secreted factors, raised the senescent cells proportion in normal and tumoral conditions and also induced tumoral cell proliferation. The NF-kB blockage by BAY alone or in combination with LPS increased cellular senescence and showed a dual role toward proliferation control in tumoral cells. These data suggest that IL-6 might play a relevant role in the pituitary tumor growth control, and NF-kB may act as a key regulator between pituitary cell proliferation and senescence in tumorigenesis.

CB-P11
CALCIUM-SIGNALING MICRODOMAINS DURING EARLY PHASE OF ENDOPLASMIC RETICULUM STRESS

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The Endoplasmic Reticulum (ER) is a multi-functional organelle that plays a critical role in a variety of processes, where the ER Ca2+ acts as a key player. Under resting conditions, the luminal Ca 2+ concentration reflects a balance between active uptake by Ca2+-ATPases and passive efflux pathways, of which the translocon play a prominent role. This is an aqueous pore, primarily formed by the Sec61α complex spanning the ER lipid bilayer, that is blocked by the ribosome on the cytosolic side and by the ER chaperone, BiP, on the luminal side. We hypothesize that during the acute phase of the UPR (Unfolded Protein Response), the Ca2+ ER eflux through the translocon is increased. To test this mechanism of action, we performed cytosolic Ca2+measurements in primary cultures of astrocytes, expressing the Ca2+ indicator GCaMP6 tethered to the ER membrane, after induction of the UPR by Tunicamycin (Tm). We observed focal release of Ca2+ in stressed astrocytes that was significantly inhibited by translocon blockers (emetine or anisomycin). In addition, pre-treatment with AB5 toxine, which specifically hydrolyses BiP, amplified the Tm-induced Ca2+ signal. Finally, co-immunoprecipitation studies showed that Tm treatments disrupted the interaction between Sec61α and BiP. Overall these data, strongly suggest that the chaperone is dissociated from the translocon increasing Ca2+ permeability.

CB-P12
GM2-GANGLIOSIDE ACCUMULATION MEDIATES ER CALCIUM DEPLETION AND PERK SIGNALING ACTIVATION

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The accumulation of misfolded proteins within the endoplasmic reticulum (ER) triggers a cellular process known as the Unfolded Protein Response (UPR), which attempts to restore ER homeostasis. If ER damage is persistent, an apoptotic response is initiated. PERK is an early ER stress sensor that attenuates protein synthesis and cell viability, which is enhanced by Calcineurin (CN) in a calcium dependent manner. But PERK signaling, including pro-apoptotic transcription factor CHOP, persists activated under prolonged stress. GM2-gangliosidosis are characterized by a progressive neurodegeneration due to deficiency in β-hexosaminidase activity. We propose to analyse if PERK signaling is involved in the neuronal cell death triggered by GM2-accumulation. N2A cells were either loaded with GM2 or treated with ShRNA specific for β-hexosaminidase. The GM2 accumulation in the ER was assessed by thin layer chromatography and immunocytochemistry. We observed, by western blotting, that the GM2 accumulation in the ER induces PERK activation, and provokes up-regulation of either CN or CHOP, at different time points. Moreover, calcium depletion, as well as CHOP level increase, were enhanced by MBCD, an agent that increases ER ganglioside delivery. This finding suggests that varied time course of the individual PERK signaling elements influence the cell's fate in response to abnormal GM2 accumulation.

CB-P13
RIBOSOME BIOGENESIS IN ZEBRAFISH DEVELOPMENT: NEW APPROACHES TO STUDY RIBOSOMOPATHIES

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Collectively, ribosomopathies are caused by defects in ribosome biogenesis. Although these disorders encompass deficiencies in a ubiquitous and fundamental process, the clinical manifestations are highly variable and typically display tissue specificity. An example is the Treacher Collins Syndrome (TCS), a craniofacial development disorder due to mutations in the TCOF1 gene, which encodes the protein TREACLE. Previously we have developed a zebrafish TCS-like model through the injection of translation-blocking morpholinos against the TCOF1 ortholog (nolc1). Our aims here were: i) to develop anti-nolc1 antibodies to deepen our studies in zebrafish TCS model, ii) to design an efficient and simple method to measure ribosome biosynthesis in TCS-like fish embryos. Zebrafish N-terminal nolc1 was cloned in the pRSET A plasmid, expressed and purified by Ni-agarose chromatography. The rabbit polyclonal antibodies obtained were highly specific and allowed us to corroborate a diminished translation of the homolog of TREACLE. We designed specific primers for the 5'ETS and ITS1 regions of the 47S pre-rRNA. rRNA transcription was estimated by RT-qPCR at different developmental stages in normal and TCS-like zebrafish embryos. Our results highlight the necessity to perform a scan along embryo development to better detect the ribosomal biosynthetic performance under a pathological condition as TCS

CB-P14
MOLECULAR DETERMINANTS THAT REGULATE DE EFFICIENCY OF PROTEIN N-GLYCOSYLATION

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N-glycosylation is one of the most frequent protein modifications in eukaryotes. Nearly one quarter of eukaryotic proteins are N-glycosylated as they enter the lumen of the endoplasmic reticulum through the SEC61 translocon. This modification is carried out by the oligosaccharyl transferase enzyme (OST), an oligomeric complex associated with the SEC61. OST transfer a high mannose glycan to the lateral chain of ASN residues within the context ASN-X-SER/THR (where X cannot be PRO). This consensus sequence is known as N-glycosylation sequon. N-glycans fulfill several biological roles. For example, they assist the protein folding pathway in the ER and they can participate in several molecular recognition events on the cell surface. The presence of an N-glycosylation sequon does not guarantee its full occupation by the OST. For this reason, a protein may present a mixture of partially occupied sequons, a phenomenon known as N-glycosylation macroheterogeneity. It is known that some sequons are poorly recognized by the OST, for instance those displaying acidic residues in the middle. Nevertheless, the molecular determinants for this behavior are largely unknown. Here we study how the protein biosynthesis dynamics modulate this complex process.

CB-P15
THE TWO ISOFORMS OF UGGT ARE DIFFERENTIALLY EXPRESSED DURING MOUSE MAMMARY GLAND DEVELOPMENT

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UGGT (UDP-Glc::glycoprotein glucosyltransferase) is the key component of the quality control mechanism of glycoprotein folding. There are two isoforms of UGGTs (UGGT-1 and UGGT-2) in vertebrates and nematodes and both of them showed canonical UGGT activity in *in vitro* assays, but it seems that they play different biological functions. As previous results showed that progesterone regulates the expression and the activity of the two UGGTs isoforms in a mouse hybridoma, we decided to analyze if these enzymes were hormonally regulated during mammary gland development. We determined by qPCR the level of expression both isoenzymes in mammary glands of C57/BL6 mice in different stages: virgin, pregnant, breastfeeding, post-lactational involution 24 h, 48 h and 72 h after weaning, and found that both enzymes were differentially regulated. The expression of uggt1 was higher than uggt2 during breastfeeding and mammary involution; but in virgin and pregnant mice uggt2 expression levels were at least 2 fold higher than that of uggt1. Pregnant mice have increasing levels of circulating progesterone along pregnancy; we analyzed the expression of both enzymes at day 7, 15 and 17 and results showed a correlation between uggt-2 expression and progesterone level. We also treated T47D cells for 24 h with increasing concentrations of progesterone and found that uggt2 expression is also regulated by progesterone.

CB-P16
REDOX PROTEIN IMPORT TO EUKARYOTIC CELLS BY THE CELL PENETRATING PEPTIDE TAT

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Cell penetrating peptides (CPPs) are short peptides (<30 aa) that cross cellular membranes with low cytotoxicity carrying drugs, proteins, nucleic acids or nanoparticles. Tat peptide, corresponding to HIV-1 Tat protein, has been studied for years for that purpose. Leptospira Interrogans Heme-oxygenase (LepHO) and Ferredoxin-NADP+ reductase (LepFNR) collaboratively catalyze heme degradation. HOs and FNRs have reported cell protection in oxidative challenging conditions. We analyzed the translocation to eukaryotic cells of recombinant proteins containing TAT CPP fused to two bacterial redox enzymes in search of a biological tool to alleviate oxidative stress damage. LepHO and LepFNR fusion proteins containing an N-terminal His-TAT extension were expressed, purified, and heme turnover determined to confirm that CPP extension does not block electron transport between the enzymes. His-TAT-LepHO and His-TAT-LepFNR were imported to SHSY5Y human neuroblastoma cells in a concentration dependent manner up to 4 μM. After 15 min both proteins were detected inside the cells and accumulate up to 1 h. Furthermore, both fusion proteins were efficiently delivered together at the same rate as if they were delivered alone.

Cells treated with both fusion proteins were challenged to survive an insult. The obtained results open the possibility of using this system as a tool for tissue preservation

CB-P17
HALOARCHAEAL BACTERIORUBERINS IMPROVE CRYOPRESERVED RAM SPERM PARAMETERS

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Sperm cryopreservation has a significant impact in the livestock reproductive field. Cryopreserved ram sperm is highly sensitive to freezing/thawing, thus, both half-life and fertilizing capacity of sperm are reduced. These events are associated with higher ion permeability, an increase in ROS production affecting membranes stability, among others. It was reported that addition of antioxidants, as vitamins E and C, to freezing extender increases sperm motility and membrane stability after thawing. In humans, carotenoids supplied to diets led to an increase in sperm motility and also ameliorated capacitation when added in vitro. Nevertheless, carotenoids on cryopreservation have not been evaluated. In our lab, a conditional Lon mutant (HVLON3) was constructed in the haloarchaeon Haloferax volcanii, that over-produces bacterioruberins. In this work, we studied the effect of these carotenoids on cryopreserved sperm. Ram sperm was thawed and treated with different concentrations of H. volcaniibacterioruberin extract (BE). Viability, progressive (PM) and total motility (TM) were analyzed. Higher percentages of viable cells were detected after 2h of treatment. Additionally, 20 µM BE significantly improved both PM and TM throughout the time evaluated (2h). Our results suggest that addition of H. volcaniibacterioruberins reduces membrane damage and improves motility of cryopreserved sperm.

CB-P18
DIFFERENTIAL INTRACELLULAR TRAFFIC OF LRP1 INDUCED BY ALPHA 2-MACROGLOBULIN AND INSULIN

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Low density lipoprotein receptor-related 1 (LRP1) is an endocytic and signaling receptor. Alpha 2-Macroglobulin-protease complex (α2M*) is a LRP1 ligand, which internalizes by endocytosis and degrades in lysosomes. α2M* also induces the LRP1 trafficking to plasma membrane (PM) through non-characterized exocytic route. Glucose transporter type 4 (GLUT4) is an insulin-regulated glucose transporter expressed in adipose and muscle cells, which is stored in specialized GLUT4 storage vesicles (GSVs). Insulin stimulation induces GSVs traffic to PM by exocytosis, with increased GLUT4 expression and glucose uptake at cell surface level. The failure of GLUT4 traffic is involved in Type 2 Diabetes Mellitus. LRP1 is the main protein component of GSVs, although its function in these vesicles is not completely understood. Herein we study if LRP1 and GLUT4 share the same exocytic route in HeLa and MIO-M1 cells cultured in the presence of α2M* (60 nM) or insulin (100 nM) at different times at 37 °C. By confocal microscopy we found LRP1 and GLUT4 colocalization in intracellular vesicles suggesting GSVs in both types of cells. By biotin-labeling protein assay, we showed that LRP1 translocates to PM with α2M*or insulin. The α2M*-induced LRP1 traffic was abolished by PD98059 but not by LY294002. These results suggest that α2M* and insulin induce the LRP1 sorting to PM by different exocytic routes.

CB-P19
REGULATION OF *Staphylococcus aureus* INDUCED AUTOPHAGY BY PROTEIN KINASE C

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Autophagy is a degradative cellular process in response to stress or infection with certain pathogens. Some pathogens are able to modify this pathway in order to survive and replicate in the host cell. We have previously demonstrated that the virulence factor alfa-hemolysin (Hla) is responsible of the autophagic response induced by Staphylococcus aureus. This toxin is used by the pathogen for escaping from its containing phagosome labelled with the autophagic protein LC3. We have found that the autophagic response induced by this bacterium is independent of the PI3K/Beclin1 pathway and it is, instead, regulated by an AMPc/EPAC/Rap2b pathway. It's known that species of Staphylococcus segregates PLC that generates DAG, which can recruit PKC from the host cells triggering a

signalling pathway. In our current study we have found that certain PKCs isoforms regulate the autophagic response induced by S. aureus. Noteworthy, both a classical and a novel PKC isoforms are able to inhibit the recruitment of LC3 to the bacterial phagosome, altering the intracellular replication of the pathogen. In addition, we have found that one of these isoforms is recruited to the S. aureus-containing phagosome in a Hla-dependent manner. Taken together our results strongly suggest that S. aureus modulates the association of PKCs to generate a more propitious replication niche before escaping to the cytoplasm.

CB-P20
Mycobacterium tuberculosis ACTIVATES AUTOPHAGY BY A PI3K INDEPENDENT PATHWAY

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Mycobacterium tuberculosis (Mtb) is the etiological agent of tuberculosis. During macrophage infection with Mtb the autophagy pathway is activated. Activation of this pathway can limit replication, but the mechanism remains unclear. The secretion system named ESX-1 is responsible for the secretion of 6 kDa early secreted antigenic target (ESAT-6) and 10 kDA culture filtrate protein (CFP-10). ESAT-6 is a hemolysin that is secreted in a homodimeric complex with CFP-10. We observed the recruitment of the autophagic protein LC3 to phagosomes containing wild type Mtb, but not to a mutant deficient for the ESAT-6 protein. To study this in more detail we generated and purified a fusion protein CFP-10/ESAT-6. A significant increase in the autophagy levels was observed in a Raw 267.4 cell line. DQ-BSA and dextran assays show a normal transport and degradation of vesicles content excluding the possibility that autophagy increase is due to interference in vesicular traffic. Interestingly our results indicate that the increment observed in autophagy is independent of PI3K but related to the EPAC pathway, as autophagy levels decreased by the use of a specific stimulator of EPAC protein. All together these results are intended to elucidate the effect of the antigenic protein ESAT-6 at the molecular level and could help to understand the role of this protein in the Mtb infection process.

CB-P21
STABILITY OF *RCSC11* MUTANT OF *Salmonella typhimurium* UNDER DIFFERENT GROWTH CONDITIONS

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Salmonella is a great prevalence pathogen in the population of Argentina. Its infection depends on the modulation of the pathogenicity islands genes expression, controlled by different regulatory systems. The RcsCDB system regulates the biosynthesis of colanic acid and flagella, and the virulence genes, between others. In the *rcsC11* mutant the system is constitutively active, resulting in a mucoid phenotype and attenuated virulence. The aim of this study was to investigate the stability of the *rcsC11* point mutation under different conditions that mimic the host environment, which was evaluated by the viability and reversion of mucoid phenotype. Our results demonstrated that, from all conditions tested, only the acid condition modified the *rcsC11* mucoid phenotype. In addition, such reversion was observed after the fifth sub-culture at pH 4.5. To confirm the phenotype reversion, mucoid and "non mucoid" colonies were transduced with a chromosomal *lacZ* fusion to the RcsB-reporter *flhC* gene. Our results demonstrated that “non mucoid” colonies displayed similar β-galactosidase activity levels than the wild type strain, supporting that the *rcsC11* point mutation was reverted in all of them. Even so, our results suggest that the *rcsC11* strain is an excellent candidate for the attenuate vaccines development, because the mucoid phenotype reversion was detected only in later stages of the assay.

CB-P22
LRP-1 TRAFFICKING IS MODIFIED BY HEMIN IN ERYTHROLEUKEMIA CELLS

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Hemin is a natural compound which is necessary to form hemoglobin, the major blood protein. It is known that hemin induces erythroid cell differentiation but the molecular and cellular mechanisms involved in this process are still debated. The transmembrane low density lipoprotein receptor related protein 1 (LRP1), is the scavenger for the hemin-hemopexine complex that allows its internalization. Autophagy is a lysosomal-degradative process necessary

for the final erythroid differentiation, leading to the degradation of non necessary organelles. Our objective was to elucidate the role of hemin-autophagy induction in LRP1 gene expression and trafficking. To determine these, we performed qRT-PCR in hemin-stimulated k562 cells. Interestingly, hemin caused an increased level of LRP1 and autophagic genes (i.e. MAP1a1b, Beclin1, Atg5). Moreover, to characterize the distribution of this receptor, different endosomal markers as Rab5, Rab7, LBPA and lysotrakcer were tested by inmunofluorescence. Confocal imaging has shown that LRP1 is mainly localized in autofagosomes (LC3 positive structures), late endosomes and lysosomal structures under hemin conditions. In general our results suggest that hemin induces autophagy, enhancing LRP1 and autophagic gene expression. Likewise, hemin would be targeting the LRP1 receptor to degradative compartiments, regulating its amount and activity.

CB-P23
POTENTIAL ROLE OF RAB21, RAB32 AND VARP IN THE AUTOPHAGIC EXOCYTOSIS

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Autophagy is a degradation process, being LC3 the main autophagosomal membrane protein. Rab32, a GTP-ase involved in the cellular transport, is related to autophagy. We described that some autophagosomes redistribute to the cell periphery and they also fuse with plasma membrane in a VAMP7 (a v-SNARE) dependent manner. This protein can interact with different proteins, like Rab21, its GEF (VARP) and Rab32. We found that Rab21 and LC3 colocalize at the cell periphery after autophagic activation. The aim of this work is to evaluate the role of Rab21, Rab32 and VARP in the redistribution of exocytic autophagosomes after autophagic stimulus. HeLa cells were transfected with GFP-Rab21 (wt or T33N), RFP-LC3 or YFP-Rab32 and then were incubated in starvation, rapamycin or resveratrol for 2-4 hours. We detected Golgi (GM130) or VARP proteins by immunofluorescence. We observed the presence of two populations of Rab32 vesicles: One is perinuclear and colocalizes with the Golgi marker; the other one colocalizes with VARP peripherically. We also found that upon autophagic stimulation, LC3-Rab32 or LC3-Rab21wt positive vesicles changed their distribution toward the periphery while Rab21 mutant still colocalizes with Golgi. Our result suggests that Rab32, Rab21 and VARP could be implicated in the autophagosomes' trafficking to the cell periphery, possibly favoring the autophagic exocytosis.

CB-P24
MACROPHAGES INDUCE ENDOCRINE RESISTANCE IN BREAST CANCER CELLS

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Despite the clinical benefit of endocrine therapies for breast cancer, a significant proportion of patients develop resistance. Tumor associated macrophages (TAMs) promote tumor growth, but it is not known if they play a role in endocrine resistance. Here we report that TAMs promote proliferation, migration and invasiveness of breast cancer cells. TAMs also generate resistance to estrogen withdrawal or treatments with SERMs as tamoxifen or ICI in estrogen-dependent breast cancer cells. Furthermore, was observed in mice that the microenvironment formed by macrophages also increased breast tumor growth and tamoxifen resistance. We showed that both signaling pathways NF-κB and IL-6 are required for these responses, which include macrophage-mediated phosphorylation of the estrogen receptor alpha (ER-α), and the activation of proliferative and pro-inflammatory genes in breast cancer cells. Strikingly, the knockdowns of any of the three transcription factors STAT3, ER-α or NF-kB in MCF-7 cells inhibit proliferation and macrophages-induced endocrine resistance. Therefore, targeting both the ER and NF-κB pathways blocks macrophages-mediated endocrine resistance in breast cancer, offering a novel mechanism and great opportunities for use in prevention and treatment of refractory breast cancer.

CB-P25
CHARACTERIZATION OF MUTANT P53-BASED MOLECULAR MECHANISMS OF TUMOR AGGRESSIVENESS

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Most human cancers inactivate the tumor suppressor p53 through missense mutations, leading to the expression of full-length point mutants. p53 mutants not only lose the tumor suppressor function of the wt protein but may also acquire novel activities, that promote aggressiveness and metastasis. We have previously found through microarray analysis that mutant p53 alters the expression profile in MDA-MB-231 breast cancer cells. We confirmed by qPCR that some genes are upregulated while others are downregulated, indicating that they may be novel targets of transcriptional regulation by mutant p53 proteins. Within these groups, there are genes whose function was related to cancer biology, as well as others whose role in cancer has been less studied. We hypothesized that upregulated genes may promote oncogenic mechanisms while downregulated ones may cooperate with tumor suppression. To study the role of mutant p53 target genes, we generated stable cell lines overexpressing candidate proteins that where evaluated in different tumorigenic assays. The effect of different environmental conditions that may affect the effect of mutant p53 on the expression of these genes was also studied. To characterize the effect of mutant p53 on transcription we generated reporters containig the promoters of candidate genes and performed luciferase assays. * These authors have contributed equally to this work.

CB-P26
GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE 2 (GPAT2) IS REQUIRED FOR MOUSE SPERMATOGENESIS

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Glycerol-3-phosphate acyltransferase 2 (GPAT2) differs from the other GPAT family members (GPAT1, 3, and 4); it is expressed almost exclusively in spermatic cells while the other 3 isoforms are expressed in lipogenic tissues. We have determined that mouse Gpat2 mRNA is expressed transiently in one step of spermatogenesis, the pachytene spermatocytes. To understand its transcriptional regulation, we bisulfite-sequenced the genomic DNA of male germ cells at different ages and found a global hypomethylation of Gpat2 promoter at 11 days of age, when meiosis starts, indicating that DNA methylation governs Gpat2 mRNA expression. To further understand the role of GPAT2 in spermatogenesis, we performed an in vivo lentivirus-mediated Gpat2 silencing in mouse testis. Histological analysis showed both a strong pre-meiotic arrest and a severe decrease in the number of mature sperm cells. We studied the effect of GPAT2 on male fertility and found that the Gpat2-silenced mice showed a diminished number of births compared to the control mice group. Also, we studied the expression of target genes which are differentially expressed during different phases of the spermatogenesis by qPCR and determined that post-pachytene gene expression was lower when Gpat2 is silenced. These results demonstrate that GPAT2 is important for the normal progress of spermatogenesis in post-natal testis development.

CB-P27
A NEW ROLE OF CDKS IN THE CONTROL OF REPLICATION FORK PROGRESSION BY CHECKPOINT KINASE 1

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The encounter of replication forks with DNA lesions activate Checkpoint Kinase 1 (Chk1), which stabilizes forks and inhibits origin firing. While the mechanisms that underlie Chk1-dependent inhibition of origin firing have been elucidated, the downstream effectors of Chk1 that control replication fork progression remain largely unexplored. We observed that roscovitine, a CDK (cyclin-dependent kinase) inhibitor, alleviates the exacerbated origin firing and impaired fork progression phenotypes caused by Chk1 depletion. Others have proposed that roscovitine restores the progression of active forks by reducing origin firing, thereby making the nucleotide pool available for DNA elongation. However, we provide evidence that this is not the case since the reduction in origin firing back to wild-type levels in Chk1-depleted cells (by means other than roscovitine) does not translate into normal DNA elongation rates. Reinforcing the notion of a direct role of CDKs in fork progression, we show that such roscovitine-induced elongation of DNA in Chk1-deficient cells requires Polη, an alternative polymerase that copies damaged DNA.

Indeed, the DNA elongation defect caused by Chk1 depletion is rescued by forcing Polη to replication forks. In conclusion, our study suggests that Chk1 signals via CDKs not only to inhibit origin firing but also to promote Polη-dependent fork stabilization.

CB-P28
NEUREGULIN-1 ATTENUATES MYOCARDIAL INJURY IN ANTHRACYCLINE-MEDIATED CARDIOTOXICITY IN MOUSE

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Neuregulin-1 (NRG1) signaling through the tyrosine kinase receptors erbB2 and erbB4 is required for cardiac morphogenesis, and plays an essential role in maintaining the myocardial architecture during adulthood. Targeted immunotherapies blocking the survival of erbB2+ cancer cells revealed that an impaired NRG1 signal under anthracycline chemotherapy may lead to dilated cardiomyopathy in a subpopulation of treated patients. The ventricular-specific deletion of Erbb4 (erbB4-KO) manifested dilated cardiomyopathy, aggravated by the administration of anthracyclines (doxorubicin). The exacerbated toxicity, in the combined treatment, induced genes of the ubiquitin-proteasome system and autophagy. Myofibril proteins were largely ubiquitinated with the commonality of a subgroup of proteins in the erbB4-KO and the doxorubicin mice. We aimed to investigate the activities underlying cardiomyocyte damage and moreover, to evaluate the therapeutic effect of recombinant NRG1β peptides. We first examined biomarkers of apoptosis and autophagy (e.g. active caspase3, p62 and LC3II/I), then characterized the ubiquitination profile of myofibrils in 2D gels towards the monitoring of the rNRG1β effect through the reversion of the molecular modifications observed in cardiotoxic conditions. We have identified new consistent biomarkers of pathology and conclude that rNRG1β protects from cardiotoxic injury.

CB-P29
DECIPHERING ROLES OF PPGALNAC-TRANSFERASES IN CELLULAR NUCLEUS

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Polypeptide GalNAc-transferases (ppGalNAc-Ts) catalyze the covalent linkage of α-GalNAc to Ser/Thr in initiation of O-GalNAc glycosylation in Golgi apparatus. The aim of the present work was to study subcellular ppGalNAc-T localization as well as nuclear O-GalNAc glycosylation. The presence of ppGalNAc-T3 in nucleus of several human cell lines was analyzed by confocal microscopy and subcellular fractionation. Purified ppGalNAc-T3 was immobilized in a chromatographic column and interaction with nuclear proteins was observed by SDS-PAGE, and identified by mass spectrometry. In order to elucidate nuclear O-GalNAc glycosylation by endogenous ppGalNAc-Ts, we purified cellular nucleus that conserve their integrity and are functionally active. Nuclei were incubated with UDP-GalNAc and nuclear protein glycosylation was analyzed by Western Blotting employing HPA lectin to detect GalNAcαSer/Thr terminals. We observed high levels of O-GalNAc glycosylation which were reduced when this sample was treated with α-GalNAc glycosidase. UDP-GlcNAc was assayed as control. In conclusion, we observed nuclear localization of ppGalNAc-T3 that can interact with nucleoproteins and nuclear protein O-GalNAc glycosylation. This posttranslational modification as well as the interaction of ppGalNAc-Ts with histones and ARN related proteins could have relevant roles in transcriptional regulation

CB-P30
SET-UP OF A FLUORESCENT ALTERNATIVE SPLICING REPORTER FOR HIGH-THROUGHPUT SCREENINGS

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Alternative splicing (AS) is the way by which two or more different mRNAs can be produced from a single gene and is the main way of expanding protein diversity in eukaryotes. Splicing is coupled to transcription, and transcription kinetics as well as factors interacting with the transcriptional machinery can influence AS decisions. We have seen that DNA damage produced by ultraviolet (UV) radiation induces a systemic transcriptional response in cells, resulting in a reduction of RNA polymerase II (RNAPII) elongation rate that leads to changes in AS patterns.

Nevertheless, the factors mediating the pathway from the DNA lesions to the transcriptional machinery are still elusive. To identify these factors, we obtained a stably-transfected HeLa Flp-In T-Rex cell line with a fluorescent reporter of AS. This reporter expresses two different fluorescent proteins depending on the inclusion or exclusion of an exon cassette. We showed that this reporter changes its AS pattern in response to UV light and thus constitutes an appropriate tool for deciphering the pathway. With this tool, we plan to perform a non-biased high-throughput analysis using an siRNA platform in order to identify factors involved in the UV-AS response. Additionally, this tool could be useful for studying AS regulation at single-cell level.

CB-P31
RNA POL II ELONGATION AND SPLICING OF THE ALTERNATIVE EXON 33 IN THE HUMAN FIBRONECTIN GENE

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Alternative splicing (AS) of the fibronectin exon 33 (E33 or EDI) is regulated by Pol II elongation, with lower elongation favouring E33 inclusion. We measured elongation rates in Hep3B, HCT116 and HEK293 cells and found slightly faster elongation in Hep3B cells, where skipping predominates. By RNA-protein immunoprecipitation, we found enhanced recruitment of the splicing factor U2AF65 to E33 when elongation is inhibited in Hep3B cells but not in HEK293 cells, where inclusion is higher. We conclude that slow elongation allows more time for the recruitment of U2AF65 to the weaker E33 3'SS before synthesis of the competing strong exon 34 3' SS. Consistently, E33 inclusion increases after knockdown of the elongation factor TFIIS. Alternatively, high Pol II processivity produces abundant E33 and E34 3'SS, with the stronger site titrating U2AF65, while low processivity decreases the amount of competing 3' sites, allowing for more inclusion. Accordingly, we found decreased pre-mRNA levels at both 3' SS after inhibition of elongation. Overall, our results indicate that low elongation/processivity favors recruitment of U2AF65 and rule out that elongation factors such as TFIIS act as recruitment seeds for splicing factors.

CB-P32
A ROLE FOR EIF4E-3 AND ME31B IN *Drosophila melanogaster* SPERMATOGENESIS

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Translational control is a key regulatory mechanism for germ line development and it is modulated at the initiation step by proteins that interact with eukaryotic initiation factor 4E (eIF4E).*Drosophila melanogaster* has 8 eIF4E isoforms. Their molecular function has been studied for eIF4E-1 (the canonical isoform), d4E-HP, which act as a translational repressor in early embryogenesis, and eIF4E-3. eIF4E-3 is a testis-specific isoform required during spermatogenesis for meiotic chromosome segregation and cytokinesis, nuclear shaping and sperm differentiation, thus males devoid of eIF4E-3 are sterile. Me31B is a RNA helicase that occurs in cytoplasmic processing bodies and is required during oogenesis. We showed that Me31B interacts with eIF4E-1 and eIF4E-3, both in the yeast two hybrid systems and in S2 cells by FRET. We demonstrate that Me31B is also expressed during spermatogenesis and co-localize with eIF4E-3 in the male germ-line cells. This is the first evidence showing that Me31B acts in both female and male germ line development and that it might require interaction with specific eIF4E isoforms to play its role.

CB-P33
DIFFERENT MECHANISMS OF INHIBITION BY ALUMINIUM ON CALCIUM PUMPS

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Aluminium (Al) is involved with the pathophysiology of neurodegenerative disorders, such as Parkinsonism dementia and Alzheimer's disease. The action of aluminum toxicity is linked to changes in the cellular calcium homeostasis, placing the transporting calcium pumps as potential targets. However, it has not been demonstrated that aluminium inhibits any transmembrane transporter of mono and divalent metals. The aim of this work was to study the molecular inhibitory mechanism of aluminium on Ca-ATPases like the plasma membrane (PMCA) and the sarcoplasmic reticulum (SERCA). For this purpose, we performed enzymatic measurements of the effect of

aluminium on different preparations of PMCA and SERCA. Our results show that aluminium: (1) inhibits Ca-ATPase activity of both enzymes with similar apparent affinity; (2) decreases the apparent affinity for Ca of SERCA, but not for PMCA; (3) decreases the apparent affinity for Mg of PMCA; (4) increases the phosphorylated intermediate (P) of PMCA while it has not effect on SERCA; (5) pH does not modify significantly the apparent inhibitory affinity for aluminium for both PMCA and SERCA. This work shows for the first time that aluminium inhibits this calcium transporter by different mechanisms.

CB-P34
QUERCETIN, A POTENTIAL INHIBITOR OF THE PLASMA MEMBRANE CA-ATPASE

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Flavonoids are commonly found in fruit and vegetables and these are also present in human plasma. These compounds are also believed to have cancer chemoprotective properties by triggering apoptosis via the Ca2+-dependent mitochondrial pathway which can be activated through an elevation of cytosolic [Ca2+] ([Ca2+]c). The increase of [Ca2+]c could be due to the fact that some flavonoids are able to inhibit some Ca2+ removing systems, as sarcoplasmic/endoplasmic reticulum Ca2+-ATPase and plasma membrane calcium ATPase (PMCA). The effect of flavonoids on PMCA is not yet clear.The purpose of the present work is to investigate the possible inhibitory effects of a flavonoid, quercetin, on PMCA. For this, we evaluated the [Ca2+]c in HEK293T cells and also studied the Ca2+-ATPase activity in vesicles and purified PMCA both obtained from human erythrocytes. The results show that (1) the rate of Ca2+ removal is lower in the presence of quercetin with respect to the control suggesting that the Ca2+ removing systems could be affected, including PMCA; (2) quercetin acts as inhibitor in both vesicles and purified systems in a micromolar concentration range and (3) the inhibition is noncompetitive with Ca2+. These results suggest that PMCA could be a specific target of flavonoids contributing to elevation of [Ca2+]c during cancer cells apoptosis.

CB-P35
BIOSYNTHESIS OF LIPOIC ACID IN THE NEMATODE *Caenorhabditis elegans*

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Lipoic acid (LA) is a sulfur-containing cofactor derived from octanoic acid that is required for the function of several multienzymes complexes involved in oxidative and one-carbon metabolism. It is also a potent antioxidant. As the information concerning LA metabolism in eukaryotes is scarce, we initiated the use of *Caenorhabditis elegans* as a model organism to study it. By *in silico* analyses we found several worm enzymes possibly involved in lipoylation. We have already shown that a block of the expression of M01F1.3, an homolog to the bacterial and yeast lipoate synthases, by RNA interference (RNAi) caused a development arrest at L4 larvae of the animals of the second generation. Using Western blot assays with anti-LA antibodies we proved that the treated worms showed reduced levels of protein lipoylation than the controls, confirming that M01F1.3 is involved in lipoate metabolism. The addition of neither free LA nor the products of the lipoylated enzymes reverted the phenotype, which lead us to think that synthesis of LA is essential in the worm. RNAi experiments against the others proteins possibly involved in LA metabolism were also performed, but no abnormal phenotype was observed. Mutant yeasts in LA biosynthesis were constructed to perform complementation assays, in order to confirm if the worm proteins identified by homology searches are indeed involved in lipoylation.

CB-P36
INHIBITION OF SPHINGOMYELIN SYNTHESIS CAUSES A RETURN TO THE INITIAL STAGE OF EPITHELIAL TISSUE

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Previously, we showed that adherens junctions (AJ) and focal adhesions (FA) are located in membrane lipid domains rich in sphingomyelin (SM) and inhibition of SM synthase 1 mediated SM synthesis altered cell-cell adhesion. Now, we investigated the reversibility of this alteration, and the effect of the inhibition of SM synthesis on FA and actin cytoskeleton. To this goal, 48 hs-old primary cultures of rat renal papillary collecting duct cells were incubated for 24 hs with D609, a SM synthase1 inhibitor. When cultured cells were treated with increasing concentrations of D609, the typical epithelial sheet was highly altered, accompanied with changes in the actin cortex and stress fibers remodeling, but cells dettachment was not observed. D609 induced a raise in the number of vinculin- and talin-stained-FA. Although D609 evoked loss of cell-cell adhesion no alteration on the amount of AJ proteins ocurred as denoted by Western blot, so the loss of cell-cell adhesion could be due to deficient protein delivery. AJ impairment appear to be reversible since cell-cell adhesion restoration was observed after reincubation in the absence of D609 for 24 hs. These results suggest that the inhibition of SM synthesis would cause a return to the initial stage of organization of epithelial tissue that is the formation of FA in order to maintain the cells attached to the matrix and thus remain viable.

CB-P37
RETINAL PIGMENT EPITHELIUM SIGNALING IN A CELLULAR MODEL OF DIABETIC RETINOPATHY

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Diabetic retinopathy (DR) is one of the leading causes of visual dysfunction and blindness. The aim of the present work was to set up an *in vitro* DR model and to study signaling events elicited by high glucose (HG) concentrations in the retinal pigment epithelium (RPE). ARPE-19 cells (human RPE cell line) were exposed to HG (16.5 and 33 mM) and to normal glucose (5.5 mM, NG) concentrations for 4 and 72 h in order to mimic a peak of and a sustained hyperglycemia. Osmotic controls were performed with mannitol. After 72 h of incubation, cell viability, evaluated by MTT reduction assay, was reduced by 30% in HG conditions with respect to NG. On the contrary, RPE cell viability was not affected in the osmotic controls. Western blot assays showed activation of the extracellular signal-regulated kinase (ERK1/2) and phosphorylation of protein kinase C α/βII (PKCα/βII) after 4 h exposure to both HG concentrations. Furthermore, immunocytochemistry assays showed that HG (33 mM) induced ERK1/2 and the nuclear transcription factor-κB (NF-κB) nuclear translocation. RPE is essential for the integrity and function of the retina and, in consequence, minor changes in cell viability could lead to photoreceptor damage and vision loss *in vivo*. Thus, elucidating HG effects in this retinal tissue could have potential implications in the discovery of new therapeutic targets for DR treatment

CB-P38
OOCYTE MATURATION WITH LINOLEIC ACID PRESERVES SPERM BINDING AND FUSION PATTERNS AFTER VITRIFICATION

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We evaluated sperm–egg fusion and adhesion properties to zona pellucida (ZP) in free bovine oocytes matured with linoleic acid (18:2n6) at different concentrations. In addition, the effect of cryopreservation on fertilization and caspase activation were analyzed. Oocytes were assigned to 3 experimental groups: matured without 18:2n6 (control), matured with 43µM 18:2n6 and matured with 100µM 18:2n6. Fresh and vitrified oocytes were fertilized in vitro after ZP removal. Activation of caspases in situ was evaluated using a specific inhibitor (VAD-FMK-FITC). Vitrified oocytes matured in 43µM 18:2n6 showed similar levels of activated caspases with respect to control oocytes, while incubation with 100µM 18:2n6 increased by 3-fold caspase activation. Incubation with 18:2n6 increased sperm binding and fusion in fresh oocytes. Only oocytes matured in 43µM 18:2n6 preserved a pattern of binding and fusion similar to non-vitrified control oocytes. Maturation with 18:2n6 (43µM) does not affect oocyte apoptotic status and preserves membrane functionality and ability to decondense sperm nucleus for fertilization.

CB-P39
PHOTODYNAMIC INACTIVATION OF GLIOBLASTOMA CELLS (T98G) USING ZNPC DERIVATES

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The development of novel therapeutic strategies for the treatment of tumors of the CNS is highly important. Glioblastomas are very aggressive brain tumors with poor prognostic and a very short survival period of approximately 1 year. The standard protocol for the treatment of these tumors include reductive surgery followed by radio and chemotherapy. Photodynamic therapy (PDT) has been studied for decades and several photosensitizers (PS) were synthetized and shown to be effective, in combination with light, to induce cell death to different tumor cells. Pthalocyanines are molecules with good properties as PS for PDT. The aim of this work was to evaluate the properties of Zinc pthalocyanines (ZnPc) and the derivates TAZnPc and TMAZnPc as PS for PDT in glioblastoma cells (T98G). All the PS were innocuous in absence of light at concentrations ≤ 0,5 μM. However, after irradiation, both ZnPc and TAZnPc induce cell death in a concentration and light dose dependent manner. Both PS rapidly induces phosphatidylserine surface exposure, mitochondrial membrane depolarization, DNA fragmentation and activation of caspase -3, hallmarks of apoptosis. Nevertheless, ZnPc induces apoptosis with lower doses of light in comparison to TAZnPc. These results suggest that both, ZnPc and TAZnPc are good PS to continue in vivo studies in a Glioblastoma animal model.

CB-P40
THE NUMBER OF SUPINE EVENTS CORRELATES WITH LONGEVITY IN *Ceratitis capitata*

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When adults of *C. capitata* fall, they might remain in a "supine" position (inverted on its back). The late recovery of supination (>2 seconds), apparently due to neuro-motor decoupling, was proposed as a possible indicator of early death. An experimental system was built to simultaneously record this and other behaviors. Early supine events were recorded between 5 and 10 days of age. Our hypothesis was that the number of "supine" events correlates with the underlying physiological state, thus eventually correlating with death. We demonstrated that under our experimental conditions, the number of supinations, which seems to be an indicator of impairment, was associated with early death, but not with the delay of the recovery. Several genes associated to ataxias in *Drosophila* were studied in medflies dissected brains: *tefu* (homologous to human ATM), *turtle*(homologous to vertebrate neogenin genes), and *parkin*. The expression was quantified by real time PCR. *tefu* mRNA showed an increase in "supine" groups, while the expression of MnSOD was significantly reduced. Our result suggests that supine flies may present an oxidative damage in brain. Surprisingly, the expression of turtle was strongly increased in "supine" flies, opposite to the observation in *Drosophila turtle* ataxic flies

CB-P41
PRENATAL NUTRITIONAL STRESS TRIGGERS PREMATURE SENESCENCE IN MOUSE EMBRYONIC FIBROBLASTS (MEFS)

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Malnutrition is a serious problem in developing countries affecting millions of unborn and young children during the most vulnerable stages of their development. In mammals, nutrition in early life (including in utero) can have lasting effects. Recent studies have suggested an association between an adverse intrauterine environment and premature aging. Cellular senescence is a permanent cell cycle arrest triggered by exogenous or endogenous stress and may be an important factor that contributes to the aging phenotype. In this work we studied the effect of protein malnutrition during pregnancy and its relationship to the establishment of senescence in MEF. CF-1 female mice were fed with normal (20%, NP) or low protein (8%, LP) diet from a week before mating until MEF extraction (14.5-day-old embryos). MEF from LP embryos acquired a large and flat cellular appearance and lower proliferation rates at earlier passages than MEF from NP embryos. We observed an increment in Senescence-associated β-galactosidase activity and reduced percentages of BrdU positive cells at earlier passages in LP MEF´s, suggesting the onset of a senescent phenotype. Finally, higher levels of ROS were observed in each passage comparing LP to NP MEF. We propose that

MEF from the offspring of LP mothers undergo premature senescence which would be caused, at least partially, by an increased production of ROS.

CB-P42
NITRIC OXIDE AND ABSCISIC ACID: KEY SIGNALLING MOLECULES OF NUTRITION AND IMMUNITY IN *Apis mellifera*

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Since many years, honeybee (*Apis mellifera*) colonies are threatened by different stress factors around the world resulting in a declination of their population. As a consequence, understanding honeybee defences to confront their threats is a priority. The importance of nutrition on bee's health has been demonstrated and become a topic of concern. Through a series of correlative reports, we have previously demonstrated that nitric oxide (NO) plays a role in wound healing and encapsulation responses, including the activation of melanin formation and the recognition of a foreign surface in larvae and adult honeybees. More recently, we reported that abscisic acid (ABA), a natural occurring compound in honeybees, also participates in the cellular responses of *A. mellifera* enhancing the wound healing process. Here we show that, as occur in animal cells, ABA induces the NO formation in honeybee. We also demonstrate that L-arginine (the natural substrate for NO production in animal cells) and ABA can be used as natural supplements in the honeybee diet by beekeepers improving the health condition of A. mellifera's colonies, adding further evidence on the importance of nutrition in the bee's immune fitness. We shall present results supporting a connection between NO and ABA signalling in *A. mellifera*'s immune response

CB-P43
INHIBITION OF THE PLASMA MEMBRANE CA^{2+}–ATPASE BY EPIGALLOCATECHIN 3–GALLATE

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Green tea polyphenols are natural plant flavonoids and comprise many types of catechins. Epigallocatechin 3–gallate (EGCg) is the major polyphenol component and primarily responsible for the green tea effects. EGCg beneficial effects include mainly antioxidant, anti–inflammatory and neuroprotective effects. Previous studies showed that EGCg inhibits the Na^+,K^+–ATPase activity by reducing the rate of the E1P to E2P transition through interaction with plasma membrane phospholipids. The purpose of this work is to investigate the effect of EGCg on the Plasma membrane Ca^{2+}– ATPase (PMCA) as another member of the P-type ATPases family. We examined the effects of EGCg on the PMCA activity by using vesicles from human red blood cells. The EGCg showed an inhibitory effect on the Ca^{2+}–ATPase activity in a dose-dependent manner with $K_{0.5}$ values of 3.5 µM. This value was similar to those found for Na^+, K^+–ATPase. One biological action attributed to EGCg is the ability to influence intracellular Ca^{2+} in both non-excitable and excitable cells. We characterized EGCg-regulated Ca2+ signaling by cytoplasmic free Ca^{2+} measurements using Fluo4–AM. EGCg decreased the rate of removal of Ca^{2+} in HEK293 cells with respect to the control, suggesting that those systems that remove calcium including PMCA would be affected, through a process that remains to be determined.

CB-P44
SHRNA SCREEN FOR UBIQUITYLATION GENES INVOLVED IN TUMOR-CELL INVASION AND MIGRATION REGULATION

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Despite formidable advance in the prevention, early detection and treatment of a great number of cancers, the development of metastasis foci in patients suffering from this disease still represents a significant reduction in their survival and life quality. Although metastatic cells have partially been characterized, there is yet not an effective treatment for this disease. The Ubiquitin-Proteasome System (UPS) plays a fundamental role in the maintenance of protein homeostasis both in normal and stressed conditions. This enzymatic cascade represents one of the most important metabolic protein degradation pathways and plays a fundamental role in the control of almost every single cellular process. Since alterations in the ubiquitylation cascade have been shown to be associated with malignant transformation, invasive potential of cells and metastasis, we sought to investigate the role of the UPS in the

regulation of tumor-cell invasion and migration. We performed a genetic screen using a shRNA library against UPS genes, and Boyden chambers to analyze the migrating/invasive potential of cells infected with this library. After the selection process, the non-migrating populations of cells were characterized and the shRNA present in them were determined.

CB-P45
STUDY OF THE ROLE OF UBIQUITIN PROTEIN LIGASE CRL4CDT2 IN THE CONTROL OF CHROMATIN REMODELING FACTOR

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The Ubiquitin-Proteasome System is a major coordinator of cellular physiology through the regulation of protein homeostasis and its substrate specificity is tightly regulated by the family of E3 ubiquitin ligases. CRL4Cdt2 ubiquitin ligase is emerging as a master regulator of cellular proliferation involved in multiple DNA repair processes, which is frequently over-expressed in a variety of human tumors and its expression correlates with tumor grade, metastasis and poor survival. However, despite the broad and potentially important implications for cancer biology, the precise molecular mechanisms by which CRL4Cdt2 exerts its oncogenic activity are still far from being understood. In order to broaden our understanding how deregulation of CRL4Cdt2 might contribute to cancer development, we used an affinity purification and mass spectrometry approach to identify and characterize new CRL4Cdt2 substrates. Among the most abundant putative Cdt2 protein interacting factors identified, we focused our attention on different members of a multi-protein complex implicated in the maintenance of chromatin structure. These proteins play an important role as barriers of cancer stem cell self-renewal and thus the characterization of their functional interaction with CRL4Cdt2 might unveil new potential therapeutic point of intervention in cancer treatment.

CB-P46
POLARIZING YEAST CELLS WITH ELECTRIC FIELDS

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Cells are naturally surrounded by organized electrical signals in the form of local ion fluxes, membrane potential, and electric fields (EFs) at their surface. Although the contribution of electrochemical elements to cell polarity and migration is beginning to be appreciated, underlying mechanisms are not known. Here we show that an exogenous EF can orient cell polarization in budding yeast (Saccharomyces cerevisiae) cells, directing the growth of mating projections towards sites of hyperpolarized membrane potential, while directing bud emergence in the opposite direction, towards sites of depolarized potential. Using an optogenetic approach, we demonstrate that a local change in membrane potential triggered by light is sufficient to direct cell polarization. Screens for mutants with altered EF responses identify genes involved in transducing electrochemical signals to the polarity machinery. Membrane potential, which is regulated by the potassium transporter Trk1p, is required for polarity orientation during mating and EF response. Membrane potential may regulate membrane charges through negatively charged phosphatidylserines (PSs), which act to position the Cdc42p-based polarity machinery. These studies thus define an electrochemical pathway that directs the orientation of cell polarization.

CB-P47
REGULATION OF ALDOSE REDUCTASE BY TUBULIN: EFFECT ON HEMORRHEOLOGY OF DIABETIC RATS

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The main pathogenic pathway of diabetes involved in the development of chronic complications of this disease is activation of the enzyme aldose reductase (AR). In our laboratory we describe a new regulatory mechanism of AR activity by tubulin interaction. The tubulin increased 7 times the AR activity. This activation induces changes in microtubule dynamics, inhibition of Na+,K+-ATPase and prevent the diabetic cataracts in rats. We also demonstrate that 3-nitro-L-tyrosine is able to prevent tubulin/AR association and abolish the activation of the AR by tubulin. The aim of this study was to evaluate the effect of 3-nitro-L-tyrosine on the arterial hypertension and deformability of erythrocytes in diabetic rats, which they correlated with hyperactivation of AR. The results indicate that

administration of 3-nitro-L-tyrosine decrease the arterial hypertension and increase the erythrocyte deformability in diabetic rats. This allows us to speculate that the tubulin/AR association is not only involved in the formation of diabetic cataract but also in the deformability of the erythrocytes and from there to the blood pressure.

CB-P48
ANALYSIS OF TUBULIN DURING ERYTHROID DIFFERENTIATION FROM HUMAN HEMATOPOIETIC STEM CELLS

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In previous works, we demonstrated that human erythrocytes contain a type of tubulin that we termed "sedimentable tubulin". The objective of the present study was to analyze the origin of this structure during hematopoiesisin vitro.Hematopoietic stem cells (HSC)were isolated from cord blood of healthy pregnancies and maintained in long-term culture medium supplemented with erythropoietin (EPO), SCF and interleukin 3 (IL3) for 7 days; then instem spam expansion mediumsupplemented only with EPO for 12 days, andfinally the culture was transferred to a Lab-Tek poly-D-lysine for 24 hours. We observed erythroiddifferentiation, obtaining 88% of enucleated cells at 12 days.Cells from different timepoints were fixed on coverslips and analyzed by immunofluorescence microscopy. In this work, we demonstrated the presence of tubulin throughout the differentiation pathway, from an abundant microtubule network observed in precursor cells (CPH) to a cytosolic distribution in the mature erythroid cell after enucleation, ie the formation of reticulocytes. Although not a typical structure as observed in erythrocytes (sedimentable tubulin), we speculate that the origin of this structure is the appearance of cytosolic tubulin in reticulocytes

CB-P49
MECHANISMS OF TISSUE REMODELING IN DIABETIC ENTEROPATHY

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The clinical course of diabetic enteropathy is highly heterogeneous and often unpredictable, with multiple complications as diarrhea, constipation or fecal incontinence. All these problems are manifestations of an intestinal tissue remodeling. Using an STZ-induced rat model, we showed that extracellular matrix macromolecules (ECM) such as collagen and fibronectin are progressively altered during diabetic progression in the whole intestinal wall. Increased expression of mesenchymal markers (vimentin and α-SMA) and significant up-regulation of inducers and mediators TGF-β1, TGFRs, p-Smad2/3 in the mucosa were also observed. In vivo results were correlated with increased collagen III synthesis both at the mRNA and protein levels, without alteration of collagen I production in high glucose-treated fibroblasts in culture. This suggests an active role of these cells in fibrogenic response in vivo. At the muscle layer a change from the contractile to a secretory phenotype of smooth muscle cells (SMC), an imbalance between MMPs/TIMPs activities and a deregulated TGF-β1 signaling were evident in STZ-induced rats. Taken together, these data give a window into the process of diabetic intestinal fibrosis where phenotypic changes in fibroblasts and in SMCs are responsible of the intestinal wall remodeling with a TGF signaling involvement.

CB-P50
YACON DIET SUPPLEMENTATION DECREASED COLLAGEN DEPOSITION IN NON ALCOHOLIC FATTY LIVER

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease. Excessive dietary carbohydrate consumption, especially excessive fructose consumption are linked to metabolic syndrome, being associated with hypertriglyceridemia, hypertension, insulin resistance and diabetes. Fructose is metabolized principally in the liver, where it is converted into fatty acids, which are stored in the form of triglycerides leading to NAFLD. We analyzed the hepatic effects of S. sonchifolius (yacón) roots in fructose-enriched diet. Adult male Wistar rats were maintained on a rat standard chow and then animals were randomly separated into a control group (SD: water a.l.) and a fructose-supplemented group (FED: fructose 10% w/v, a.l.). After 12 w FED rats, were randomly assigned according to the treatment: FED or a FED+Y (340mg yacon flour/kg bw). Administration yacon flour for 16 w lowered visceral fat-pad and hepatic weights compared to FED rats. Yacon supplementation was associated with a decrease in serum and

hepatic triglycerides, cholesterol, VLDL, ALT and AST activities. Moreover, FED+Y rats showed improved hepatic steatosis, inflammation and limited hepatic fibrillar collagen deposition. Levels of TGF-β and p-Smad2/3 were decreased in FED+Y livers. In summary, yacon roots diet supplementation would seem beneficial for prevention of NAFLD and its metabolic consequences.

CB-P51
ANTIOBESITY AND LIPID-LOWERING EFFECTS OF YACON ROOTS IN HIGH FAT DIET-INDUCED OBESE RATS

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Obesity is a global public health concern and the quest continues to explore natural product-based novel therapeutics. The present study investigated the anti-obesity properties of S. sonchifollius (yacon) roots and its molecular mechanism in rats under high-fat diet (HFD). After 12w on a HDF male Wistar rats were randomly assigned to one of the following groups: SD, HFD and HFD+Y (680mg yacon flour/kg bw). Administration yacon flour for 8 w significantly reduced body weight, food intake, feed efficiency and body mass index in the HFD+Y rats. Lipid profile, serum glucose and insulin were similar to SD animals. The atherogenic index decreases compared with the HFD group. Yacon treatment significantly improves pathological changes in liver, intestine and adipose tissue and decreased the relative weights of epididymal, perirenal and visceral white adipose tissues. Serum levels of leptin and adiponectin were normalized in HFD+Y rats improving insulin sensitivity. Feeding with yacon reversed the HFD-induced down-regulation of the peroxisome proliferator-activated receptor γ (PPARγ), adipocyte protein 2 (aP2) genes and elevated AMPK phosphorylation in visceral adipose tissue. These findings suggest that yacon roots administration suppresses high-caloric-diet-induced obesity and it can be developed as a potential candidate for the treatment of obesity and associated complications.

CB-P52
VASOPRESSIN ANALOGS INHIBIT CELL GROWTH IN HUMAN PROSTATE CANCER CELLS WITH NEUROENDOCRINE FEATURES

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Neuroendocrine (NE) differentiation occurs in prostate cancer (PCa) associated to androgen deprivation and castration resistance. NE tumor growth is stimulated by specific neuropeptides. Inapropiate secretion of the neuropeptide vasopressin (AVP) was reported in patients with PCa associated with therapy resistance and increased aggressiveness. Desmopressin (dDAVP) is a synthetic analog of AVP that acts as a selective agonist for the AVP V2 receptor. DDAVP shows antitumor properties in breast, colorectal and lung cancer models. In our laboratory new synthetic AVP analogs were developed presenting improved cytostatic activity. Our goal was to evaluate the antiproliferative ability of the new analogs in an aggressive human prostate cancer cell line. First, we study the presence of specific NE markers (CgA and NSE) by western blot and qRT-PCR and the AVP V2r expression by immunofluorescence in PC-3 cells. The cytostatic action of the analogs was studied in exponential growth and low cellular density. Analogs (100 nM – 1.5 μM) significantly reduced cell growth ($p<0.05$), showing a higher effect at low density. In vitro, overnight treatment with 1 μM of dDAVP decreased levels of CgA and NSE mRNA expression by qRT-PCR. These results show the antitumor properties of the AVP analogs on an aggressive cell line of castration resistant human prostate cancer with NE features.

CB-P53
COPPER OVERLOAD TRIGGERS ANTIOXIDANT DEFENSES IMBALANCE IN HIPPOCAMPAL HT22 NEURONS

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Copper (Cu)-induced oxidative stress has been involved in the pathogenesis of several neurodegenerative diseases such as Alzheimer's disease and related disorders. In this work, we characterized the response of hippocampal neurons to Cu overload and we also studied the signaling pathways involved in the regulation of antioxidant defenses and neuronal survival. HT22 hippocampal neurons incubated with increasing Cu2+ concentrations (100-250 μM) showed decreased glutathione (GSH) levels and GSH peroxidase expression, and increased expression levels of the rate limiting step enzyme for GSH synthesis, glutamate cysteine ligase (GCL). Cu-induced imbalance in antioxidant defenses generated an increase in reactive oxygen species content. As a result of the increased pro-oxidant conditions, neuronal damage was evidenced by mitochondrial dysfunction and increased lipid peroxidation levels. Mitochondrial function was even more affected by pharmacological inhibition of MAPK (U0126) and PI3K (LY294002) pathways.

Both effector kinases, ERK1/2 and Akt, respectively showed a differential neuronal localization and expression levels in neurons exposed to Cu-injury. Our results show that Cu-induced neuronal injury is generated by an imbalance in cellular GSH metabolism and that neuronal survival depends on PI3K/Akt and ERK1/2 mediated pathways.

CB-P54
EFFECTS OF METFORMIN IN COMBINATION WITH 2-DEOXYGLUCOSE IN FELINE MAMMARY CARCINOMA CELLS

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Most tumor cells metabolism rely in an exacerbation of glycolisys which increased the malignant phenotype. The aim of the present work was to investigate the in vitro effects of a combination of metformin (MET, antidiabetic drug, OXPHOS inhibitor) and 2-deoxyglucose (2DG, hexokinase inhibitor) on two cell lines, AlRB (HER2 positive) and AlRATN (HER2 negative) derived from a spontaneous feline mammary carcinoma. AlRB and AlRATN were grown as both monolayers (M) and spheroids (S). After 24 h of culture, cells were treated with 0.5 mM 2DG, 1 mM MET or a combination of both MET-2DG. Concentration-response curve were also assessed. The antitumor effects of bioenergetic inhibition were evaluated by the acidic phosphatase assay (APH), 5 days (M) or 9 days (S) after treatments. We found that both cells lines significantly decreased cell viability ($p<0.001$) in a concentration dependent manner after 2DG or MET treatments. AlRB were more sensitive to 2DG than AlRATN when cultured as monolayers ($p<0.05$). On the contrary, AlRATN where more sensitive to MET than AlRB when culture as spheroids and also compared with its respective monolayers. Finally, the combination of MET-2DG potentiated the individual effects ($p<0.01$) only in AlRB monolayers. The results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

CB-P55
TYPE I AND TYPE II BMP RECEPTOR EXPRESSION AND SIGNALING IN BOVINE OVIDUCT EPITHELIAL CELLS

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Members of the transforming growth factor beta (TGF-β) family, including the bone morphogenetic proteins (BMPs) are expressed in the mammalian oviduct epithelial cells. These signaling molecules play important roles in development and tissues homeostasis; little is known about their function in the oviduct. Modulation of TGF-β/BMP receptor activity is a critical step for signaling regulation. In the present study, RT-qPCR was used to analyze mRNA expression of BMP receptors type I (BMPR1A, BMPR1B, ACVR1, ACVR1L) and type II (BMPR2, ACVR2A, ACVR2B) in bovine oviduct epithelial cells (BOEC) isolated from ampulla and isthmus at proestrous and diestrous (n=3). Results were statistically analyzed by Student t-test. All of the mRNA BMP receptors were detected in BOEC, except ACVRL1. During diestrous, higher significant mRNA levels were found for BMPR1B, BMPR2 and ACVR2B in isthmus BOEC; and for ACVR2 mRNA in ampulla epithelial cells ($p<0.05$). Additionally, BMP signaling pathway was evaluated in primaries BOEC cell cultures. Human rBMP5 (50 ng/ml) was added into BOEC cell cultures for 1, 3, 5 and 24 hours. Smad6 (a direct target gene of BMP signaling) expression was analyzed by RT-qPCR. A significant increase of Smad6 mRNA levels was detected in cultures treated with rBMP5. Altogether, these data suggest that TGF-β/BMP act as autocrine regulators of the epithelial oviduct function.

CB-P56
THE MEMBRANE-BOUND INMUNOPHILIN ZONDA IS CRITICALLY REQUIRED IN EARLY STAGES OF AUTOPHAGY

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Autophagy is an intracellular process in which damaged organelles and protein aggregates are degraded and recycled in order to maintain normal cellular homeostasis. Defects in the autophagic machinery have been associated with numerous diseases. The first distinguishable autophagy-specific membranous structure, the omegasome, forms when the VPS34 autophagy nucleation complex mediates local deposition of phospatidylinositol-3-phospate (PI3P) in a specific domain of the ER. The molecules and mechanisms that mediate the assembly and localization of the

autophagy-specific VPS34 complex remain poorly defined. We have identified a membrane-bound immunophilin, that we have named Zonda, which plays a critical role at early stages of autophagy. Zonda operates downstream to ATG1, the first autophagy- specific kinase, and is required for autophagic activation of VPS34. Zonda displays a reticular distribution under basal conditions and nucleates in puncta when autophagy is induced. Zonda puncta colocalize with omegasome marker DFCP1 which suggests that Zonda is part of omegasomes. This is supported by observations in which ATG5 and ATG8 particles are contained within Zonda-labeled omegasomes. We concluded that Zonda is a novel component of the autophagy cascade essential for the autophagic activation of the VSP34 complex.

Enzymology

EN-P01
CHARACTERIZATION OF A NOVEL CARBOHYDRATE BINDING DOMAIN OF PUTATIVE AMYLASE FROM *Ostreococcus tauri*

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Starch Binding Domains (SBD) are one type of carbohydrate binding domains (CBM) that have acquired the evolutionary advantage of being able to interact and disrupt the substrate surface on a particular way because they present two polysaccharide binding sites . The proteins containing this class of CBMs show a strong physical association with the different substrates, increasing the rate of enzymatic reactions. These modules also occur in proteins with no hydrolytic activity, constituting a core from which the catalytic proteins are organized. We are studying the starch metabolism of the picoeukaryote *Ostreococcus tauri* because even it has a small genome, this pathway maintain a complexity similar to Arabidopsis thaliana. In the present work, we characterized a predicted CBM20 containing amylase of *Ostreococcus tauri*. We propose a possible molecular structure using bioinformatics modelling techniques, and we analyze the binding properties of this module to different insoluble substrates. The results suggest that this particular CBM has a high affinity for linear polysaccharides.

EN-P02
FUNCTIONAL ENZYMES THAT REPAIR OXIDIZED METHIONINE IN PROTEINS FROM *Leptospira interrogans*

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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. Little information is available concerning of mechanisms for repairing oxidized proteins in *Leptospira interrogans*. These mechanisms would be relevant for the survival of this pathogen under oxidative conditions. Two genes encoding for putatives *Lin*MsrA (*linmsrA1* and *linmsrA2*) and one for *Lin*MsrB (*linmsrB*) has been found in the *L. interrogans* genome project. In this work, we report the functional characterization of *Lin*MsrB. The enzyme is a monomeric protein and specific for L-Met(R)SO reduction, using DTT or TNB as the reducing substrates. Interestingly, no MSR activity (in an *in vitro* assay) was observed using leptospira TRX as reducing substrate. In addition, we found that *Lin*MsrB could compensate for MSR deficiency in yeast mutant strain lacking both MsrA and MsrB activities. The results support the occurrence of a metabolic pathway involved in the critical function of repairing oxidized macromolecules.

EN-P03
BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF A HYBRID CLUSTER PROTEIN FROM *Entamoeba histolytica*

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Entamoeba histolytica, an intestinal parasite that is the causative agent of amoebiasis, is exposed to elevated amounts of highly toxic reactive oxygen and nitrogen species during tissue invasion. Hybrid cluster proteins (HCP) contain

two types of Fe/S clusters, namely a [4Fe-4S] or [2Fe-2S] cluster and a novel type of hybrid cluster, [4Fe 2S-2O]. While it was proposed that HCP acts in some step of nitrogen metabolism in bacteria, a specific role for this protein remained unknown. In this work, we present functional data and structural properties of HCP from *E. histolytica* (*Eh*HCP). We employed an *E. coli*-based functional complementation assay to rescue phenotypes of hcp mutant strain. The complementation experiments with recombinant HCP demonstrate that the Δ*hcp E. coli* strain can suppress the sensitivity to hydrogen peroxide or hydroxylamine. Furthermore, ferredoxin from *E. histolytica* and rubredoxin from *Clostridium pasteurianum* were reducing substrates for recombinat *Eh*HCP in vitro. Altogether, the results suggest that HCP could be involved in oxidative and nitrosative stress protection in the parasite. To the best of our knowledge this is the first characterization of a eukaryotic HCP. Granted by UNL, CONICET (PIP112 2011 0100439, PIP114-2011-0100168) and ANPCyT (PICT2012-2439, PICT2013-0253)

EN-P04
INSIGHTS INTO THE INTERACTION OF B30.2 DOMAIN OF GNIP WITH GLYCOGENIN

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Glycogenin (GN) is the autoglucosyltransferase that initiates the biosynthesis of glycogen. By self-glucosylation from UDP-glucose and Mn^{2+}, GN synthesizes a maltosaccharide that acts as substrate for the full polymerization and branching produced by glycogen synthase and branching enzyme, respectively. Mammalian GNs have been extensively studied, however, little is known about their functional regulation. Searching for potential regulators using a yeast two-hybrid screen, an unknown protein was identified, later called GNIP (Glycogenin Interacting Protein). GNIP gene encodes at least three isoforms produced from alternative splicing: GNIP1, GNIP2 and GNIP3. The predicted structure of GNIP1, the largest isoform, contains an N-terminal RING finger domain, a B box domain, a coiled-coil domain and a C-terminal B30.2 domain. GNIP2, the only isoform that could be expressed in a soluble form, interacts with glycogenin and increases its self-glucosylation 3-4 fold. The interaction with glycogenin is mediated by B30.2, a domain that is present in a large number of proteins with diverse functions. Since little is known about this interaction and its consequences, we have prepared the B30.2 domain of human GNIP and analyzed its effect on glycogenin activity and the dependence of the interaction with the oligomeric state of the enzyme. Here we describe the results obtained during these studies.

EN-P05
ARGININE-KINASE OF SPIDER *Polybetes pythagoricus*: CDNA DESCRIPTION AND THE OBTAINING OF RECOMBINANT PROTEIN

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Phosphagens in invertebrates are important because of their bioenergetic function, and as allergens for human beings. Particularly in spiders, studies are scarce. The arginine kinase enzyme (AK, EC 2.7.3.3) maintains the levels of phospho-arginine phosphagen by the reversible phosphorylation of L-arginine with ATP. In the present work it was identified the coding region of AK gen of the spider *Polybetes pythagoricus* (Chelicerata, Class Arachnida) performing a cDNA library through reverse transcription of mRNA of muscle tissue to obtain data on expressed genes. Through Sanger sequencing and assembling a cDNA fragment was obtained for AK and its sequencing was completed through the technique of rapid amplification of cDNA ends. It could be determined that it shows 384 amino acids, a theoretical molecular mass of 43 kDa, and an isoelectric theoretical point of 6.37. From the bioinformatics analysis it was observed that it shows 93% of identity with AK of the spider S. mimosarum and 81% of identity with AK of horseshoe crab, considered as the phylogenetically basal chelicerate *L. polyphemus* (Chelicerata, Merostomata). By means of the construction of a synthetic gen it was possible to express the protein recombinant in *Escherichia. coli*. These studies will enable us to perform a biochemical, biophysical, and structural characterization.

EN-P06
LECTIN DOMAINS AS SCAFFOLD TO DIRECT O-GALNAC GLYCAN BIOSYNTHESIS

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O-GalNAc glycan biosynthesis occurs mainly in Golgi. Molecular organization and functional regulation of this process are not well understood. We evaluated the effect of lectin domains (β-trefoil fold) of polypeptide GalNAc-transferases (ppGalNAc-Ts) on catalytic activity of glycosyltransferases involved in the first steps of O-GalNAc glycosylation. The presence of lectin domain of ppGalNAc-T3 (T3lec) or -T4 (T4lec) during ppGalNAc-T2 and ppGalNAc-T3 catalytic reaction had a clear inhibitory effect on GalNAc-T activity. In transient transfected CHO ldlD cells a catalytically inactive mutant, mimicking a lectin domain, reduced αGalNAc incorporation in relation to a mock vector. Also, both lectin domains interacted with the catalytic domain of ppGalNAc-T2 and this interaction was not mediated by carbohydrate. Opposite to the previous results, T3lec but not T2lec and T4lec, had a clear activating effect of core 1 galactosyltransferase (C1GalT) enzyme activity. We also see an enhancing effect on C1GalT activity in presence of full-length ppGalNAc-T3. We describe for the first time a role for lectin domains that involved protein-protein interaction in the regulation of glycosyltransferases activity of O-glycan biosynthesis pathway

EN-P07
OPTIMIZATION OF A SYNTHETIC MEDIUM FOR CELLULASES SECRETION OF *Trichoderma harzianum* NATIVE OF MISIONES

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A reduction in the cost of bioethanol production can be achieved by reducing the cost of raw materials or cellulase enzymes. The aim was to optimize a synthetic culture medium to maximize the cellulases secretion of a Trichoderma harzianum strain native of Misiones. A Box-Behnken response surface design was performed to analyse carboxymethylcellulose (CMC) as sole carbon source; and urea, yeast extract and ammonium sulfate as nitrogen sources. The effects of the factor were tested at three levels and 29 tests were conducted, including a central point quintupled. The synergistic effect of the three cellulases was determined by the filter paper method (FPU). The EGs and CBHs activities were determined by the method of DNS acid, and BGLs activity was determined by the method of p-nitrophenyl-β-glucoside. Taking the days with the highest enzyme activity (without statistically significant differences), a positive influence (statistically significant) of CMC was observed in all the analysed enzymes. Also, it was observed a statistically significant and positive interaction between urea and yeast extract regarding EGs activity; and a statistically significant and positive influence of urea, as well as its interaction with CMC for FPU activity. It was determined which combination of factor's levels led to the optimization of cellulases's secretion of a T. harzianum native of Misiones.

Lipids

LI-P01
CARDIOLIPIN METABOLISM IN MICE BRAIN DURING EARLY DEVELOPMENT: SEXUAL DIFFERENCES

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During perinatal period, testosterone (Te) release in males play a role in sexual dimorphic brain development. Mitochondrial metabolism is highly active during this period, thus we aimed to study cardiolipin (CL) metabolism and the relationship with Te. C57BL/6 offspring were separated by sex at different postnatal days (PND 0-10) to obtain plasma and cerebral cortex. Te levels in plasma, the expression of enzymes involved in CL metabolic pathway and CL content and composition were assessed. We found sex-independent variation in CL content coincident with the expression of the CL de novo synthetic enzymes. CL unsaturation index (UI) was higher in males (PND 0-2) due to the high content of 20:4 and 22:6, correlated with Te levels. This could be explained by a differential expression of

enzymes involved in both CL recycling pathways. To assess if this was due to Te levels, females were androgenized with a single injection of Te propionate at PND 0. We found that the sexual differences in UI and recycling enzymes were dependent on the perinatal hormonal levels. Despite that Δ5 and Δ6 desaturases were also differentially expressed, only Δ6 desaturase depends on Te levels. The sexual dimorphism found here would be relevant for understanding the roles of lipids in sexual dimorphic brain development, and possible long-lasting consequences of an early exposure to endocrine disruptors.

LI-P02
NITRO-FATTY ACID IRREVERSIBLE INHIBITION OF PTP1B

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Insulin signaling impairment is associated with type II diabetes and obesity. This molecular pathway is tightly regulated by protein kinases and protein phosphatases activity. Lack of PTP1B activity improves insulin sensitivity and blood glucose levels. PTP1B enclose a cysteine in the catalytic domain which turns out an important modulator of its activity by reactive oxygen species (ROS). Recently, we have shown that systemic nitro-oleic acid (NO_2-OA) administration reduces insulin resistance in a diabetic (ob/ob) mouse model and increases glucose uptake. Nitro-fatty acid derived activates, in part, PPARγ dependent gene expression. However, the thiol reactivity of NO_2-OA appears to play a crucial role in modulate insulin sensitivity by inhibited PTP1B activity. Our results demonstrated that enzyme activity was dose dependent and irreversible inhibited by NO_2-OA as shown by GSH and BME competition. Furthermore, mass spectrometry analysis revealed NO_2-OA covalent adducted to different nucleophile residue on PTP1B, including the cysteine 215. In addition, extended insulin phosphorilation of AKT, and MAPK in 3T3-L1 cell pretreated with NO_2-OA compared with untreated cells. Thus, we have characterized the inhibition of PTP1B by nitro-alkylation of cys-215, a mechanism that suggests the participation of NO_2-FA in the insulin signaling pathway via PPARγ independent manner.

LI-P03
ROLE OF NITRO-FATTY ACIDS IN THE REGULATION OF SCAVENGER RECEPTORS AND FOAM CELL FORMATION

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Nitro-fatty acids (NO_2-FA) are potent lipids signaling mediators formed from nitric oxide and nitrite dependent reaction. NO_2-FA modulates signaling cascades via covalent posttranslational modification (PTM) to important regulatory proteins, thus affecting downstream signaling events, such as the NF-κB, PPARγ and Keap1-Nrf2 pathways. We have previously demonstrated that macrophages form nitro-conjugated linoleic acid (NO_2-CLA) under inflammatory condition and they exhibit important anti-inflammatory and cytoprotective actions in vitro and in vivo. In the present work we explored the ability of NO_2-FA to modulate the expression of scavenger receptors (CD36, SRA and LRP-1) on RAW264.7 and THP-1 macrophage cell line. Nitro-oleic acid (NO_2-OA) induced CD36 expression and exhibited slight effect on LRP-1 and SRA expression. This up-regulation of CD36 receptor was linked to the electrophilic activation of the antioxidant transcription factor Nrf2 by NO_2-FA. Thus, uptake of modified LDL particles in macrophage treated with NO_2-OA result in an increase lipid accumulation by appearance of Red Oil O positive lipids droplets compared to untreated cells. Our results indicate that NO_2-FA induce foam cell formation in vitro and challenged the understanding between anti-inflammatory and anti-atherogenic action of NO_2-FA during plaque formation in atherosclerosis.

LI-P04
DIETAR FATTY ACIDS AND UNSATURATED FATTY ACIDS ON TRIACYLGLYCERIDES METABOLISM IN MICE

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The amount and type of dietary fat could modulate the levels of triacylglycerol (TAG) in plasma and tissues. The aim was to investigate the effects of trans fatty acids (TFA) on TAG regulation in mice fed different unsaturated fatty acid (UFA) diets. Male CF1 mice (22 g) were fed (30 d) with a standard diets differing in dietary n-3/n-6/n-9 UFA ratios: Canola (C:1/2/6), Maize (M:1/53/31) or Olive (O:1/10/76) oils containing 0.75% of TFA (Ct, Mt and Ot, respectively) or not. Expression and activities of lipogenic and oxidative enzymes, serum and liver TAG content,

hepatic TAG secretion rate (TGSR), and adipose tissue (AT) and muscle lipoprotein lipase (LPL) activities, were assessed. TFA induced hepatic TAG accretion in Ot and Ct groups (100%) vs. their respective control group, being associated with an exacerbated expression (FAS 300%; ACC 100%) and activity (FAS 270%; ACC 140%) of lipogenic enzymes induced by the increased SREBP1a expression (800%) in Ot group. In Ct, it was related to a lower CPT-I activity (-350%). These effects, lead to an enhanced TGSR in Ot (260%) and Ct (160%) groups vs. their controls, however the LPL activity in AT (200% in Ot and Ct vs. O and C, respectively) normalize the levels of serum TAG. In Mt group no changes were observed in these parameters. In conclusion, TFA altered TAG metabolism by different mechanisms depending on the type of dietary UFA.

LI-P05
C-FOS REGULATES BRANCHING FORMATION IN NEURONS

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Neuronal differentiation is a complex event where lipid synthesis for membrane biogenesis plays a key role. As we have previously shown that c-Fos associates to membranes of the endoplasmic reticulum (ER) and activates phospholipid synthesis, this phenomenon might be associated with the molecular mechanisms that allow the higher rates of membrane genesis required for neuronal differentiation. Analysis of c-Fos localization by immunofluorescence shows it co-localizing with ER markers in the neuronal soma and mainly forming structures at the branching sites of the neuronal processes. When c-Fos expression is blocked in primary cultures of rat hippocampal neurons either using a lentiviral vector or a specific antibody, differentiation is impaired and no development of axonal processes is observed. We examined if the enzyme CTP:phosphocholine cytidyltransferase-β2 (CCTβ2), an integral enzyme of the ER membranes that plays an important role in the formation of axon branches, is activated by c-Fos. We observed co-immunoprecipitation of c-Fos with the enzyme and positive results for the interaction between these two proteins was found using FRET technique. These results indicate a possible mechanism for branching regulation and support our hypothesis of the importance of c-Fos-mediated activation of phospholipid synthesis during neuronal differentiation.

LI-P06
EFFECT OF GERANIOL ON RAT CARDIOMYOCYTES AND ITS POTENTIAL AS A CARDIOPROTECTIVE NATURAL COMPOUND

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Heart damage is nowadays one of the major causes of morbidity and mortality. We propose the monoterpene geraniol (G) as an agent that would render myocardial cells more resistant to deleterious effects such as ischemia (I) followed by reperfusion (R), high levels of reactive oxygen species (ROS) or hypertrophy. These physiological and/or pathological stressors generate many alterations in cardiac myocytes and even cause cell death. We evaluated the effect of G on myocytes induced to oxidative stress and on two key signaling pathways enzymes that mediate some cardiovascular-protective effects: AMP-activated protein kinase (AMPK) and ERK1/2. Assays were performed in neonatal rat ventricular cardiomyocytes isolated from 1–2-day-old rats. ROS levels were measured with fluorogenic probes in cells incubated with G for 24h and then subjected to oxidative stress conditions (150 μM H_2O_2) or to an IR model (hypoxia chamber 3% O_2 for 1h and normoxic conditions for 1h). Cell viability was evaluated by MTT assay and pAMPK and pERK1/2 levels were determined by Western-blot. Our results showed that G (5-200 μM) decreased cardiomyocytes ROS levels, increased pAMPK and decreased pERK1/2 revealing its actions in different biochemical mechanisms with cardioprotective effects. This study suggests that G could be a potential adjunct therapy to reduce or even prevent myocardial damage.

LI-P07
ROLE OF MEMBRANE COMPONENTS OF *Ensifer meliloti* IN THE DESICCATION TOLERANCE

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The low water availability impact mainly on biological membranes. To survive, bacteria must be able to sense environmental conditions and respond. The aim of this work was analyze the effect of drying on the viability, viable

but not cultivable state, the membrane state and fatty acids (FA) composition in *Ensifer meliloti* 1021. Cells were grown at 28°C to exponential phase and then 5 ml of culture were filtered on membranes S-Pak 0.45μm Type HA (Millipore Inc.). After filtration, the filters were placed on empty petri plates and placed in an oven at 28°C and humidity 35%. The store time was 0, 1 and 7 days. The cultivable cells was determined by counting the UFC/ml and the viable cells by LIVE/DEAD® BacLight™ kit, the phase transition temperature (Tm) determined by DSC technique and the FA composition determined by GC. We observed a gradual decrease in the survival after 1 day of drying compared to the control (time 0 days), reaching a survival of only 1% after 7 days. Desiccation promoted viable non-culturable state after 1 day drying. Exposure to 1 day of drying caused an increase in saturated FA 16:0 (65%) and 18:0 (500%) as well as a decrease the 18:1Δ11 (65%). The Tm of the cell membranes exposed to 1 day desiccation resulted in 0.5ºC greater control. E. meliloti modifies its membrane FA by decreasing the degree of unsaturation as an adaptation mechanism to tolerate desiccation.

LI-P08
Azospirillum brasilense RESPONDS TO WATER DEFICIT ADJUSTING LIPID COMPOSITION

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Azospirillum is a rhizobacteria studied for its ability to improve growth and performance of many crop by alleviating stresses. We studied *A. brasilense* tolerance to water deficit and the dynamics of adaptive process at the membrane level. *A. brasilense* was exposed to polyethylene glycol 15 mM (PEG) growth and PEG shock (10, 20, 30 and 60 min). Membrane fluidity was determined by measuring fluorescence polarization of DPH. Lipids were extracted. The fatty acids (FA) were analyzed by GC. Identification of phospholipids (PL) was performed using TLC. For its quantification, [1-^{14}C] sodium acetate was added to culture. *A. brasilense* viability was reduced in presence of PEG. Cells grown with PEG showed membrane fluidity similar to those grown without. Lipid composition was modified, increasing phosphatidylcholine and decreasing phosphatidylethanolamine amounts. The unsaturation FA degree was reduced. The fluidity decrease 20 min after the addition of PEG, indicating that the PEG has a fluidizing membrane effect. Fluidity returned to initial values after 60 min of PEG exposure. We conclude that *A. brasilense* perceive osmotic changes by changing membrane fluidity. This effect is offset by changes in PL and FA composition, contributing to the homeostasis of membrane fluidity under water deficit. This knowledge can be used to develop new *A. brasilense* formulations applied to water deficit.

LI-P09
BEHAVIORAL EFFECTS OF THE MODIFICATION OF FATTY ACID METABOLISM IN *Caenorhabditis elegans*

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Downregulation of the expression of long chain fatty acid metabolism enzymes LET-767, ELO-5 and SPTL-1 by the RNA interference approach leads to an increase in the aversion behavior of wild-type *Caenorhabditis elegans* N2 nematodes towards the normally palatable food *Escherichia coli* HT115. Rescue of this aversion phenotype by supplementation with different lipids such as arachidonic acid and its endocannabinoid derivatives is carried out, together with treatment with monoacylglycerol lipase inhibitor JZL 184. Finally, study of the aversion behavior of fatty acid metabolism mutant nematodes *fat-3(ok1126* and *elo-5(gk208)* is assayed, raising the possibility of a communication between different lipid metabolism pathways, known to regulate postembryonic development and behavior. These observations suggest the existence of a neuroendocrine signal linking lipid metabolism in the intestine with the neurons that modulate behavior and development in *Caenorhabditis elegans*.

LI-P10
SPHINGOMYELIN SYNTHESIS IS INVOLVES IN DE-DIFFERENTIATION PROCESS IN MDCK CELLS

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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 to differentiate switch to mesenchymal phenotype thus performing an epithelial to mesenchymal transition (EMT). To study the sphingolipid metabolic

pathway involved in such process, confluent MDCK cells were subjected to hypertonicity and concomitantly SMS was inhibited by pharmacological and knockdown strategies. Both strategies showed alteration of polarized phenotype with acquisition of mesenchymal phenotype. The phenotype alteration was accompanied with alteration in plasma membrane SM distribution, suggesting an alteration in cell polarization. To evaluate the EMT, different markers were performed. Results showed an increase in mesenchymal marker, citoskeleton reorganization and loss of the epithelial marker. Moreover, SM inhibition induced an increase in lectin BSL-1 expression (mesenchymal marker) and a decrease in lectin DBA (collecting duct cell marker). It has been reported that these cell could suffer a trans-differentiation to myofibroblast, however, no increase in alpha-smooth muscle actin was observed in our model. These results suggest that the inhibition of SM synthesis induces the de-differentiation of MDCK, thus suggesting the implication of SM in EMT.

LI-P11
DISTINCTIVE REGULATION OF SREBP1 AND SREBP2 BY HYPERTONICITY

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We previously showed that environmental osmolarity regulates phospholipid synthesis and fatty acid storage in (TG) molecules. It is known that both phospholipid and fatty acid synthesis can be regulated by the transcriptional activation of their biosynthetic enzymes which may be mediated by the transcription factor sterol response element binding protein (SREBP). Two isoforms have been reported: SREBP1 and SREBP2. In the present work, we wanted to evaluate whether changes in environment osmolarity can modulate SREBP1 and/or SREBP2 activities by measuring their mRNA levels, protein expression and localization in renal cells. To do this, MDCK cells were grown in isotonic (298 mOsm/Kg H2O) and NaCl-hypertonic (520 mOsm/Kg H2O) media. The results showed that both SREBP1 and 2 are expressed in MDCK cells in basal conditions. Hypertonic medium increased SREBP1 and SREBP2 mRNA levels showing the highest levels at 24 h. SREBP2 protein also showed an increase at 24 h of treatment. By fluorescence microscopy, we found that SREBP1 and SREBP2 are located in different subcelular compartments. Such a distribution was dependent on MAPK pathways since pretreatment with U0126 changed the location pattern of SRBP1 and U0126, SB202190 and SP600125 changed SREBP2 localization. This different behavior may be responsible for lipid regulation in hypertonicity. *Both are considered first authors

LI-P12
STUDY OF RENAL LIPID METABOLISM IN RATS SUBMITTED TO WATER DEPRIVATION

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Previously we showed that hyperosmolarity activates lipid metabolism in renal cell cultures (in vitro). We aimed to evaluate whether such mechanisms are activated in vivo. Thus, we evaluated if water deprivation (that leads to renal interstitial tonicity increase in vivo) affects lipid metabolism in renal tissue. Adult male Sprague–Dawley rats were randomly divided into two groups: euhydrated (EU), allowed free access to water, and 72 h water-deprived (WD), without access to water for 72 h. All rats had access to food ad libitum. Rats were sacrificed, both kidneys removed and cortex, medulla and papilla dissected in order to evaluate lipid composition and metabolism. Lipids from each zone were extracted by Bligh & Dyer method, separated by TLC and quantified. The results showed that in WD rat-papilla, phospholipid content and synthesis increased by 47% and 43 %, respectively. WD rat-papillary triacylglycerides (TAGs) decreased by 20 % but synthesis increased by 60% while medullary and cortical TAGs increased by 90 and 72 %, respectively. These preliminary results go along with those previously obtained in vitro. Further experiments are necessary to explain the relationship between lipid metabolism and osmotic stress. *Both are first author; **Both are last author

LI-P13
HIGH OSMOLARITY INDUCES ENDOPLASMIC RETICULUM STRESS IN RENAL MDCK CELLS

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Renal medullary collecting duct cells are physiologically exposed to high and variable concentrations of NaCl involved in urinary concentrating mechanism. Despite such adverse environment, renal cells activate protective and survive mechanisms. In these sense, our lab demonstrated that hyperosmolarity increased lipid biosynthesis to protect membrane integrity. It is also known that the transcription of osmoprotective genes such as organic osmolyte and urea transporters, COX2, AQP 2 and chaperones Hsp70, Osp 94 are induced. The increase in the protein synthesis rates could cause endoplasmic reticulum (ER) stress. In the present work we evaluated whether high osmolarity induces ER stress by evaluating three ER stress markers, XBP-1, CHOP and BiP proteins. To do that, MDCK cells were subjected to isosmolality or hyperosmolality (512 mOsm/kg H2O) for different times and the expression of ER markers were evaluated by RT-PCR. Results showed that there are not significant changes in BiP expression but CHOP mRNA showed an increase at early stages of NaCl treatment. XBP-1 mRNA levels showed an increase at 12h and remained constant until 48 h. These results showed that high NaCl treatment induced ER stress in MDCK cells. The XBP-1 activation could be related to the lipid synthesis increase which is necessary for membrane expansion to alleviate the ER stress. *Both are considered last authors.

LI-P14
TL(I) AND TL(III) INDUCE ALTERATIONS IN LIPID METABOLISM IN DIFFERENTIATED MDCK CELLS

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Thallium (Tl) is a toxic heavy metal that contaminates the environment and affects human health. Tl intoxication affects several organs and tissues, being the kidney a main target of Tl toxicity. However, the molecular mechanisms are still poorly understood. Tl has two oxidation states, the monovalent (Tl(I)) and trivalent (Tl(III)) cations. Since most heavy metals disturb cell lipid homeostasis, in the present work we studied if Tl may affect lipid metabolism in differentiated renal epithelial (MDCK) cells. Confluent MDCK cells were differentiated in hypertonic medium for 72 h and further incubated for 72 h in the absence or presence of Tl(I) or Tl(III) (10 or 100 μM). After incubation, cells were collected, counted and lipids were extracted. Chloroformic extracts were resolved by TLC, and phospholipids (PLs), cholesterol (Cho) and triacylglycerides (TG) contents were analyzed. Both Tl(I) and Tl(III) significantly increased PLs and Cho contents. Accordingly, microscopy images showed increased cell size. Together, results could suggest an expansion of membranes. Also, Tl(I) and Tl(III) significantly increased TG. Further experiments are needed to assess if such alterations in lipid metabolism are involved in Tl-mediated damage. This work was supported by grants of FONCyT (PICT 2013-1132) and University of Buenos Aires (20020130100195BA).
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LI-P15
EVALUATION OF THE ANTIOXIDANT EFFECTS AND POTENTIAL ANTIATHEROGENIC PROPERTIES OF MONOTERPENES

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Monoterpenes (MTs) present in essential oils of citric fruits and aromatic plants exert an antioxidant effect that could diminish the cellular oxidative stress and prevent plasma LDL oxidation thus reducing atherogenic risk. D-limonene (L) is one of the most frequent MTs in nature and the majority constituent of mandarin (*Citrus reticulata*) essential oil (MEO). Our objective was to study the impact of L and MEO on the redox balance in tumor (A549) and normal (rat cardiomyocytes) cell cultures, and to analyze its capacity to prevent human plasma LDL oxidation. The effect of L and MEO (10-250 μl/L) on lipid peroxidation in A549 and LDL was evaluated (in presence or absence of $CuSO_4$) through the colorimetric quantification of TBARS. Also it was determined the activities of dismutase superoxide, catalase, and glutathione S- transferase. In cardiomyocytes treated with L (2-647 μl/L) incubated in conditions of normoxia, and hypoxia and reoxygenation, the reactive oxygen species (ROS) were determined through fluorescence. Cell viability was evaluated by the MTT assay. It was demonstrated that L and MEO have an antioxidant effect

because lipid peroxidation and ROS levels diminish and the activity of antioxidant enzymes increases. Our results show that MTs induce mechanisms that contribute to minimize oxidative processes associated to pathogenesis of several diseases such as atherosclerosis.

LI-P16
PHYTOSTEROLS FROM *Haematococcus pluvialis*: POTENTIAL ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES

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Haematococcus pluvialis is an oleaginous microalga proposed as a source of triacylglycerol (TAG) for biodiesel production. The aim of this work was to describe the effect of light-induced stress on lipid accumulation in a native strain. For this end, *H. pluvialis* CCALA 1081 from Bahía Blanca (Arg.) was grown under control conditions or light-induced stress (300 µmol $m^{-2}s^{-1}$) for 3, 6 and 12 days. Then, the lipid profile was analyzed in order to determine TAG and phytosterol (PHS) contents, the fatty acid profile of TAG and the composition of the PHS fraction. After 3 days of light-induced stress, TAG and PHS contents significantly increased with respect to control (210% and 1251%). Palmitic, oleic, linoleic and α linolenic fatty acids were increased whereas caproic acid content diminished in *H. pluvialis* under stress. Light-induced stress also reduced β-sitosterol content while clerosterol, brassicasterol and Δ7-campesterol augmented in the PHS fraction. No significant levels of lipid peroxidation were detected after 3 days of light-induced stress. Finally, PHS fraction displayed increased antioxidant capacity and, also, antiproliferative effect in human neuroblastoma cells. Our results show that light stress induces PHS accumulation in *H. pluvialis* with potential application as antioxidant and antiproliferative agents.

LI-P17
PIP_2SIGNALING: A TWO-BRANCHED PRO-LIFE RESPONSE AGAINST OLIGOMERIC AMYLOID-β PEPTIDE

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We have previously demonstrated the ability of Aβ peptide to trigger local synaptic signaling. In this work, we characterized hippocampal neuronal response to nanomolar concentrations to Aβ oligomers. Even though lipid peroxidation and mitochondrial dysfunction were not detectable in Aβ-treated neurons, the activation of phosphoinositide-dependent cellular signaling was observed. Akt and ERK1/2 showed to be activated in a phosphatidylinositol 3-kinase (PI3K)-dependent manner. Moreover, Akt was found to translocate from the cytoplasm to the nucleus whereas FoxO3a and phospho-ERK1/2 were found to move in the opposite direction. In the presence of Aβ, PKC underwent phosphorylation and maturation, both being required for its activation. PKC priming process showed to be abolished by a PIP2-PLC inhibitor and neomycin (a phosphoinositide chelator). On the other hand, cell viability was found to be finely tuned by phosphoinositide availability in the presence of Aβ, since both chelation of phosphoinositides and inhibition of PIP2 hydrolysis diminished mitochondrial function. In summary, our results show a key role for a two-branched pro-life PIP2-dependent signaling pathway with consequences in mitochondrial function and cell survival: i) the conversion of PIP2 to PIP3 with FoxO3a inactivation, ii) the hydrolysis of PIP2 which triggers a multi-step signaling involving ERK1/2 activation.

LI-P18
STUDY OF ENTEROCYTE FATTY ACID BINDING PROTEINS SPECIFIC FUNCTIONS IN CACO-2 CELL MODEL

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Two isoforms of Fatty Acid Binding Proteins (FABPs) are abundantly expressed within intestinal epithelial cells: liver FABP (LFABP) and intestinal FABP (IFABP). They are associated with intracellular dietary lipid transport and trafficking towards diverse cell fates. But still their functions are not well understood. By means of Caco-2 intestinal cell model, we addressed the matter following two pathways: protein interaction and FABP ablation. Firstly, intestinal FABPs interaction study revealed two candidates for LFABP: HSP60 and calreticulin, by Far Western Blot-MS. IFABP interaction with PPARγ was found by Co-immunoprecipitation. Currently, our efforts are focused on

recombinant PPARγ purification to analyze the interaction with IFABP *in vitro* and studying changes in the expression of PPARγ downstream genes *in cultivo*, using inhibitors and activators of FABPs and PPARγ, alternatively. Secondly, LFABP ablated Caco-2 cells by mRNA antisense strategy showed interesting modifications in lipid uptake, distribution and secretion. LFABP expression was reduced up to 85% in different cell populations and no compensatory increase in IFABP was observed. Nowadays, we are studying the role of LFABP on membrane phospholipid synthesis and β-oxidation. In summary, our work improves existing knowledge of FABPs functions, enforcing the idea of differential roles for I and LFABP in these cells.

LI-P19
STARD7 DEPLETIONINDUCES ENDOPLASMIC RETICULUM STRESS (ER) AND GOLGI APPARATUS FRAGMENTATION

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Lipid and protein transport between organelles is an essential process in the organization of the different cell compartments. StarD7 is an intracellular lipid transport protein, member of the START domain superfamily, which is involved in many physiological processes such as lipid transfer, metabolism, and modulation of signaling pathways. StarD7facilitates the delivery of phosphatidylcholine to the mitochondria and previous results indicated that StarD7 silencing decreased ABCG2 multidrug transporter level, cell migration, proliferation, and phospholipid synthesis. Also, StarD7 silencing produced an increase in basal ROS as well as in H_2O_2-induced ROS levels. Here, we report that HepG2 cells transfected with StarD7 siRNA during 72 h and analyzed by transmission electron microscopy showed altered mitochondria and ER morphology. These changes were accompanied with an ER stress response measured by augmented expression levels of inositol-requiring enzyme 1 (IRE1α), calnexin, glucose regulated protein 78/immunoglobulin heavy chain-binding protein (Grp78/BiP), and protein kinase-like ER kinase (PERK). In addition, immunofluorescence assay revealed that StarD7 knockdown induced Golgi apparatus fragmentation, labeled by anti-Giantin. In summary, our results provide novel evidence for an important role of StarD7 in maintaining cell homeostasis. Supported by FONCyT, CONICET, SECyT-UNC.

LI-P20
CADMIUN ALTERS THE LIPID METABOLISM IN RAT MAMMARY GLAND. EFFECT OF SOY PROTEIN

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We studied the effect of different diets on the lipid profile of rat mammary gland (MG). We also looked at the potential protective outcome of those diets under exposure to Cadmiun (Cd), which is a toxic element and an important environmental contaminant.4 female lots Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control) and the other, 15 ppm of Cd in the drinking water for 60 days. Lipids were extracted by Hexano:Isopropanol:BHT(3v:2v:1%.) solvent. Total cholesterol (TC) and phospholipids (PL) were determined by colorimetric assay. Fatty acids (FA) were subjected to gas chromatography-mass spectrometry. HMG CoA Reductase (HMGCoAR) protein expression was determined by Western blot. Interestingly, our results show that Soy diet decreases TC and tend to increase PL in relation to Cas. Cd does not influence the level of TC and PL in either group. HMGCoAR expression is decreased by Cd in CAS fed rats as well as on both group of Soy diet. Regarding to the FA composition we observed that Cd decreases the amount of 18:2 y 18:3n3 in CAS group, which may suggest that Soy prevents the effect of Cd. Simultaneously, Soy decreases 16:0 compared to Cas. These findings indicate that Cd exposure alters the composition of lipids in MG and Soy protein diet may regulate these effects.

LI-P21
MODULATION OF CHOLESTEROL SYNTHESIS IN THE HIPPOCAMPUS BY CADMIUM AND SOYBEAN-DIETARY PROTEINS

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Cadmium (Cd) neurotoxicity may be related to its effects on lipid metabolism. Soybean-dietary proteins have a number of health-beneficial effects due to their antioxidant properties. Herein, we aimed at testing whether Cd in the drinking water can modify lipid metabolism in the hippocampus (Hp), and if so, whether this effect can be modulated

by dietary soybean-proteins. We fed 4 groups of female Wistar-rats for 60 days as follows: casein-based diet + tap water (CBD); CBD + tap water with 15 ppm Cd (CDB-Cd); soybean-based diet + tap water (SBD); and SBD + tap water with 15 ppm Cd (SBD-Cd). In relation to the CBD group, the CBD-Cd group showed decreased body-weight gain, increased systolic-blood pressure, decreased diastolic-blood pressure, increased triglycerides and total cholesterol—increased LDL-c but decreased HDL-c. These parameters were not affected in the SBD-Cd group. Previously, we show that in the Hp, Cd increases the content of pro-inflammatory fatty acids (C18:00, C20:4, C22:5(n-3)) in both diets (CBD and SBD). Herein we show that with respect to CBD, Cd increased fatty-acid synthase and DGAT-2, but no changed HMG-CoA reductase mRNAs (RT-PCR). Whereas, SBD reduced cholesterol content and also HMG-CoA reductase mRNAs in Hp. Our findings suggest that Cd modifies cholesterol synthesis in the Hp, and that these effects are prevented by soybean-dietary proteins.

LI-P22
DOES TUBULIN REGULATE PHOSPHOLIPIDS ASYMMETRY IN THE PLASMA MEMBRANE OF MAMMALIANS CELLS?

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Phospholipids asymmetry in the plasma membrane is kept by lipid flippases and scramblases. The flippases are P4-ATPases that transport phosphatidylethanolamine and phosphatidylserine (PS) from the extra to the intracellular leaflet. While PS exposition is an apoptotic signal in mammalian cells and is a key event in erythrocytes and platelets during clot formation, this lipid in the inner leaflet regulates the recruitment of polarity factors and cytoskeletal components. Erythrocytes from hypertensive patients have increased tubulin in the plasma membrane, what results in intracellular Na+ accumulation (because tubulin inhibit de Na+/K+ pump) and gives less deformable erythrocytes, one of the causes of arterial hypertension. As tubulin regulates several P-ATPases, in this work we investigated the possible regulation of flippases by tubulin. Here, we developed a method to assay flippase activity in vitro. Our results show that: i- rat brain tubulin inhibits flippase activity in human ghost erythrocytes, and ii- the induction of tubulin association to the plasma membrane by carbachol in COS cells was correlated with a decrease of flippase activity, similar results were observed when the cells were treated with nocodazole. These results suggest that tubulin would regulate the flippase activity in these cells and propose tubulin as one of the first described flippase regulators.

LI-P23
ROLE OF LIPOVITELLINS IN THE EMBRYO DEVELOPMENT OF THE SPIDER *Schizocosa malitiosa*

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In oviparous species, the only source of nutrients is the egg vitellus. In arthropods vitellus, it is usual to find carbohydrates, protein, and lipid which are being consumed throughout the development. These macromolecules can be found freely or as lipovitellins. In the present work, we studied the contribution of the lipovitellins as energetic resource and their consumption throughout the three stages of development utilizing the spider *Schizocosa malitiosa* as a model of study. From this analysis we could observe that the proteins of the lipovitellins remained constant throughout the three stages, representing in all the cases 20% of the proteins of the total homogenate of eggs. With respect to the lipid content it was observed that in early stages of development the lipids associated to lipovitellins represent approximately 25% of the lipids of total homogenate, whereas in advanced stages only 3% of the lipids of total homogenate correspond to lipovitellins. This decrease is mainly due to the consumption of TAG and PL that diminished 90% between 5 and 25 days of development, possibly to be used as energy source and for membrane formation of the embryo under formation. These basic results are necessary in order to understand the structure/function relationship of the macromolecules involved in the reproduction and development of such a numerous Order as that of Araneae.

Microbiology

MI-P01
ANTIVIRAL ACTIVITY OF ANDEAN POTATO POLYPHENOLIC EXTRACTS AGAINST HEPATITIS B VIRUS

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Hepatitis B virus (HBV) causes acute and chronic liver infections. 400 million people are chronically infected worldwide. Natural polyphenols are a promising source of new antiviral compounds. The aim is to study the antiHBV activity of andean potato polyphenolic extracts (PPE). PPE were obtained from the andean variety CL658 and HepG2 2.2.15 cells (producing infectious HBV) were used to test antiviral activity. Cells were treated with different PPE concentrations, incubated for 6 d. The maximum non-cytotoxic concentration (MNCC) was determined by MTS assay, trypan blue (TB) and ethidium bromide/acridine orange (EB/AO) stainings. Changes in cellular morphology were studied by phase contrast and electron microscopy. HBsAg was assayed as a measure of viral replication. RESULTS: PPE showed cytotoxicity, dependent on concentration and incubation time. MNCC was determined at 50 μg/mL. The number of apoptotic cells increased at concentrations higher than MNCC. EPP treatment at MNCC did not changed cellular morphology at different incubation times. A dose and time dependent reduction in HBsAg levels was detected, suggesting an inhibition of the viral replication. Antiviral activity is also studied by HBeAg and HBV-DNA quantifications. Molecular mechanisms of action will be determined. CONCLUSION: PPE exert antiHBV activity and is a potential source of new drugs for the treatment of HBV.

MI-P02
TWIN ARGININE TRANSLOCATION IN *Mesorhizobium loti*: ROLE IN SYMBIOTIC PROCESS WITH LOTUS

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In rhizobia-legumes symbiosis atmospheric nitrogen is fixed in a process known as biological nitrogen fixation. This process is performed in specialized structures in legume roots and is fundamental for sustainable agricultural practices. Nodule formation is characterized by a complex exchange of signals between the plant and rhizobia; bacterial secretion systems are responsible for protein secretion to the media or to the host cell during the interaction. In this work we proposed to analyze the role of the TAT system of *Mesorhizobium loti* in the symbiosis with *Lotus* spp. We have constructed mutants of *M. loti* deficient in *tatC* gene (one of the translocases in TAT system). By SDS-PAGE analysis we have observed a major number of proteins in Δ*tatC* culture supernants, which could be related to a defect on cell membranes, as seen in SDS sensibility assays. *Lotus tenuis* plants inoculated with *tatC* mutant produced a major but smaller and colorless number of nodules in roots, and plants presented a little but significant difference in dry weight and height. These results suggest that TAT system is not essential (as in other rhizobia) but has an effect in symbiosis. In silico analysis using TatFIND and PredTAT programs identified a list of possible candidates to be secreted by TAT; some interest proteins are being analyzed for further characterization of this system

MI-P03
ELIMINATION OF *Pseudomonas aeruginosa* THROUGH EFFEROCYTOSIS

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Pseudomonas aeruginosa (PA) is an environmental bacterium that is capable of causing acute infections in individuals with wounds and chronic infections in Cystic Fibrosis (CF) patients. For opportunistic pathogens such as PA, the mucosal barrier represents a formidable challenge for colonization and bacterial-mediated damage. Infections develop only in patients with altered epithelial cell barriers. In CF patients the respiratory tract is characterized by the presence of hyper-viscous mucus, robust airway inflammation and accumulation of apoptotic cells. We have recently reported that PA interacts with polarized epithelial barrier adhering as aggregates at very localized spots of the apical surface. We demonstrated that aggregates are formed *in situ* and that they can be rapidly internalized into epithelial

cells in a Lyn-PI3K dependent manner.In this study we show those localized spots where PA aggregates correspond to sites of multi-cellular junctions formed by four or more cells. We further show that in those sites PA attaches to extruded apoptotic cells or cell bodies. PA is then internalized through engulfment of apoptotic cells (a process called "efferocytosis") by surrounding epithelial cells. Inside these cells bacteria inhabit, along with apoptotic cell debris, an efferocytic phagosome that acquires lysosomal features. PA is eliminated intracellularly upon 24 hours.

MI-P04
STUDY OF A PROTEASE INHIBITOR AND ITS ROLE IN ORAL *Brucella abortus* INFECTION

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Brucellosis is a world widespread zoonotic disease. Oral infection is one of the ways in which the disease is acquired. Previous results demonstrated that a purified recombinant *Brucella* spp. protein called U-Omp19 is able to inhibit *in vitro* the activity of main gastrointestinal proteases. In this work, we performed kinetic assays using trypsin-, α-chymotrypsin- and pancreatic elastase-specific substrates and demonstrated that U-Omp19 inhibits the three serin proteases by a mixed noncompetitive mechanism with Ki values in the μM range. To test if Omp19 in the *Brucella* membrane protects it against the bactericidal activity of the main gut proteases, an Omp19-deficient strain (Δomp19 Ba) and the complemented mutant (Δomp19-omp19 Ba) were generated. In contrast to WT Ba, Δomp19 Ba was susceptible to elastase bactericidal activity (P<0.001 vs WT Ba and Δomp19-omp19 Ba). Trypsin and α-chymotrypsin did not kill any Ba strain. Finally, BALB/c mice were intragastrically inoculated with $1x10^8$ CFU of either Δomp19 Ba or the parental WT strain. Twenty days later there was a significant reduction in the CFU in the spleens of Δomp19 Ba in comparison with WT-inoculated mice (P<0,0001 vs WT Ba). Altogether our results indicate that Omp19 plays a role in *Brucella*´s ability to withstand the harsh environment of the gastrointestinal tract and establish a chronic infection after oral infection.

MI-P05
IDENTIFICATION OF A PHOSPHATASE AS AN INTERACTING PARTNER OF THE *Brucella abortus* EFFECTOR BPE123

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Brucella abortus is an intracellular pathogen whose virulence depends on a type IV secretion system (VirB). This system translocates proteins into the host cell to modulate the intracellular fate of the bacterium in order to establish a secure niche where it actively replicates. BPE123 (BAB2_0123) is a protein of 17 kDa highly conserved in all sequenced *Brucella* species, and it is translocated into the host cell cytoplasm in a VirB-dependent manner. In order to identify proteins that interact with BPE123, that may constitute a VirB substrates or proteins required for the translocation process, we have performed a bacterial two hybrid screen. We demonstrate that BPE123 interacts with a phosphatase named SerB (BAB1_1410), classified as a member of the phosphoserine phosphatase subgroup of the HAD (haloacid dehalogenase) family of hydrolases. These results were further confirmed by co-immunoprecipitation experiments. SerB was expressed in *Escherichia coli* as a histidine-tagged fusion protein (His_SerB) and the purified protein exhibited a phosphatase activity towards p-nitrophenyl phosphate (Km= 1.53 mM). Optimal activity was observed at pH of 7, with a strong preference for Mg^{2+} over Mn^{2+}. His_SerB activity was inhibited by sodium pyrophosphate and EDTA. Further experiments are in progress to determine whether SerB is a VirB substrate itself and to analyze its role during infection.

MI-P06
Brucella abortus CYCLOPHILIN B IS TRANSLOCATED INTO HOST CELLS IN A T4SS–DEPENDENT MANNER

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Brucella is an intracellular bacterial pathogen that causes the worldwide zoonotic disease brucellosis. *Brucella* virulence relies on its ability to transition to an intracellular lifestyle within host cells. *Brucella abortus* has two cyclophilins, CypA and CypB. They were induced during *B. abortus* intracellular life and they play an important role in stress adaptation and virulence. Interestingly, while CypA shared homologies with cyclophilins of Gram-negative

bacteria, CypB has a primary protein structure characteristic of eukaryotic cyclophilins. The relation with pathogenesis coupled with the sequence similarity to eukaryotic cyclophilins is strongly suggestive of CypB being deployed by *B. abortus* as an effector that mimics the host cyclophilins. Interestingly, using the *Bordetella pertussis* adenylate cyclase fusion assay, we have determined that while *Brucella* CypB is translocated to host cell cytosol, CypA is not. In addition, CypB translocation was reduced in a *virB* mutant strain, concluding that CypB is translocated into host cells in a T4SS-dependent manner. We confirmed VirB-mediated translocation of CypB by immunofluorescence confocal microscopy. Moreover, CypB ectopically expressed in mammalian cells co-localized with cortical actin, suggesting a role for CypB in targeting and regulating actin cytoskeleton dynamics during *B. abortus* infection.

MI-P07
INVERSE REGULATION BETWEEN T3SS EXPRESSION AND SWIMMING MOTILITY IN *Mesorhizobium loti*

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Type three secretion system (T3SS) is a multi-protein bacterial complex that delivers effectors proteins into the host cell. In several rhizobia T3SS participates in the determination of nodulation competitiveness. *Mesorhizobium loti* MAFF303099 has a functional T3SS. Flavonoids together with the transcriptional factor NodD induce the expression of rhizobial T3SS components. We have observed that in the inducing condition of rhizobial T3SS, an inhibition of bacterial swimming motility in soft-agar (0.2%) occurs. Analysis of this inhibition was made also with two T3SS *Mesorhizobium* mutants. RhcN mutant, affected in the ATPase system, presents T3SS complex at membranes but is unable to secrete pili components and effectors. The other mutant strain, *y4yS*, is affected in the assembly of the T3SS complex. Motility experiments showed that *y4yS* mutation have reverted the inhibition phenotype in motility, however *rhcN* mutation does not altered the wild type phenotype. T3SS inducing conditions result in minor levels of flagellin protein and lower expression levels for the transcriptional factor VisN (global regulator of flagellar genes in rhizobia). The results indicate that *M. loti* T3SS complex formation at membranes negatively regulates the swimming motility in soft-agar at a transcriptional level. It remains to be determined the molecular mechanism involved in this regulation.

MI-P08
THE RV2577 GENE OF *Mycobacterium tuberculosis* IS A VIRULENCE FACTOR IN TUBERCULOSIS PATHOGENESIS

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Mycobacterium tuberculosis is the causative agent of Tuberculosis. The outcome of the host-pathogen interaction depends on the bacterium's virulence factors and host immune system. Our group has studied a large number of mycobacterial virulence factors among them the *Rv2577* gene. This gene is up-regulated in a *M. bovis* hyper virulent strain and also, an Rv2577 mutant strain presented an attenuated phenotype of virulence. In this study we continue the characterization of *Rv2577* gene and its relevance in virulence. For this end, we analyzed the intracellular trafficking of *M. tuberculosis* wild type (wt), an Rv2577 mutant and a complemented strain by confocal microscopy. We observed the mutant co-localized with the LAMP2 protein, a marker of late phagosome. The wt phenotype was completely restored in the complemented strain. An *in silico* analysis demonstrated Rv2577 has a Tat signal sequence, suggesting its translocation out the cell. In fact, a subcellular screening by western blot performed with a polyclonal anti-Rv2577 made in this study, shown Rv2577 is localized in the cell wall fraction. Also, Rv2577 has a metallophosphoesterase domain, suggesting a phosphatase activity, yet undetermined. The results suggest Rv2577 is involved in phagosome maturation arrest, possibly by an intimal interaction with phagosomal proteins, suggesting a relevant role in the virulence of *M. tuberculosis*.

MI-P09
PATHOGEN EXCLUSION AND IMMUNOMODULATORY EFFECT OF S-LAYER PROTEIN OF *Lactobacillus acidophilus*

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Lactobacilli are found in human intestine and some strains are considered probiotic. Surface layer (S-layer) protein constitute in some species their outermost cell envelope and have been considered to be involved in modulating the host immune system and inhibit pathogenic bacteria adhesion which has recently gained attention as a way to prevent infection. Here, we investigate *L.acidophilus* ATCC4356 adherence to epithelial cell lines and ability to interfere with *Pseudomonas aeruginosa* by plate counts. The S-layer modulation of cytokines production in the murine macrophage cell line J774A.1 and Caco-2 cells was also studied by ELISA and qRT-PCR.Interaction with mucins, immobilized on a PVDF membrane was assayed.*Lactobacilli* showed lower adherence to lung A549 cells than intestinal Caco-2 cells ranging from 0.5 to 10% depending on the number of input bacteria and day-old Caco-2 monolayers. We found that S-layer protein was also able to produce pathogen exclusion against *Pseudomonas* when added purified; however, live lactobacilli with or without S-layer did not. Moreover, in macrophages co-incubated for 24 h with purified S-layer, IL-6 and TNF-α secretion was significantly increased. Likewise, purified S-layer led to an elevated expression peak of TNF-α; at 4h of incubation in Caco-2 cells. Interaction with mucins was detected with both protein and cells.

MI-P10
A GENOMIC GLANCE AT THE CHEMOSENSORY SYSTEMS OF A POLYAROMATIC-HYDROCARBON CHEMOTACTIC STRAIN

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Halomonas sp. KHS3 is a hydrocarbon-degrading microorganism isolated from seawater of Mar del Plata harbor. This strain is able to grow on aromatic hydrocarbons and shows chemotactic responses to these compounds. The whole genomic sequence of this strain was recently obtained (Gasperotti et al., 2015). In order to identify the proteins involved in the detection of hydrocarbons, the chemosensory systems present in the genome of *Halomonas sp.* KHS3 were analyzed. Based on the preliminary annotation of the genome in the RAST server, we found 24 chemoreceptors (MCPs), and two different copies each of genes coding for chemotaxis proteins. These genes were found to be organized in two clusters: one showing a gene organization similar to that described for the well-known *E. coli* chemotaxis cluster and the other showing a different organization. This second cluster includes an MCP of different class than the other MCP genes and a diguanylate cyclase with a receiver domain, suggesting involvement of this system in c-di-GMP levels control. A comparative study between 49 *Halomonas* available genomes showed that half of them have similar organization of chemosensory clusters. The predicted ligand-binding domains of the 24 MCPs were subjected to structural modeling and their genomic context was analyzed, in order to get information about their likely participation in hydrocarbon detection.

MI-P11
CLONING, EXPRESSION AND CHARACTERIZATION OF TWO CHEMORECEPTORS FROM *Halomonas* sp. KHS3

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Halomonas sp KHS3 was isolated from a hydrocarbon contaminated area from Mar del Plata harbor. This strain is able to grow on aromatic hydrocarbons and exhibits chemotactic responses towards these compounds. The analysis of its whole genomic sequence showed the presence of twenty-four chemoreceptors (MCPs), including at least one that is presumably involved in the specific responses to hydrocarbons. Based on their genomic context, two MCPs were chosen for cloning and expression in *E. coli* cells, named PEG_925 and PEG_4119. PEG_925 is located within a cluster of chemotaxis genes, and is possibly involved in general nutrient chemotaxis response, while PEG_4119 is found in a cluster containing genes of the salicylate pathway. Flagellar rotation assays in *E. coli* cells expressing PEG_925 or PEG_4119 indicated that they are able to activate and control the kinase CheA. Using the same assay, we explored possible ligands and found that PEG_925 responds to aspartate as an attractant. PEG_4119, on the other hand, responded to lactic acid as an attractant. Higher order organization was analyzed through subcelullar localization assays using YFP fusion proteins and crosslinking assays, indicating a proper interaction between these MCPs with other components of the chemotactic signaling complex of *E. coli*.

MI-P12
FERULOYL ESTERASE ACTIVITY IS INFLUENCED BY BILE, PROBIOTIC INTESTINAL ADHESION AND MILK COMPONENTS

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Feruloyl esterases (FE) are bacterial and mammalian enzymes that catalyze the hydrolysis of hydroxycinnamate esters releasing antioxidant hydroxycinnamic acids. Administration of *Lactobacillus fermentum* (*Lf*) CRL1446, FE-producing strain, increased intestinal FE activity and improved oxidative status in mice. The aim of this work was to evaluate the effects of bile, adhesion to intestinal epithelium and milk components on FE activity. Effect of bile was evaluated by incubation of cells in PBS+Oxgall. FE activity was determined in supernatants and cells by incubation in PBS containing 1 mM methyl ferulate. Released HA was quantified by HPLC. Adhesion was evaluated *ex vivo* by incubation of *Lf* suspensions (10^9 cfu/ml) with intestinal tissue fragments (ITF) and exfoliated intestinal epithelial cells (IEC). Effect of milk components on FE activity was assessed by incubation of ITF (with/without adhered *Lf*) in presence of milk fat and calcium salts. Incubation with bile increased FE activity in whole cells and supernatants (2–fold), compared to controls. *Lf* was able to adhere to ITF (10^6 cfu/mm^2), and when evaluated in IEC, a 17% adhesion index was observed. Higher activities were detected in ITF with adhered bacteria and incubated in presence of milk fat and calcium salts. These results support the potential use of *L. fermentum* CRL1446 for the development of new functional foods.

MI-P13
LAPA IS A NOVEL ADHESIN INVOLVED IN THE DEVELOPMENT OF BIOFILM IN *Bordetella bronchiseptica*

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Bordetella bronchiseptica (*Bb*) is a gram negative pathogen which causes respiratory infections in a broad range of mammals. It naturally infects and colonizes upper respiratory tract for their lifetime. It is known that biofilm formation ability is an essential feature for the establishment of those infections. We have previously demonstrated that a c-di-GMP (cdG) is involved in biofilm formation. However, protein factors involved remains to be revealed.We identified three candidates that could be part of the biofilm control mechanism in a cdG-dependent manner. Those proteins were first identified on *Pseudomonas fluorescens* and named Lap (large adhesion proteins). We previously described that LapG overexpression reduces biofilm formation on two different backgrounds (RB50 and 973 strains). In this work we are reporting that Lap´s genes are transcribed from the same operon. We also engineered *Bb* knocked-out mutants on LapG and LapA. Accordingly with our proposed model Δ*lapG* shows enhanced biofilm formation and Δ*lapA* lower biofilm capability on crystal violet microplate dishes assays and over glass surfaces. This is to our knowledge the first full description of a Lap system in a pathogenic bacteria. We showed that Lap proteins are involved in biofilm formation, a phenotype essential for pathogenesis of *Bordetella*. Further research is ongoing to elucidate Lap proteins role *in vivo*

MI-P14
A MANNOSE-BINDING LECTIN DISPLAYS *Candida* GROWTH INHIBITION AND ADHESION REDUCTION TO HOST CELLS

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Candida cell wall consists of a skeleton of glucans and chitin, with an outer layer enriched in mannoproteins. Lectins can show antimicrobial abilities through their interaction with glycoconjugates on pathogen surfaces. We have described a sunflower mannose-binding lectin (Helja), which inhibits the growth of *C. parapsilosis*. The aim of this work was to assess the Helja effect on *C. guillermondii* (*Cg*) growth and its adhesion to host cells, and to explore the interaction of the lectin with the outer *N*- and *O*-mannoproteins, using yeast mutants with specific mannosylation defects. Helja (100 µg/mL) was able to reduce by 75 % the growth of the wt strain. To determine the contribution of the fungal mannosylation on Helja effect, we used a *Cg pmr1*Δ null mutant, which displays both *O*- and *N*-mannan severely truncated. This strain showed similar growth inhibition in the presence of Helja than wt yeast. The lectin was also tested for its effect on *Cg* adhesion to buccal epithelial cells, detecting a significant binding reduction of wt strain to these cells. However, the *pmr1*Δ mutant showed similar levels of adhesion both in the presence and absence of

Helja. Thus, the nature and extent of *Cg* mannosylation appear to be essential for Helja interference on fungal adhesion to host cells, but the mannan defect assessed is not sufficient to bypass the growth inhibition displayed by this lectin.

MI-P15
Clostridum septicum AND *Clostridium chauvoei* INDUCE DIFFERENT TLRS EXPRESSION

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Toll-like receptors (TLRs) are the best characterized innate immune receptors. Upon ligand binding, TLRs activate a signaling pathway that enhances expression of immune factors such as cytokines, provoking inflammation and communication with the adaptive branch of immunity. *Clostridium chauvoei* and *Clostridium septicum* are Gram positive anaerobic flagellated pathogens, with 99% genetic similarity but different immunity. Both pathogens are causative agents of myonecrosis, which is a deadly disease affecting cattle, man and many warm-blooded animals. The aim of this work was determine TLRs expression induced by *C. chauvoei* and *C. septicum* in peritoneal mouse macrophage. Macrophage (1 x 10^6 cell/well) were co-cultured with *C. chauvoei* and *C. septicum* cells obtained in log phase culture at different multiplicity of infection (MOI) for 4, 12 and 24h. RNA was reverse transcribed into cDNA and used for RNA amplification with specific primers, using RT-PCR. The bands were analyzed by Scion image software. The results showed that *C. chauvoei* cells induces 1.4-2 times increase of TLR 5 expression early in time, even at low MOI; while *C. septicum* induced TLR 2 expression, 2 times higher than that of control at 4 h post-infection. In conclusion, specific TLR2 would be singled out as a dominant receptor for *C. septicum* and TLR5 as a dominant receptor for *C. chauvoei*.

MI-P16
INFLAMMATORY RESPONSE TO *Clostridium chauvoei* INFECTION. TLRS AND INTERLEUKINS EXPRESSION.

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Clostridium chauvoei is the causative agent of blackleg, a deadly infection that affects cattle and sheep worldwide. The symptoms are generalized fever and inflammation. Activation of Toll-like receptors (TLRs) by bacterial antigens induces an inflammatory response in which several pro-inflammatory and anti-inflammatory interleukins are involved. The aim of this study was to determine the dominant TLRs, activated when cells encounter *C. chauvoei* cells, and the associated expression of pro-inflammatory (IL1β, IL6 and TNFα) and anti-inflammatory (IL4 and IL10) interleukins in mouse peritoneal macrophages. The expression of TLRs and interleukins was analyzed by semi quantitative RT-PCR. The results showed a significant increase in expression of TLR5 at 4 and 10h post-infection, a 10 times increase of TNFα and 3 times increase of IL1β and IL6 expression at 24h. IL4 increased 5 times compared to the control at 10h. Conversely, the expression of IL10 decreased significantly ($p < 0.05$). The results indicate that infection with *C. chauvoei* induces the expression of TLR5 (which recognizes flagellin, the main protective antigen) and pro-inflammatory interleukins, also inhibits the response of IL-10, which would explain the acute inflammation observed in the blackleg disease.The early expression of IL-4 would be associated with the induction of a characteristic Th2 type humoral response.

MI-P17
LEISHMANIASIS: A BIOINFORMATIC APPROACH FOR NOVEL ANTIGENIC PROTEINS

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Leishmanisis comprises a group of parasitic diseases caused by the flagellated protozoan from genus *Leishmania*. Nowadays there are not effective vaccines or diagnosis methods against these diseases, thus it is necessary to search for new parasite antigens that are able to generate the adequate immune response. Bioinformatic programs for antigenic prediction are promising tools to facilitate this search. In this study we analyzed two *L. braziliensis* proteins (A and B) using epitope prediction programs for lymphocyte B (LB), T (LT) and MHC from Immune Epitope Database (IEDB). The analysis demonstrated that A and B bear available epitopes able to be recognized by LB and LT-MHC. Protein A showed a higher epitope number and antigenicity for LT rather than for LB, and less IgG inductive epitopes in respect to protein B, pointing a potential cell response. Meanwhile, B exhibited epitopes related

to the contrary effect, with potential to lead a humoral response, which would involve a Th2 path. These data corresponded to the empirical results observed for both proteins. Regarding these results we conclude that epitope prediction software may be very useful for the pre-selection of novel antigenic proteins against leishmaniasis and other diseases.

MI-P18
PROTEOMIC ANALYSIS OF *Bordetella bronchiseptica* WITH HIGH INTRACELLULAR C-DI-GMP CONCENTRATION

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Bordetella bronchiseptica is a bacterium that causes respiratory infections in a variety of hosts. We have already described second messenger c-di-GMP (cdG) role in motility and biofilm formation through both overexpression and deletion of the diguanilate cyclase BdcA. We previously showed that over expressing BdcA triggers high c-di-GMP levels. Hence, proteins positively regulated by cdG were expected to be overrepresented in BdcA over expressing strain (Bb-BdcA). We performed quantitative comparative proteomic to find proteins involved in cdG control of known and possibly new phenotypes. Cytosolic fractions of Bb-BdcA were compared to wild type (WT). More than 850 proteins were identified by this method. Among them a group of 13 proteins were only present in Bb-BdcA. Interestingly, expression of a putative RNA-binding regulatory protein, Hfq, is strongly enhanced by BdcA, suggesting interplay between these regulatory networks. Only three proteins were found to be absent in Bb-BdcA and present in WT. One of them is a putative hidrolase previously described by us and regulated by BvgR, a *Bordetella* response regulator. This work presented the quadrupole Orbitrap mass spectrometer technique to analyze bacterial proteomes. Many proteins showed significantly differences expression profiles, encouraging us to elucidate their role in cdG regulon and *Bordetella* pathogenesis.

MI- P19
CHARACTERIZATION OF THE CDF TRANSPORTERS IN *Pseudomonas aeruginosa*: NEW ROLES IN PROTEIN ASSEMBLY

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Pseudomonas aeruginosa is an opportunistic pathogen prevalent in cystic fibrosis patients. Transition metals (TM) (Zn^{2+}, Fe^{2+}, Cu^{+}, etc.) are essential protein co-factors. Their levels in bacteria are controlled by TM-responsive transcription factors, TM-chaperones, and TM-transporters. Transporters of the cation diffusion facilitator (CDF) family form dimers that export TM from the cytosol. *P. aeruginosa* presents three homologous CDF genes, PA0397 (PaCzcD), PA1297 (named here CotP) and PA3963 (PaYiiP). In inhibition halo experiments, ΔPaCzcD and ΔPaYiiP deletion strains showed a lower sensitivity to Zn^{2+}. However, in iron-rich medium and in the presence of Zn^{2+} the strains secreted higher levels of the TM chelator pyoverdine. The non-pyoverdin producer double mutants, Δ*pvdA*-ΔPaCzcD and Δ*pvdA*-ΔPaYiiP, displayed increased Zn^{2+} and Ni^{2+} sensitivity compared to the single mutant Δ*pvdA*. In a plant infection model only ΔPaYiiP and ΔPaCzcD had a reduced fitness. Most importantly, a 4-fold change in sensitivity to imipenem was observed in the strains ΔPaCzcD and ΔPaYiiP. Distinct from PaCzcD and PaYiiP, CotP appears to have a role in Co^{2+} homeostasis as the mutant strain is more sensitive and accumulate this ion. We hypothesize that PaCzcD and PaYiiP function as a heterodimer exporting Zn^{2+} to the periplasm for metallo-β-lactamase assembly, while CotP is a Co^{2+} efflux transporter.

MI- P20
A SIMPLIFIED CULTURE MEDIUM FOR AN ANTIINFLAMMATORY SOLUBLE FACTOR PRODUCTION BY *Lactobacillus. reuteri* CRL 1098

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Some specific soluble factors secreted by probiotic lactic acid bacteria are able to modulate the immune response. Our previous studies demonstrated that a *Lactobacillus reuteri* CRL 1098 soluble factor identified as a 5785 Da peptide, present in the supernatant (LrS), modulates the inflammatory response triggered by LPS *in vitro* and in a mice model. LrS was produced under stress conditions by starved cells after 4h incubation in RPMI 1640 medium, where the bacteria maintained alive but did not proliferate. The aim of this work was to improve LrS production. Two

steps will be performed: 1) Design of a simplified and low cost medium for optimal LrS production. Casitone medium, PBS, TRIS, citrate buffer with and without glucose were assayed. Bacteria survival was evaluated by cell counting in MRS agar. LrS cytotoxicity was determined by Trypan blue and MTT assay. *L. reuteri* only maintained alive in casitone medium and citrate buffer with 4 g/l glucose. These LrS diminished TNF–α and nitric oxide production in both mouse peritoneal macrophages and RAW 264.7 cells stimulated with LPS. 2) Optimization of medium components (MRS) for *L. reuteri* biomass production, using a factorial statistical experimental model, is in progress. Application of an economic and more competitive medium for LrS production by *L. reuteri* CRL 1098 represents an important step for its enforcement at pilot scale.

MI-P21
REGULATION OF LPXO1 AND LPXO2 AND ITS INVOLVEMENT IN POLYMYXIN B RESISTANCE IN *Serratia marcescens*

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The lipopolysaccharide (LPS) lipid A is recognized by the innate system. The gram-negative bacteria modify the LPS to avoid host immune system and to resist killing by antimicrobial peptide. *S. marcescens* is an enteric bacterium that can function as an opportunistic pathogen within immunocompromised hosts. This pathogenic bacterium contains two putative *lpxO* genes homologues (*lpxO1* and *lpxO2*). LpxO is an oxygenase that 2-hydroxylates specific acyl chains in the lipid A. In this work, we analyzed the regulation of *lpxO1* and *lpxO2* and its role in antimicrobial peptide resistance. We constructed *lpxO1* and *lpxO2* mutants in *S. marcescens* and determined their resistance to polymyxin B. The results show that LpxO1 is necessary for polymyxin B resistance, while LpxO2 is dispensable. In addition, *lpxO1* mutant exhibited less swarming motility, suggesting that LpxO1 modification plays a role in swarming. In many bacterial species, modifications in the LPS that confer resistance to antimicrobial peptides are regulated by the PhoP/PhoQ system. Furthermore, in *Klebsiella pneumoniae* RamA, a transcriptional regulator, functions as an alternate regulator of certain lipid A biosynthesis genes. The contribution of PhoP and RamA in the expression level of *lpxO1* and *lpxO2* was analyzed by RT-PCR. Our results show that, in *Serratia*, PhoP and RamA are involved in *lpxO1* and *lpxO2* transcriptional regulation.

MI-P22
REGULATION OF *NarZ* GENE EXPRESSION BY RcsCDB/RstAB SYSTEMS IN *Salmonella typhimurium*

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In order to survive at the environmental changes *Salmonella* Typhimurium is able to utilize specialized mechanisms to control the gene regulation. The RcsCDB system responds to stress conditions and it is involved in the controls of *Salmonella* virulence. The *narZ* gene encodes a nitrate reductase and its expression is required to support carbon source starvation and anaerobic growth in *Salmonella typhimurium*. The aim of this work was investigated the mechanism implicated in the control of the *narZ* gene transcription mediated by RcsCDB and its effect in the intestinal lumen establishment. Our results showed that the RcsCDB system controls negatively the *narZ* transcription. The bioinformatic and gel shift analysis demonstrated that the *narZ* gene promoter region contains an RcsB-binding site and that this regulator exerts their effect by directly binding to this conserved box. In addition, was reported that the RstAB system down regulated the *narZ* expression. According this, we also investigated a putative interaction between the RstAB and RcsCDB systems in the control of the *narZ* gene transcription, under the bacterial growth in carbon source starvation and anaerobic conditions. In this study we described that the *narZ* gene expression is modulated by a mechanism in which RcsB and RstA compete by the control of the gene transcription.

MI-P23
THE CbrAB/Crc SYSTEM CONTROLS THE BACTERIOCIN PRODUCTION IN THE PGPR *Pseudomonas fluorescens* SF39A

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P. fluorescens SF39a secretes a bacteriocin that inhibits the growth of phytopathogenic strains. The knowledge about the regulation of this bacteriocin is relevant because this compound could be applied as biocontrol agents. The aim of this work was to study the role of the CbrAB/Crc system in the bacteriocin regulation in the strain SF39a. CbrAB/Crc pathway is composed by the CbrAB two component-system, the sRNA CrcZ and the protein Crc. Crc acts as a translational repressor by binding to target mRNAs. When the CbrAB is activated, CbrB activates the transcription of *crcZ* and this sRNA counteracts Crc function by sequestration of the protein. In this study, mutants of SF39a in the *cbrA*, *cbrB*, *crcZ* and *crc* genes were obtained. CbrA, CbrB and CrcZ mutants showed an increase in bacteriocin production, while Crc mutant displayed a decrease in bacteriocin production with respect to the wild-type. These results suggest that CbrAB/Crc pathway controls the bacteriocin production in strain SF39a. Probably, Crc could repress the translation of mRNAs encoding a repressor of the bacteriocin or directly activate bacteriocin genes. Moreover, the CbrA, CbrB, CrcZ and Crc mutants were less competitive than the wt strain in the wheat rhizosphere. In conclusion, the CbrAB/Crc system affects bacteriocin production and it is required for a successful establishment of the bacteria in the environment.

MI-P24
REGULATION OF THE TYPE VI SECRETION SYSTEM OF *Serratia marcescens*: THE ROLE OF THE Rcs SYSTEM

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Serratia marcescens is an opportunistic human pathogen that represents a growing problem for public health. It has been previously reported that the type VI secretion system (T6SS) of *S. marcescens* has a role in bacterial competition. This characteristic may provide an important competitive advantage against other bacteria in the host or in environmental niches. However, little is known about its regulation. In this work, we analyze the regulation of the T6SS of *S. marcescens*. We propose that this system is regulated by the Rcs system. We found that the *rcsB* mutant strain shows a lower antibacterial activity than the wild type strain. This observation is also supported by immunodetection assays, which shows lower levels of Hcp (a structural component of the T6SS) in the *rcsB* mutant. In addition, we have found a putative RcsB binding site in the promoter region of the cluster that codes for the components of T6SS. Using a fusion to GFP as a reporter of the transcriptional activity of that promoter, we have determined that the level of transcriptional activity in the *rcsB* mutant strain is lower than in the wild type strain. In agreement with this result, we have done real-time PCR assays and we have found that the level of transcript of T6SS is lower in the *rcsB* mutant. Together, our results show that, in *S. marcescens*, the Rcs system regulates the T6SS at the transcriptional level.

MI-P25
THE TWO-COMPONENT SYSTEM PrrBA FROM *Brucella melitensis* IS INVOLVED IN OXIDATIVE STRESS RESISTANCE

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Brucella is the causative agent of the zoonotic disease brucellosis, which is endemic in many countries around the world. The success of *Brucella* as pathogen resides in its ability to adapt to the harsh environmental conditions found in mammalian hosts, including oxidative and nitrosative stress, low pH and low oxygen tension. The two-component system PrrBA from *Brucella* is involved in redox sensing, in the adaptation to low oxygen tension and in the establishment of a chronic infection in mice. We have shown that a *prrB* mutant strain in *B. melitensis* is more sensitive to the oxidative stress caused by the reactive oxygen species O_2^- and H_2O_2 than the parental strain. Also, we have shown that *prrB* gene expression is induced under the presence of O_2^- and H_2O_2. Several enzymes have been described in *Brucella* that participate in the detoxification of reactive oxygen species, such as: the Cu-Zn and the Mn superoxide dismutases encoded by *sodC* and *sodA* genes, respectively; the catalase encoded by *katE* and the alkyl-hydroperoxide reductase encoded by the *ahpCD* operon. Performing qRT-PCR assays we have shown that the PrrBA system is involved in the induction of *sodA* and *ahpC* genes under oxidative stress. The results presented in this work

suggest that the PrrBA two-component system is involved in coping with the oxidative stress found inside the mammalian hosts during *Brucella* infection.

MI-P26
REGULATION OF LIPID BIOSYNTHESIS IN GRAM-POSITIVE BACTERIA

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A crucial but often overlooked aspect of cell biogenesis and survival is the need for a precise coordination between the biosynthesis of its macromolecular constituents. In the case of bacterial cells, this means producing appropriate amounts of four classes of macromolecules: nucleic acids, proteins, lipids and the polysaccharides of the cell wall. In many cases, the molecular basis of this coordination is still mysterious. The acetyl-CoA carboxylase (ACC) is a key enzyme of the fatty acid biosynthetic pathway as it catalyzes the formation of malonyl-CoA, a precursor of this metabolic route that has a fundamental regulatory role in an important group of Gram-positive bacteria. To date, a detailed analysis of the catalytic activity of ACC and its regulation in Gram-positive bacteria is still missing. In this work, we focused on determining the kinetics of the ACC enzyme of the model Gram-positive bacterium *Bacillus subtilis* and also investigated if the protein YqhY, that is encoded in the *accB-accC-yqhY* operon and belongs to the family of proteins without known function Asp23, is capable of modulating its activity. Our results contribute to the understanding of how this key enzyme of lipid biosynthesis works and its activity is controlled, shedding light on the coordination of lipid and membrane synthesis with the other biosynthetic activities in Gram-positive bacteria.

MI-P27
STUDY OF THE REGULATION OF OPERONS INVOLVED IN LIPID METABOLISM BY MCE3R

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The *mce* operons constitute four homologous regions in the *Mycobacterium tuberculosis* genome. Although the function of the Mce protein family has not been clearly established, its members are believed to be membrane lipid transporters. Based on microarray and qRT-PCR studies, in a previous work we found that the regulator of the *mce3* locus, Mce3R, negatively regulates the expression of the Rv1933c–Rv1935c and Rv1936–Rv1941 transcriptional units (Santangelo et al. 2009). These operons are adjacent to one another and divergently transcribed. Here we proposed to confirm the regulation of these operons by Mce3R using promoter-*lacZ* fusions in *M. tuberculosis*. To perform the β-galactosidase assay the Rv1933c–Rv1935c and Rv1936–Rv1941 promoter region (PechA) was fused in both orientations to the *lacZ* reporter gene in the expression vector pYub178 and the obtained constructions were used to transform *M. tuberculosis* H37Rv and a strain where the Mce3R regulator has been eliminated (Δmce3R). We found that the presence of Mce3R abolished the expression of the *lacZ* gene with one orientation of the promotor but not with the other, thus confirming the regulation of one of these operons by Mce3R (Rv1936-Rv1941). Likely, Mce3R needs additional cis-regulatory elements, not included in the PechA promotor region herein studied, to repress the Rv1933c–Rv1935c operon.

MI-P28
CHARACTERIZATION OF THE LONG-CHAIN ACYL-COA CARBOXYLASE OF *Mycobacterium tuberculosis*

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The two Fatty Acid Synthase (FAS) systems of the human pathogen *Mycobacterium tuberculosis* work in concert to synthesize mycolic acids. FAS I builds a long-chain acyl-CoA (C24-CoA) and FAS II generate a very long-chain fatty acid (meromycolic acid). A long-chain acyl-CoA carboxylase (LCC) activates the C24 acyl-CoA, and the acyl-AMP ligase FadD32 activates the meromycolic acid chain. These two chains are later condensed by Pks13 to yield the final mycolic acid. Besides its importance in mycolic acid biosynthesis, there were no conclusive results of the subunit composition of the acyl-CoA carboxylase responsible to generate the long-chain carboxyacyl-CoA. Previous results suggested that the subunits AccA3 and AccD4 were part of this LCC complex. Analysis of an *accD5-accE5* conditional mutant in *M. smegmatis* allowed us to speculate that the subunits AccD5 and AccE5, that are part a of well-characterized propionyl-CoA carboxylase, are also involved in the long-chain acyl-CoA carboxylation. To solve this issue, we developed an *in vitro* assay for measuring LCC activity in the presence of its purified subunits. By

using this assay we demonstrated that the LCC complex is formed the subunits AccA3, AccD4, AccD5 and AccE5. The specific role of AccD5 and AccE5, and the kinetic characterization of this enzyme complex are now under study.

MI-P29
Arachis hypogaea PGPR FROM ARGENTINE SOIL MODIFIES ITS LIPIDS COMPONENTS IN RESPONSE TO TEMPERATURE

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A Gram-negative, aerobic, motile by subpolar flagella, rod shaped bacterium was isolated from *Arachis hypogaea* rhizosphera from a Río Cuarto soil. L115 strain was identified as *Ochrobactrum intermedium* (16S rRNA gene analysis). Mechanism related to plant growth promotion were analyzed. L115 synthesize siderophores (Chrome Azurol S agar), produce indol acetic acid (Salkowski colorimetric method) and presented 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (medium supplemented with ACC). L115 increased the peanut shoot length and root dry weight (plant growth chamber inoculation test) indicating a PGPR role. Considering that the peanut seeding temperature range of soil varies from 16-18°C, we evaluated the effects of 18°C on bacterial growth in respect to 28°C. A decrease in viability was observed, although L115 was able to adapt. To understand the mechanisms of adaptation, after 24 h of incubation in medium YEM, total fatty acids (FA) were extracted and analyzed by GC. Phospholipids (PL) were separated by TLC after incorporating [1-^{14}C] sodium acetate to culture. At 18°C, it was observed an increase of 18:1Δ11 FA and a decrease of 16:0 and 18:0 FA. The most important change in PL was an increase in phosphatidylcholine and a decrease in phosphatidylethanolamine. The results suggest that L115 adapt to low grown temperature by modifying FA and PL membrane composition.

MI-P30
THE DIGUANILATE CYCLASE BdcA SENSE DIVALENT CATIONS IN *Bordetella bronchiseptica*

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Bordetella bronchiseptica is a pathogenic bacterium that causes respiratory infections in a variety of hosts. We have already described c-di-GMP second messenger role in motility and biofilm formation through both overexpression and deletion of the diguanilate cyclase BdcA (*Bordetella* diguanilate cyclase A).We observed that overexpression of BdcA significantly impaired movement and enhanced biofilm formation. C-di-GMP regulates multiple levels of flagellum-based motility. To address how c-di-GMP regulates motility in *Bordetella*, we performed semiquantitave western blot analysis of different mutant strains. We could detect that overexpression of BdcA not only reduced bacteria motility but also abolished flagellin expression. Interestingly we observed that PilZ, a c-di-GMP receptor that also modulates *Bordetella* motility, is not involved in BdcA mediated motility inhibition as described in other bacteria. Besides domain responsible of DGC activity, BdcA has a predicted calcium binding domain. We hypothesized that calcium or divalent cations would modulate BdcA activity. We analyzed different divalent cation effects on motility and biofilm formation. Our results strongly suggest that Ca^{+2} and Cu^{+2} stimulate BdcA activity that enhance biofilm formation. This result reports the first environmental signal that induces modifications in c-di-GMP network in *Bordetella*.

MI-P31
COMPENSATORY MUTATIONS IN C-DI-GMP PATHWAYS UNDERLIES *Pseudomonas aeruginosa* ADAPTATION TO BIOFILMS

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Pseudomonas aeruginosa is able to grow in communities known as biofilms. Small Colony Variants (SCV), one of the adapted morphotypes that arise from biofilm cultures, have been characterized as small size colonies formed by hyperadherent and highly biofilm producer cells. When SCV are grown on solid media, wild type-like morphotypes emerge at the edge of the colonies, which also show reversion of SCV phenotypic traits. In this work, we analyzed the adaptability of *P. aeruginosa* by carrying out a long-term assay of experimental evolution followed by whole-genome sequencing. Three lines of *P. aeruginosa* PA14 were subjected to alternating and successive cycles of biofilm growth (conversion) and growth in solid media (reversion). In each line, whole genomes of the ancestral and of the most evolved clones were sequenced. We observed the acquisition of one non-synonymous mutation per round

of evolution. Strikingly, most of these mutations were found in genes of the *wsp* and *yfi* operons, coding for chemosensory systems involved in biofilm formation through the modulation of c-di-GMP levels. So far, our results indicate that, in our experimental evolutionary assay, the adaptability of *P. aeruginosa* to repeated cycles of SCV conversion/reversion is based on compensatory spontaneous mutations that produce variations in the intracellular levels of c-di-GMP.

MI-P32
STABILITY OF *RcsC11* MUTANT OF *Salmonella typhimurium* UNDER DIFFERENT GROWTH CONDITIONS

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Salmonella is a great prevalence pathogen in the population of Argentina. Its infection depends on the modulation of the pathogenicity islands genes expression, controlled by different regulatory systems. The RcsCDB system regulates the biosynthesis of colanic acid and flagella, and the virulence genes, among others. In the *rcsC11* mutant the system is constitutively active, resulting in a mucoid phenotype and attenuated virulence. The aim of this study was to investigate the stability of the *rcsC11* point mutation under different conditions that simulate the host environment, which was evaluated by the viability and reversion of mucoid phenotype. Our results demonstrated that, of all tested conditions, only the acid condition modified the *rcsC11* mucoid phenotype. In addition, such reversion was observed after the fifth sub-culture at pH 4.5. To confirm the phenotype reversion, mucoid and "non mucoid" colonies were transduced with a chromosomal *lacZ* fusion to the RcsB-reporter *flhC* gene. Our results demonstrated that “non mucoid” colonies displayed similar β-galactosidase activity levels than the wild type strain, supporting that the *rcsC11* point mutation was reverted in all of them. Even so, our results suggest that the *rcsC11* strain is an excellent candidate for the attenuate vaccines development, because the mucoid phenotype reversion was detected only in later stages of the assay.

MI-P33
EVIDENCE SUPPORTING A CONNECTIVE ROLE OF A PROTEIN INTERACTION SURFACE WITHIN CHEMOTACTIC ARRAYS

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The molecular basis of bacterial chemotaxis relays on chemoreceptors detecting and transmitting signals to the kinase CheA through the adaptor protein CheW. These core proteins interact between each other forming large interconnected arrays. In addition to the known interaction surface between CheW and CheA, involved in CheA activation, current models of the organization of receptor complexes propose the existence of a novel interaction surface between these proteins. The models are based on cryotomographic studies and suggest a role for the new interface in the connection of functional units within the array. In order to test the validity of this proposal, we carried out all-codon mutagenesis at three CheW residues located in the postulated surface. Only few replacements affected the chemotactic ability of the cells as assessed in soft-agar plates, indicating that this interface tolerates modifications. Characterization of functionally defective mutants by FRET assays showed that they were still able to mediate CheA activation. Some of them were unable to respond to an attractant stimulus inhibiting CheA activity. Others, including truncated variants at this interface, responded in a notably less cooperative way. Since cooperativity is indicative of signal spread, our results support that this contact between CheW and CheA might connect signaling units within the array.

MI-P34
REVEALING THE PROPERTIES OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM *Ruminococcus albus*

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ADP-glucose pyrophosphorylase (ADPGlcPPase) from Firmicutes (low G+C Gram-positive bacteria) is enconded by two genes, *glgC* and *glgD*, leading to a heterotetrameric protein structure, unlike other prokaryotic ADPGlcPPases. The enzyme from different Firmicutes sub-groups presents dissimilar kinetic and regulatory properties, as shown in Bacillales and Lactobacillales groups. So far no details are known about the ADPGlcPPase from Clostridiales, a third

group of Firmicutes. To further explore structure-function relationships of this enzyme from Firmicutes, we performed the molecular cloning of the *glgC* and *glgD* genes encoding the ADPGlcPPase from *Ruminococcus albus*. Results show that the recombinant *R. albus* GlgC subunit expressed in *Escherichia coli* is active. Conversely, the GlgD subunit was found inactive when expressed alone; although its co-expression with GlgC increase by 2-fold the activity. These results place the ADPGlcPPase from *R. albus* in between those from Bacillales and Lactobacillales, supporting the phylogenetic analysis that groups GlgC and GlgD subunits in distinctive clusters respect to the Firmicutes groups. The complete characterization of regulatory and structural properties of *R. albus* ADPGlcPPase is critical to establish evolutionary relationships of this enzyme in Firmicutes and other Gram-positive and Gram-negative prokaryotes.

MI-P35
CHARACTERIZATION OF THE HIGHLY ACTIVE CATALASE FROM THE POLYEXTREMOPHILIC STRAIN *Acinetobacter* Ver3

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The Andean Altiplano is a sedimentary–volcanic plateau at an average altitude of 4,000 m. It is characterized by extreme environmental conditions such as high UV radiation, low nutrient concentration, the presence of heavy metals, high salinity levels and large daily temperature fluctuations. *Acinetobacter* strain Ver3 isolated from Verde Lake displayed high UV tolerance and resistance to pro-oxidants, features related to its single catalase species, 15-times more active than the total enzyme observed in control collection strains. The gene encoding the highly active catalase KatA -from the HPII type- was identified after the complete sequencing of the *Acinetobacter* Ver3 genome. The enzyme was cloned and expressed in *E. coli* using pET28 vector, and the recombinant enzyme was purified following ammonium sulfate precipitation of soluble extracts and subsequent anion-exchange chromatography. The absorption spectrum of KatA exhibited a typical Soret band at 408 nm observed in heme-containing catalases, with a 1:1 stoichiometry. Kinetic measurements revealed a high structural stability with no loss in activity between pHs 6 and 12 and tolerated temperatures between 0° and 60° Celsius. Kinetic measurements provided values of k_{cat} and k_{cat}/Km of $5{,}624.10^7$ and $1{,}286.10^7$ respectively, which are among the highest parameters reported for bacterial catalases.

MI-P36
AMINOACID SEQUENCING AND SUGAR COMPOSITION OF S-LAYER PROTEINS FROM *Lactobacillus kefiri* STRAINS

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Surface layers are (glyco)proteinaceous cell envelopes ubiquitously found in different bacterial species, including *L. kefiri*, a potentially probiotic microorganism. Previous mass spectrometry analysis showed a great structural heterogeneity among glycosylated S-layer proteins (SLP) from different *L. kefiri* strains, some of them share several fragments with the SLP from phylogenetically related lactobacilli. Based on this information, specific primers were designed in order to amplify SLP gene's fragments by PCR. A fragment of 340 bp length was amplified from DNA of 22 strains of *L. kefiri*. Multiple alignments of the deduced amino acid sequences demonstrated a strong sequence conservation of this fragment in all SLP studied, however it was possible to group strains in five different sets. The glycosylation signature motif SSASSASSA, recently described in *L. buchneri* CD34, was also found in all the strains. Glyco-SLP from four strains were selected and glycans obtained by β-elimination were further hydrolyzed and analyzed by HPAEC-PAD. All of them presented glucose as main component, although differences in minor components were detected. This is the first approach on characterization of primary structure of glycosylated SLP from *L. kefiri* strains. The results obtained are essential to understand the functional properties of these proteins as surface components of bacterial cells

MI-P37
IDENTIFICATION OF NON-SYNONYMOUS MUTATION BETWEEN PROSPEROUS AND A NON-PROSPEROUS XMDR *Mycobacterium tuberculosis*

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About 4.5% of new tuberculosis cases have Multi-drug-resistant tuberculosis, which are resistant to the first-line antibiotics. Some MDR-TB strains have the ability to evade the host human defences and that may be one of the reasons of the high mortality rates of people infected with MDR-TB. In particular, strain M is highly prosperous in the Argentine and is able to build up further drug resistance without impairing its ability to spread. The main goal of this project is to identify the genes involved in this immune evasion strategy. To carry out this project we will take advantage of having a M-highly genetically related MDR Haarlem strain, the 410 strain, that produced only one recorded case in the last two decades. This M-highly related MDR Haarlem strain was used as contrasting strain in order to find the genomic particularities of M that could be involved in its interaction with the human immune system. Strains M and 410 were sequenced up to 99 %. We found that at least 13 genes carrying non-synonymous mutations between M and 410 strains. Some of these genes are involved in the metabolism of complex lipids and others in drug resistance. Interestingly, a SNP in a gene encoding a putative transglycosylase produces a truncated protein in M.

MI-P38
RESISTANCE TO MICROCIN J25 BY TARGET MODIFICATION. IS THERE A SECOND-STEP MUTATION?

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Bacterial RNA polymerase is the target of the peptide antibiotic microcin J25 (MccJ25). Long ago, we isolated a strain (SBG231) with a point mutation in the *rpoC* gene of RNA polymerase (RNAP), which confers high-level resistance to the antibiotic. The mutated residue is located at the entry of the RNAP secondary channel. A plasmid, called pDJJ12, carrying the *rpoB* and *rpoC* genes of RNAP, complements the mutation and turns SBG231 cells again sensitive to MccJ25. A vector with *rpoB* only has no effect. To our surprise, in the present study we found that a plasmid bearing the *rpoC* gene alone does not complement the mutation. We also determined that RNAP mutants emerge much less frequently than non-target mutants. An attractive hypothesis to explain these observations is that there is a need for two mutations in RNAP for MccJ25 resistance development. Perhaps the second hit is in the *rpoB* gene. A closer look at the pDJJ12 plasmid used in complementation assays showed that in addition to the RNAP genes it includes a short ORF of unknown function called *yjaZ*. This could be a likely candidate for the second hit. However, against this possibility there is the fact that a deletion of the entire ORF did not affect the sensitivity to MccJ25. We are currently searching for the putative second mutation by using transduction analysis and nucleotide sequencing of the *rpoB* gene in SBG231.

MI-P39
GENETIC AND PHYSIOLOGICAL STUDIES OF AN *Escherichia coli sbmA tolC* DOUBLE MUTANT

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We have previously shown that the combined loss of the inner membrane protein SbmA and the outer membrane protein TolC in *Escherichia coli* K-12 results in an inability to form colonies at 42°C in LB medium. Each mutation alone does not exhibit heat sensitivity. To test whether this phenotype is strain-dependent, in the present study we introduced both mutations in another background and found that the newly constructed mutant displays the same phenotype. Interestingly, we found that *E. coli* K-12 strains produce a diffusible factor that restores the ability of the *sbmA tolC* mutant to form colonies at 42°C. This factor is secreted both in solid and liquid culture media. *E. coli* K-12 strains with mutations blocking the synthesis of the siderophore enterobactin (*aroB* and *entE*) do not produce the stimulating factor, suggesting that it could be enterobactin. Mutants in *entS* and *tolC* (in which enterobactin excretion is highly reduced) are also capable of promoting the growth of the double mutant at 42°C. This could be explained by assuming that the degradation products of enterobactin, whose excretion is not affected in *entS* and *tolC* mutant strains, also have factor activity. Our results suggested that the thermosensitive phenotype of the double mutant *sbmA*

tolC may result from iron limitation. This is supported by the observation that addition of exogenous iron relieves the phenotype.

MI-P40
ENTEROBACTIN PROTECTS *Escherichia coli* FROM THE ROS-MEDIATED TOXIC EFFECTS OF COPPER

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Siderophores are metal-chelating agents produced by bacteria under Fe-limiting conditions. Its primary function is to chelate the ferric iron and therefore make it available. Recent reports indicate that siderophores have wider and more complex functions than those known. We previously reported the requirement of enterobactin, a catechol siderophore produced by *E. coli*, for colony development in minimal media (M9) and proposed a new function as an intracellular oxidative stress protector. Now, we analyzed the role of enterobactin in copper toxicity. We showed that the lack of enterobactin increased copper cell sensitivity. Addition of enterobactin to the media protected cells; on the other hand, supplementation with iron didn't impact on the sensitivity to copper. Besides, we observed that enterobactin must to be internalized and hydrolyzed to confer a full protection against copper damage. Also, *entE* expression increased in cells growing in M9 and LB supplemented with copper. Finally, we found that the addition of copper to *entE* mutant increased levels of reactive oxygen species, which were lowered in the presence of exogenous enterobactin. Results suggest that the copper toxicity in enterobactin deficient mutants could be due to the oxidative stress generated by this metal and support our hypothesis implying cathecols siderophores in an oxidative stress protection function.

MI-P41
METABOLIC AND STRUCTURAL CHARACTERIZATION OF ENTEROCIN CRL35-RESISTANT *Listeria monocytogenes* CELLS

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Enterocin CRL35 is a subclass IIa bacteriocin produced by *Enterococcus mundtii* CRL35 that is active against the foodborne pathogen *Listeria monocytogenes*. Resistant cells, that can be isolated with low frequency, constitute a great opportunity to better understand the mechanism of action of this bacteriocin. Therefore, the goal of the present work was to compare phenotypic characteristics between the sensitive strain and two resistant cells recently isolated in the lab. This study shows that mutants have a slower growth rate and lower glucose consumption, which correlated with a less pronounced acidification of the medium. Interestingly, lysozyme sensitivity was different among the cells under study. Therefore, ultrastructure was analyzed by Transmission Electron Microscopy. On the other hand, monolayer studies with purified lipid extracts indicated a more fluid and expanded state for the sensitive strains compared to resistant ones. However, no differences in the perpendicular molecular dipole of the lipids of both sensitive and resistant cells could be measured. The stiffer films formed by lipids of the resistant strains would be due to differential fatty acid compositions. Taken together, these results indicate that both metabolic and structural modifications are involved in the resistance of *Listeria* cells towards enterocin CRL35.

MI-P42
CLONING OF HYBRID BACTERIOCINS WITH DIFFERENT HINGE REGIONS

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The ribosomally synthesized antimicrobial peptides produced by bacteria are called bacteriocins. Generally the ones produced by Gram (-) are active against other Gram (-), in the same way, the ones secreted by Gram (+) bacteria are active against other Gram (+) phylogenetically related to the producing strain. The aim of this work was to produce hybrid bacteriocins active against both kinds of bacteria. To achieve it, we conducted transcriptional fusions between the structural genes of different bacteriocins produced by lactic acid bacteria, and the gen of microcin V (MccV), a bacteriocin produced by *Escherichia coli*. Genes were fused by asymmetric PCR reactions, intercalating different coding sequences for a region called "hinge", in order to obtain broad spectrum recombinant chimerical bacteriocins. The fused genes were cloned into the expression vector pET 28 to produce the fusion proteins. All constructs were expressed in *E. coli* BL21 (DE3) after IPTG induction. The antimicrobial activity of different cell extracts and partially purified preparations of hybrid bacteriocins were tested against Gram (-) and Gram (+) reporter strains.

Bacteriocins resulting from the fusion between enterocin CRL35 and MccV with different hinges were active against both, *Listeria spp*. and *E. coli*.

MI-P43
ROS PRODUCTION INDUCED BY MICROCIN J25 IS MEDIATED BY CYTOCHROME *bd*

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Microcin J25 (MccJ25) is a plasmid-encoded lasso peptide secreted by *Escherichia coli* that displays antibiotic activity against a range of food-borne Gram-negative pathogens including *Escherichia coli*, *Salmonella* and *Shigella*. The peptide is a potential candidate for a number of applications including food preservation because its resistance to proteolytic degradation and extreme temperatures. The membrane respiratory chain is one of the targets of MccJ25. In fact, this peptide inhibits the oxygen consumption and increases the superoxide production. As a matter of fact, wild type *E. coli* C43 strain was sensitive to the peptide whereas the Δ*bd*I Δ*bd*II double mutant became resistant. Since we had previously demonstrated that MccJ25 inhibited the cytochrome *bd*I oxidoreductase activity, we decided to investigate *in vitro* ROS production with membranes of these mutant strains. The cytochrome *bd* double mutant membrane produced the lowest ROS level, indicating a possible role of this oxidoreductase in the MccJ25 mechanism of action. Moreover, we evaluated the sensitivity of different *E. coli* strains in the presence of the antioxidant ascorbic acid, confirming that the peptide activity was lower when this compound was present.

MI-P44
CHARACTERIZATION OF PHAGE-LIKE PYOCINS FROM *Pseudomonas* SF4C

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Bacteriocins are protein produced by bacteria that can kill closely related species. *P. fluorescens* SF4c is a plant-growth promoting bacterium that synthesizes bacteriocins (phage-like pyocins), which inhibit the growth of the phytopathogenic strain *Xanthomonas axonopodis pv vesicatoria* Xcv Bv5-4a. The aim of this work was to characterize the pyocins secreted by the strain SF4c. The complete cluster of pyocins was identified in the genome of the strain SF4c, finding structural genes of the pyocins R and F, as well as, regulatory and lytic genes. The structure of the bacteriocin and its mechanism of action on the sensitive strain *X. axonopodis pv vesicatoria* Xcv Bv5-4a was studied by atomic force microscopy (AFM). The pyocins presented a structure similar to the bacteriophage tails and they were attached to the surface of the cell to exert their antimicrobial action. The action of the pyocins on the cell was evidenced by a diminution of their volume, observed as a decrease in the height of cell Xcv Bv5-4a in the AFM image. These results suggest that the mechanism of action of these pyocins involve the damage of the membrane of susceptive cells, probably by formation of pores. The characterization of this class of antimicrobial compounds is relevant because of their potential application as biocontrol agent.

MI-P45
DEVELOPMENT OF A SUICIDE PROBE BASED ON PEDIOCIN PA–1 THAT IS ACTIVE AGAINST *Escherichia coli*

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Pediocin PA-1 is a subclass IIa bacteriocin produced by *Pediococcus acidilactici*. This group of peptides acts on the cell membrane of Gram (+) bacteria by forming pores. Apparently, these peptides bind to a membrane specific receptor. The theories on pore formation mechanism by bacteriocins suggest that they would induce conformational changes on the receptor to form a channel that remains open or, they would just use the receptor as an anchor and then form the pore. In both cases, pore formation causes leakage of ions, dissipation of proton motive force and release of essential metabolites. In order to study the mechanism by which Pediocin PA-1 induces the loss of membrane integrity, we led the bacteriocin to the cell membrane of *E. coli*, by a transcriptional fusion of the peptide with EtpM, a protein of the type II secretion system from *E. coli* O157: H7. In this work we constructed the fusion *etpM-pedA* under the tight control of P_{BAD} promoter (repressed by glucose and induced by arabinose). In addition we carried out the co-expression of EtpM-PedA with the Pediocin PA-1 immunity protein. The results show that Pediocin PA-1 is capable of acting on Gram-negative bacteria when it is anchored in the plasmatic membrane,

independently of its specific receptor. This suicidal probe will allow a deeper study of the mechanism of action of bacteriocins and its immunity proteins, using an *in vivo* system.

MI-P46
THIONIN-LIKE ANTIMICROBIAL PEPTIDE: INHIBITORY ACTIVITY AND SYNERGISM EFFECT AGAINST *Fusarium solani*

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We have previously isolated a thionin-like antimicrobial peptide, CaThi, from *Capsicum annuum* fruits. The aim of this work was the investigation of the mechanism of action from CaThi and additionally its combinatory effect with fluconazole against *Fusarium solani*. As method, CaThi was assayed for antimicrobial activity, plasma membrane permeabilization ability, induction of intracellular reactive oxygen species and localization of CaThi tagged with FITC in *F. solani* cells. To determine the possible synergistic effect, *F. solani* was grown in the presence of CaThi in combination with fluconazole. After this assay, viability was determined. Our results showed that CaThi inhibits 82% of growth, prevents the formation of *F. solani* hyphae from spores, changes their membranes, induces production of hydrogen peroxide, and it is localized intracellularly. The assay with CaThi in combination with fluconazole reaches 100% inhibition and causes 100% viability loss. In conclusion we show that CaThi has strong activity against *F. solani*, inhibits the spore germination and induces its permeabilization and hydrogen peroxide accumulation. The results suggest the occurrence of an intracellular target for CaThi. Combination of fluconazole and CaThi increased the inhibitory activity, suggesting that a synergistic mechanism causes spores 100% viability loss.
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MI-P47
ANTIMICROBIAL ACTIVITY OF *Adenanthera pavonina* DEFENSIN APDEF1 AGAINST *Saccharomyces cerevisiae*

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The aim of this work was to evaluate the antimicrobial activity of the plant defensin ApDef1 which was obtained from *Adenanthera pavonina* seeds, against the yeast *Saccharomyces cerevisiae*. As methods, peptides were extracted from the seeds and submitted to ammonium sulfate fractionation, dialysed against distilled water and then submitted to chromatographic methods. A 7 kDa peptide was sequenced and tests were performed to evaluate its antimicrobial activity by minimum inhibitory concentration (MIC) determination, membrane permeabilization and induction of hydrogen peroxide capability. The primary structure of this peptide showed homology with plant defensins, which was named ApDef1. ApDef1 was able to inhibit the growth of *S. cerevisiae*, presenting MIC of 40 µg/mL and fungicide activity, it required a maximum of 18 h to cause the unfeasibility of cells and it was able to modify the yeast plasma membrane. The treatment with the 2.5 µg/mL of ApDef1 induced the production of hydrogen peroxide after 18 h of assay. In conclusion, ApDef1 presents a strong antimicrobial activity against cells of yeast, killing them in 18 h. ApDef1 permeabilizes yeast membranes and causes increasing of hydrogen peroxide. Supported by: UENF, CNPq, CAPES, FAPERJ.

MI-P48
ROLE OF BOTRYDIAL AND BOTCININS IN THE INTERACTION BETWEEN *Botrytis cinerea* AND COMPETING FUNGI

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Aim: To analyze the fungitoxic potential of botrydial and botcinins produced by *B. cinerea* and to determine if these compounds play a role in the antagonism between this phytopathogenic fungus and competing microorganisms. Methodology: Dual culture assays were used to screen a collection of microorganisms isolated from natural environments for the presence of fungi inhibited by wild-type (WT) *B. cinerea*. These fungi were designated as FIBBC. *B. cinerea* mutants unable to produce botrydial and botcinins were also analyzed for their ability to inhibit the abovementioned fungi. Botrydial and botcinins were purified from WT *B. cinerea* cultures and their inhibitory activity on conidia germination and germ-tube growth of FIBBC was analysed. Results: Fungal strains of the genus *Trichoderma* inhibited in dual cultures by WT *B. cinerea* were identified. *B. cinerea* mutants that produce no

botrydial and botcinins still antagonized the abovementioned FIBBC. Botrydial and botcinins inhibited conidia germination and germ tube growth of FIBBC. Conclusion: Botrydial and botcinins are toxic to potential fungal competitors of *B. cinerea*. However, the relevance of each of these compounds in the inhibition activity of *B. cinerea* towards these fungi is difficult to be estimated on the basis of mutant analysis, probably due to functional redundancy between the different toxins.

MI-P49
CELLULAR DAMAGE INDUCED BY HARMOL ON *Penicillium digitatum* AND *Botrytis cinerea*

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P. digitatum and *B. cinerea* are postharvest fungal phytopathogens, causal agents of green and grey molds, respectively. To control them, treatments with fungicides are used. Due to resistant strains proliferation, development of alternatives for decay control becomes important. β-Carboline (βC) are plant alkaloids with a wide spectrum of antimicrobial properties. Here, inhibitory action of the βC harmol (Hol) at different concentrations in 24h-incubations was tested against both phytopathogens. After incubation with 1 mM Hol at pH 5, UFC/ml decreased from 10^6 to 10^4 for *P. digitatum*, while no viable conidia were observed for *B. cinerea*. In contrast, CFU/ml remained unmodified at pH 9 for both fungi. To analyze membrane integrity, conidia exposed to Hol were incubated with Sytox Green, and broad dye incorporation was observed for both pathogens. The intracellular ROS production was determined using the H_2DCFDA probe. For *B. cinerea* treated with Hol, a ROS increment detected, while they were not generated in *P. digitatum*. In addition, ultrastructural changes in conidia were analyzed by TEM, revealing severe cellular damage. Our data show that Hol at pH 5 alters conidia ultrastructure and membrane integrity of both fungi, while increased ROS production was only observed in *B. cinerea*. These findings represent the first insights to elucidate Hol mode of action against phytopathogen fungi.

MI-P50
EFFECT OF *Curcuma longa* AND *Origanum vulgare* EXTRACTS ON *Clostridium perfringens* BIOFILM

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Clostridium perfringens is an anaerobic gram-positive pathogen causative agent of gas gangrene and food transmitted infections in humans and animals. Curcuma and oregano have been used with medicinal purposes because of their antibacterial, anti-inflammatory and pro-apoptotic activity. The aim of this work was to study the induction of morphological changes and antimicrobial activity of extracts of curcuma and oregano in *C. perfringens* biofilm and plantonic cells. Two food isolated strains were used, an enterotoxigenic and a non-enterotoxigenic. The antimicrobial activity was determined by treatment with extracts and antibiotics at 50 and 100 µg/ml at 24 and 48h. The effect of extracts in plantonic cell was determined by absorbance decrease at OD 580nm. The antimicrobial activity against biofilm was determined by most probable number technique (MPN). Morphological changes were observed by optical and scanning electron microscopy and the expression of virulence factors *cpe* and *ccpA* was measured by RT-PCR. Plantonic cells growth was inhibited by oregano. In biofilm was observed a significant growth inhibition with both extracts. The extracts induced significant morphological changes. RT-PCR indicated that the expression of *cpe* and *ccpA* was not modified by extracts. These results showed that curcuma and oregano extracts have significant antimicrobial properties against *C. perfringens*.

MI-P51
MOLECULAR TYPING USING RAPD-PCR IN GROUP B STREPTOCOCCAL ISOLATED FROM NEWBORNS IN MISIONES

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Streptococcus agalactiae, Group B *Streptococcus* (GBS) is considered to be the major cause of neonatal sepsis and meningitis of bacterial origin. The Random Amplified Polymorphic DNA analysis (RAPD) is an accessible and sensitive method based on the use of arbitrary primers to amplify polymorphic segments of DNA and a powerful method to differentiate GBS strains. The aim of this study was to determine if there clonal relationship between the

strains of group GBS isolated from newborns in the province of Misiones, Argentina. For this purpose eleven strains of GBS invasive isolated from blood cultures and cerebrospinal fluid (CSF) were studied by RAPD using three primers, designated OPS11, OPB17, and OPB18. The PCR mixture consisted of buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl; 2.5 mM MgCl2), 100 mM each of the four deoxynucleoside triphosphates (INBIO, Argentina), 0.4 mM primer and 2.5 U of Taq DNA polymerase (INBIO, Argentina) in a total volume of 25 ml. Eleven different band patterns were obtained by RAPD-PCR with significant bands ranging from 100-5000 bp. In conclusion, the RAPD-PCR assay showed that each of the isolated clones belonged to a different RAPD genotype, revealing that neonatal invasive GBS infections were not epidemiologically related. The genetic diversity studies provide insight into the regional epidemiology of this disease and adapt prevention strategies.

MI-P52
CLINICAL *Acinetobacter baumannii* ACCESSORY GENOME DIFFERENCES REVEALED BY WHOLE GENOME SEQUENCING

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The study of the *Acinetobacter baumannii* pan-genome, and in particular the accessory genome of phylogenetically-related multidrug-resistant (MDR) clinical strains provides insight into the evolution of antimicrobial resistance in this healthcare-associated opportunistic pathogen. We have conducted a whole genome sequence analysis (WGS) of the phylogenetically related MDR *A. baumannii* strains Ab244 and Ab242 belonging to ST104 to determine differences in the accessory genome organization which could account for differences in their imipenem susceptibility phenotypes. Ab242 and Ab244 contigs order were inferred using MAUVE with *A. baumannii* references genomes as blueprints. Both strains shared a high degree of chromosome synteny, but showed differences in the accessory genome. These including i) a 25-kbp plasmid harboring $bla_{\mathrm{OXA\text{-}58}}$ and *aphA6* genes conferring carbapenem and aminoglycosides resistance, respectively, present only in Ab242; ii) differences in prophage gene content, iii) an island harboring heavy metal resistance genes only in Ab244; and iv) different *carO* variants encoding outer membrane proteins associated to imipenem uptake. Overall, these observations give evidence of differential gene transfer events and genome reorganization between these strains providing insight into the evolution of resistance determinants among this nosocomial pathogen population.

MI-P53
EXPRESSION AND BIOGENESIS OF *Acinetobacter baumannii* OXA-58 IN *Escherichia coli*

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We studied the biogenesis of the carbapenem hydrolyzing class D β-lactamase (CHDL), OXA-58 from *Acinetobacter baumannii* in *E. coli* as a model system. The mature $bla_{\mathrm{OXA\text{-}58}}$ sequence was fused to a *pelB* signal sequence in a pBAD-derived plasmid and expressed in this host. OXA-58 biogenesis was studied following β-lactam resistance, level of protein production, and subcellular localization. High levels of a 29 kDa OXA-58 band were observed in whole *A. baumannii* and *E. coli* cell extracts which correlated with resistance to imipenem only in *A. baumannii*, although in both cases resistance to ampicilin was obtained. Subcellular fractionation showed that OXA-58 accumulated in both species mostly in the periplasmic fraction, although a significant percentage was also associated to membranes. *In vitro* enzymatic assays revealed imipenemase activity in the soluble periplasmic fraction of *A. baumannii* cells but much lower activity in those of *E. coli*. The presence of molecular crowders such as PEG, ficoll or PVP did not affect substantially the imipenemase activity of the extracts, ruling out in principle an effect of macromolecular crowding in the regulation of this activity in the periplasm. Overall these results suggest that different post-traductional events occur in *A. baumannii* and *E. coli* which may differentially affect substrate specificity towards β-lactam substrates.

MI-P54
PHOTOTHERMAL THERAPY OF THE PATHOGENIC BACTERIA WITH POLYANILINE NANOPARTICLES

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Increases in the prevalence of antibiotic resistant bacteria require new approaches for the treatment of infectious bacterial pathogens. It is now clear that nanotechnology has driven a new approach in the using nanoparticles to destroy pathogenic bacteria. A new therapeutic modality is called photothermal therapy. This therapy is based in the use of conductive nanoparticle that absorbed in the range of near-infrared (NIR) radiation and can transfer this energy into the surrounding environment as heat that could cause irreparable cellular damage. In this work were performed experiments to determine if these polyaniline nanoparticle (PANI-Np) dispersed in polivinylpirrolidone (PVP) in combination with NIR irradiation could be used to destroy *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) cells. The bacteria were incubated with PANI-Np and irradiated during 15 min with focused laser (785nm, 500mW) and post-treatment was observed bacterial damage and was verified by viability testing. In both microorganisms the viability reduction was about 10%, compared to control conditions. This mechanism of cell death was observed by DNA fragmentation. These results reveal that the PANI-Np, following NIR exposure can be used for killing bacteria because was observed a significant decrease in cell viability triggering cell death.

MI-P55
ISOLATION AND STUDY OF *Pseudomonas putida* STRAINS ADAPTED TO SALINE STRESS

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In this work we used a "fast selection" method to obtain good root-colonizing and salt tolerant bacteria, by successive and alternating rounds of growth in saline conditions of corn plants inoculated with the wild type strain *Pseudomonas putida* KT2440. We isolate two bacteria adapted to saline conditions (C2 and C3) after 7 cycles in corn. To evaluate salt tolerance of these bacteria, we performed growth curves in saline and non-saline conditions. We observed greater tolerance of C2 and C3 strain, compared to the WT strain, after 8 hours of growth (68% and 74% of higher growth for strains C2 and C3, respectively). In addition, the two adapted bacteria showed better growth promotion effect both on corn and soybean plants in saline conditions (70-90 mM NaCl), comparing with the parental strain, both *in vitro* and *in vivo* assays. When corn plants were inoculated with C2 and C3 strains and grown in saline conditions showed 61% and 51% longer roots length, respectively, compared with plants inoculated with the WT strain. In soybean inoculated with C3 strain (*in vitro*), 48% longer roots were obtained, whereas in *in vivo* assays 45% higher plants were obtained with C2 strain. This work shows an interesting method to get adapted bacteria without the need for genetic tools. A hopeful solution to the cultivation in saline soils could be proposed by using these adapted bacteria.

MI-P56
CHARACTERIZATION OF THE UBIQUITIN-DOMAIN CONTAINING PROTEIN NMAG_2608 OF THE ARCHAEON *Natrialba magadii*

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Although Ubiquitin is restricted to eukaryotes, ubiquitin-like proteins/domains (Ubl/Uld) are found in all domains of life. They do not share high sequence identity but display the β-grasp fold and often exhibit the C-terminal di-glycine motif characteristic of Ubiquitin.Nmag_2608 is an ubiquitin-like domain (Uld) protein from the haloalkaliphilic archaeon *Natrialba magadii* expressed and secreted to the extracellular medium at early stationary phase. Its Uld, P400, has been previously identified and characterized. The aim of this work was evaluating the physiological role of Nmag_2608 and the importance of P400 in this role. For this, P400 was heterologously expressed in *E. coli* and purified. Given Nmag_2608 localization, a possible role in nutrient uptake was explored by evaluating the interaction of P400 with different aminoacids at several ratios (1:10;1:50). Different peaks and their UV spectrum obtained from HPLC analysis of the mixtures were compared with that of sample containing only p400r or amino acids. Differential peaks were collected and interaction confirmed by Western blot assays with Anti-P400. Results showed that P400 interacts only with tryptophan. HPLC assay showed a new pattern appears with characteristic absorbance spectrum

which corresponds with interacting P400r-Trp. This interaction could be necessary for the activity of P400 in the extracellular medium.

MI-P57
LIPID BODIES PRODUCTION IN THE UNICELLULAR ALGAE *Scenedesmus oblicuus* IS INFLUENCED BY SORROUNDING B

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The production of biodiesel from microalgae is currently seen as a possible solution to the exhaustion of fossil fuels. Upon nutrient starvation, microalgae undergo growth arrest and lipid accumulation into newly synthesized oil bodies. Several microalgal species synthesize and respond to indole-3-acetic acid (IAA), but its physiological role remains unclear. *Azospirillum brasilense* is a rhizobacteria that enhances plant growth by the production of IAA, cytokinin, gibberellin, ethylene and nitric oxide. The aim of this work was to determine the role of IAA in microalgae-bacteria communication on mixed consortia with rhizobacteria. Our results show that *Scenedesmus oblicuus* was impaired in lipid accumulation upon nutrient stress on mixed cultures with the *A. brasilense* mutant strain Faj009, with a 90% reduction in IAA production. Conversely, co-inoculation with *A. brasilense* wild type or with the Faj009pipdC strain, carrying a plasmid with the wild type version of the *ipdC* gene, resulted in lipid accumulation upon nutrient stress and oil bodies' production. On the other hand, *S. oblicuus* cells cultured with the Faj009 strain remain unaltered, with intact chloroplasts and lacking of oil droplets. These results suggest that algae-bacteria communication influence algae physiology, and might have implications for extensive culture of microalgae.

MI-P58-
Bifidobacterium ENZYMES INVOLVED IN LIPIDS METABOLISM: IMPACT ON THE DEVELOPMENT OF FUNCTIONAL FOODS

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Few studies showed the influence of bifidobacteria over lipids derived from milk products, especially fatty acids (FA) hydrolysis by esterase and lipases enzymes and production of CLA and CLNA by isomerases with health–promoting properties. The aim of this work was to determine enzymes activities by 3 bifidobacteria: INL2, ZL1228 and LM7a, isolated from breast milk and cultured in MRS–cys at 37 °C, 48 h under anaerobic conditions. Esterase activity was measured in cell–free extracts by PAGE using acrylamide gels at 12% without SDS and incubated in presence of α–naphtil derivates from FA (C2–C12) and Fast Red TR as developer. To evaluate bioactive lipids production by GC bacteria were cultured in MRS–cys and skim milk 10% with LA and LNA. The highest level of esterase activity was detected against α–naphtil acetate, propionate y butyrate. Low levels were obtained in presence of C10 and 12 for LM7a and ZL1228. Electrophoretic zymograms showed 4 esterase enzymes with different electrophoretic mobilities and substrate specificity. No lipase activity was detected. All strains produce CLA in MRS–cys and skim milk. INL2 produced the highest levels of CLNA in MRS–cys while ZL1228 resulted the best producer in skim milk. This study shows bifidobacterias with esterase and isomerase activity, both important for the development of functional foods with desirable organoleptic characteristics.

MI-P59
FUNCTIONAL GENOMIC ANALYSIS OF THE ADAPTIVE RESPONSE OF *Enterococcus mundtii* CRL35 IN A MEAT MODEL

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During the last decades, reports about enterococci used as starter cultures or co-cultures (adjuncts) in the production of artisanal fermented products, have increased significantly. Previous studies showed that *Enterococcus mundtii* CRL35 (CRL35) was able to grow in culture media containing meat extract. Here, its growth (OD_{600}, UFC/ml and pH), antimicrobial activity and global stress response (2D electrophoresis), in a chemically defined medium supplemented with meat proteins (CDMP) in comparison with a non-supplemented (CDM), were first evaluated. As a result, it was found that CRL35 was able to grow properly reaching the late log phase after 14h at 25 ºC, with 6.60×10^8 UFC/ml and pH=6.09 in CDMP, and 5.90×10^8 UFC/ml and pH=6.15 in CDM. Additionally, in both conditions, CRL35 was capable of inhibit the pathogen *Listeria monocytogenes* by the action of Enterocin CRL35. Secondly, a gene expression analysis was performed in a sterile meat extract, through RNAseq. Consistent with the expected

metabolic adaptations, genes related to proteins and carbohydrates metabolism, and to stress response, were identified. These results demonstrated that CRL35 is well adapted to the meat environment, representing a promising functional adjunct culture able to retain its technological and bioprotective properties, to ensure the sensory and hygienic quality of the final product.

MI-P60
FUNCTION OF *Trypanosoma cruzi* RNA-BINDING PROTEINS

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In Trypanosomes, the regulation of gene expression depends almost exclusively on the modulation of mRNA abundance and translation, which are regulated by RNA-binding proteins (RBPs). Despite their significant roles as master regulators of gene expression, information on RBP function is lacking. To answer this, we developed an in vivo reporter system to tether RBPs to the 3' untranslated region (UTR) of an mRNA coding for luciferase in *Trypanosoma cruzi*. With this setup we can analyze the effect of any given RBP on the abundance and translation efficiency of the reporter mRNA, by measuring reporter mRNA levels and luciferase activity. As a baseline control we used a construct expressing GFP. As expected, the polyA binding protein TcPABP1 showed to increase the reporter's abundance and its translation by 8 and 6 fold when compared to the baseline, serving as an internal functional control. Experimentally, we found that TcUBP1 and TcZFP3 showed to repress translation of the reporter mRNA, while having no significant effect on mRNA abundance. TcRBP4 showed to stabilize the reporter mRNA with a concomitant increase in luciferase activity. TcZFP2 had no significant effect on mRNA abundance or translation. Our results remark the effect a single RBP can exert on a target mRNA, highlighting translational repression as an important regulatory mechanism of gene expression in *T. cruzi*.

MI-P61
CONSTRUCTION AND ANALYSIS OF A FAS I CONDITIONAL MUTANT IN *Mycobacterium smegmatis*

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In order to dissect the global network involved in the regulation of FAS I and FAS II systems and elucidate how they crosstalk to keep lipid homeostasis in mycobacteria, we constructed a *M. smegmatis* conditional mutant in the *fas* operon, consisting of *fas* and *acpS* genes. The mutant strain was constructed using the Tet-Pip OFF system which replaces the endogenous P_{fas} promoter and as the result of this replacement, the *fas* operon is under the control of P_{ptr} promoter, which is regulated by the addition of anhydrotetracycline (ATc). As expected, the *fas* operon is essential for viability. The conditional mutant obtained allowed us to analyze the impact of reduced levels of *de novo* fatty acid biosynthesis on the synthesis of the exquisite repertoire of lipids present in mycobacteria cell envelope using TLC and LC-MS. These results represent an important step towards understanding the complex regulatory network involved in maintaining lipid homeostasis in mycobacteria.

MI-P62
BIOSYNTHETIC PATHWAY AND IMPROVE PRODUCTION OF VITAMIN B12 BY *Lactobacillus coryniformis* CRL 1001

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Cobalamin (CBL) is one of the most complex non-polymeric macromolecules synthesized by *Eubacteria* and *Archaea*. The biosynthesis de novo of CBL is highly complex and involves about 30 enzymes. We demonstrated that cell extract of *Lactobacillus* (*L.*) *coryniformis* CRL 1001 is able to correct the coenzyme B12 requirement of *Salmonella* Typhimurium AR 2680 (*metE-cbiB-*) in minimal medium. The aim of this study was to assess the genetic organization of vitamin B12 biosynthesis in CRL1001 genome and establish its biosynthetic pathway. In silico analyzes reveal that this strain possesses a coenzyme B12 gene cluster encoding the gene sets (*cbi*, *cob*, and *hem*) for CBL biosynthesis. Interestingly, CRL 1001 genome lacks *cobT*. Instead, *cblS* and *cblT*, α-ribazole kinase and its transporter respectively were found. These genes are present in CBL-producer *Listeria* strains and in *L. rossiae* DSM 15814 but both are absent in *L. reuteri* CRL 1098, a cobalamin producer. To validate the genetic findings, we investigated the CBL synthesis in the presence of key intermediates: DMB (5,6-dimethylbenzimidazole) and L-Thr (Threonine) (separately and together). DMB increases the corrinoid compound production but the higher

concentration of CBL was obtained with L-Thr. These results provide valuable evidence of the cobalamin synthesis pathway and its regulation in *L. coryniformis* CRL 1001, a potential probiotic strain.

MI-P63
EFFECT OF PROBIOTIC ADMINISTRATION ON BIOCHEMICAL PARAMETERS AND INTESTINAL MICROBIOTA IN CALORIC RESTRICTION

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Effects of caloric restriction diet and *Lactobacillus fermentum* (Lf) CRL1446 and *L. casei* (Lc) CRL431 (probiotic strains) administration on metabolic parameters and intestinal microbiota was studied. Balb/c mice were divided into the following groups: normal diet (ND), caloric restriction diet (CR), CR diet plus Lf and CR plus Lc. CR diet was administered during 45 days and strains were given in the dose of 10 cells/mL/day/mouse. Triglyceride (TG), total cholesterol (TC), glucose and leptin were determined in plasma. Gut microbiota was evaluated by high-throughput sequencing of 16S rRNA gene amplicons. Glucose, TC, TG and leptin levels were decreased in CR groups. Lc administration increased glucose and leptin levels. Lf decrease TC and leptin levels, showing lower values than in CR group. Gut microbiota studies, showed that CR diet increased abundance of Bifidobacterium and Lactobacillus genus and improved Bacteroidetes:Firmicutes ratio. Lc administration increased abundance of Lactobacillus genus. However, Lf administration increased abundance of Bifidobacterium genus and was able to maintain Lactobacillus genus during time of treatment. Probiotic strains improved metabolic parameters modulating intestinal microbiota differently. Lc and Lf increased dominance of certain species reducing biodiversity. L. fermentumexerted a bifidogenic effect under CR conditions.

MI-P64
ISOLATION OF PATHOGENS ENTEROBACTERIA FROM AGRO-INDUSTRIAL WASTE OF FAMATINA VALLEY-LA RIOJA

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The Famatina Valley´s agroindustrial activity displayed a significant increase in the level of production and in the new company's establishment, such as wine and olive oil industries. Consequently, this growth was accompanied by the need to expand areas of cultivation, manufacturing plants and urbanization of the region to contain the worker and their family. These environmental changes increase the risk of the water and soils sources contamination, which could displace the autochthonous microbiota and promote the emergence of humans and farm animal's pathogens. In this work we characterized the microbiota present in samples obtained from different sources: i) grape marc of La Riojana Co wine producing; ii) effluents water samples from Agroarauco Co (olive oil producer) and iii) effluents wastewater treatment "Aguas de La Rioja". In the samples from the source i, ii and iii, processed in selective media, we identified a variable number of species belonging to the enterobacteria group, representing a 3.3, 20.6 and 45% of the total cultivable microbiota obtained in LB media (per ml of sample), respectively. Moreover, we detected *Salmonella* and *Shigella* strains at a variable number. The presence of these pathogens represents a potential hazard for the human health, suggesting that the increase these industries production will aggravate this risk if not properly controlled.

MI-P65
ANTIFUNGAL ACTIVITY AND CELL DIFFERENTIATION IN *Bacillus subtilis* INDUCED BY *Setophoma terrestris*

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Onion is the second vegetable crop produced worldwide. Pink root is among the major limiting diseases of onion. Since its causal agent – *Setophoma terrestris* (St) – is one of the most severe pathogens in Argentina, we decided to address the biocontrol as a mean to control it. We have previously isolated bacteria with antagonistic activity against St from soil samples under continuous onion culture. The most efficient isolate in controlling St was identified by MALDI-TOF as *Bacillus subtilis* subsp. *subtilis* (Bss). Bss showed a strong capacity of fungal growth inhibition on co-culture plates. Interestingly, this inhibition, that was determined by the release of diffusible bacterial metabolites, could be observed on plates containing cell-free supernatant of Bss which had been previously exposed to the fungus

(Bss post-St). In contrast, no significant differences in the fungal growth were observed between control plates and plates containing cell-free supernatant from Bss grown without previous contact with St. In addition, an enhanced biofilm forming ability and the formation of a highly structured pellicle were observed in Bss post-St compared to Bss grown without previous contact with St. Consistently, Bss post-St showed an inhibition of swarming motility. These results suggest an association between biofilm formation pathways and the release of biocontrol compounds.

MI-P66
WHOLE-GENOME SEQUENCING ANALYSIS OF A CARBAPENEM-RESISTANT *Acinetobacter bereziniae* CLINICAL STRAIN

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Acinetobacter bereziniae is rarely found in healthcare-associated infections. We recently isolated a carbapenem-resistant *A. bereziniae* strain (HPC229) from a leukemia patient hospitalized in Rosario, Argentina, and recently reported the complete sequence of a $bla_{\text{NDM-1}}$-harboring plasmid pNDM229 present in this strain (Brovedan *et al*., AAC July 2015, in press). Here, we describe relevant traits of HPC229 through whole-genome sequencing studies (WGS). Phylogenetic analyses based on 16S rRNA, *gyrB*, *rpoB*, and *rpoD* genes showed the closest relationship of HPC229 with the carbapenem-resistant *A. bereziniae* CHI-40-1 among other related strains. In addition, the corresponding bla_{OXA229}-like chromosomal genes typical of this species were 99.3 % identical between these two strains. HPC229 and CHI-40-1 harbored $bla_{\text{NDM-1}}$ genes in pNDM-BJ01-like plasmids displaying distinctive features between them. Chromosomal *carO* genes coding for outer membrane proteins associated to imipenem uptake displayed only 77.8 % identity between both strains revealing distinct *carO* variants. Lack of congruence between phylogenetic trees based on housekeeping and *carO* genes indicated exchange of *carO* among the *A. bereziniae* population mediated by horizontal gene transfer. WGS analyses may thus help to better understand the evolution and dissemination of carbapenem resistance among the *Acinetobacter* global population.

MI-P67
SECRETED PROTEOME OF THE PSYCHROTOLERANT YEAST *Güehomyces pullulans* IN DIFFERENT CARBON SOURCES

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Our research main focus is the biotechnological potential of yeasts isolated from Patagonia extreme environments. In this work we have studied the secreted proteins of the psychrotolerant basidiomycetous yeast *Güehomyces pullulans*. First, the genome of *G. pullulans* strain CRUB1754 was sequenced with a Miseq, resulting in a mean coverage of 10 and a genome size of 22.6 Mb. An *ab initio* search for coding sequences predicted about 7000 putative CDS, which allowed building a protein database to interrogate with mass spectrometry data. For the secretome characterization, yeasts were grown on media containing glucose, starch or cellobiose as main carbon sources. Proteins from culture supernatants were concentrated by ultrafiltration. A proteomics LC-MS shotgun strategy was used to study 3 biological replicates of each condition. A total of 85 proteins were identified in all samples, from these 47 were present in at least two replicates. Proteins with the highest detection rates included alcohol oxidase, glycoside hydrolases, alpha amylase and a xylanase, the last one was the most represented protein in all conditions. An aspartic protease was exclusively identified in the presence of starch whereas the alpha amylase was highly induced. As a conclusion, a number of proteins with biotechnological potential were found and their enzymatic capabilities will be subject to future analysis.

MI-P68
PROTEOMIC ANALYSIS OF *Rhizobium* sp. LPU83 UNDER ACID STRESS USING AN ORBITRAP MASS SPECTROMETER

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Oregon-like rhizobia are acid-tolerant bacteria, very competitive for the nodulation of alfalfa in acid soils although inefficient for biological nitrogen fixation. These features place these rhizobia as a potential risk factor in agricultural soils when compete with efficient symbiont *Ensifer meliloti*. In this work we studied the global proteome responses of *Rhizobium* sp. LPU83 under acid stress. Initially, by evaluation of growth kinetics over pHs ranging from 4 to 7,

the acidic condition was chosen. Proteome was analyzed by nano-flow ultra-high-performance liquid chromatography coupled to a quadrupole Orbitrap mass spectrometer for identifying proteins possibly involved in acid tolerance. A total of 864 proteins were identified. Fifty-one of them were significantly up-regulated and eighteen were down-regulated. *In silico* characterization of these proteins revealed differences between stressed and non-stressed cells. A group of up-regulated proteins might be involved in alanine, aspartate and glutamate metabolism, and another group in oxidative phosphorylation, stimulating energy production. These proteomics-based results could help to improve our understanding in the acid tolerance in rhizobia.

MI-P69
GENOMIC COMPARATIVE ANALYSIS OF FOUR *Enterococcus faecium* STRAINS ISOLATED FROM CHEESE

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Enterococcus faecium is a controversial microorganism used in food industry due to its role in human infections. Still its presence in cheese microbiota as non-starter lactic acid bacteria contributes to flavor development. From the laboratory culture collection four cheese isolated *E. faecium* strains (IQ110, IQ23, GM70 and GM75) previously identified by 16S RNAr gene sequencing were selected for whole genome sequencing in order to provide genetic information about technological properties and putative pathogenic determinants. The genome size of these strains ranged from 2.7 to 3.1 Mbp. Gene prediction and annotation were performed using RAST server. Genome level phylogenetic analysis performed with Gegenees indicated that IQ110, GM70 and GM75 strains presented over 63% similarity with *E. faecium* strains whereas IQ23 presented 87 and 92% with *E. durans* strains. These results were confirmed by ANI, *in silico* DDH and MLSA analysis. These strains were also analyzed on their technological properties: aroma compounds production and citrate fermentation. Three strains (IQ23, GM70 and GM75) are capable of fermenting citrate while one is defective in this phenotype (IQ110). IQ23 and GM70 showed identical *cit* cluster. Bioinformatic and biochemical approaches could become complementary valuable tools to select *Enterococcus* strains that could be used as adjunct in cheese industry.

MI-P70
RECLASSIFICATION OF A CYCLODEXTRIN GLYCOSYLTRANSFERASE-PRODUCING BACTERIUM OF INDUSTRIAL APPLICATION

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Cyclodextrin Glycosyltransferase (CGTase), a glycoside hydrolase, catalyzes starch conversion into cyclodextrins and other industrial products such as maltooligosaccharides. We have isolated a CGTase from a soil bacterium which had been classified as *Bacillus circulans* based on its phenotypic profile. The 16S rRNA gene is widely used for phylogenetic studies; however, its use is limited due to intragenomic heterogeneity and low discriminative power at related genus. In addition, housekeeping genes are used. The aim of this work was to identify this CGTase-producing bacterium by molecular phylogenetic analysis of the 16S rRNA gene and the housekeeping genes *dnaK*, *gyrB*, *recA*, *rpoB* and *tufA*. All genes were amplified by PCR and sequenced. The substitution model was inferred with jModeltest. Phylogenetic trees were reconstructed by Maximum Likelihood (ML) and Bayesian Analysis methods using PAUP* and Mr. Bayes, respectively. For ML, the robustness was estimated by 500 replicates of non-parametric bootstrap. According to the results, the strain of *Bacillus circulans* was grouped within the genus *Paenibacillus*. In addition, *Paenibacillus* molecular signatures in the 16S rRNA and *rpoB* genes were found in the respective sequences of *Bacillus circulans*. We conclude that the bacterium under study must be reclassified within the *Paenibacillus* genus, with subsequent definition of specie.

MI-P71
Halomonas sp. KHS3 AS A POTENTIAL TOOL FOR TREATING HYDROCARBON CONTAMINATED EFFLUENTS

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Finding hydrocarbon (HC) degrading microbes with new and versatile skills in extreme environments, is a challenge in the wastewater treatment process. We have isolated an *Halomonas* sp. KHS3 strain from Mar del Plata port

seawater. The entire bacteria genome sequence was recently published (Gasperotti et al., 2015), as well as its ability to grow in gasoil (D´Ippólito et al., 2011). In this work we report the *H.* sp. KHS3 skill to use different HC for growing. The growth of *Halomonas* sp. in minimal medium with the addition of different HC, as the sole source of carbon and energy, was followed by turbidity at 600 nm. The bacteria exhibited improved growth in several polyaromatic HC: phenanthrene, naphthalene, fluorene and in benzoic acid; although, no significant growth was observed in aliphatic HC (hexane, ciclohexane). Analysis of the genome for identifying possible metabolic degradation pathways of *H.* sp. KHS3, allowed identifying 74 genes related to the metabolism of aromatic compounds. Most of these genes would be involved in catechol and protocatechuate branches of b-ketoadipate pathway. Furthermore, genes of salicylate, gentisate and homogentisate pathway were found. These results, coupled with the plasticity of this bacterium to grow at different temperatures (4-37ºC), pH (6-10) and salinities (0.5-12% w/v), highlights its potential use in bioremediation of hydrocarbon-contaminated salty places.

Neuroscience

NS-P01
MODIFICATION OF THE CELL CYCLE KINETICS IN *C-FOS -/-* NEURAL STEM/PROGENITOR CELLS

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Neurons of the mammalian CNS are originated from progenitors dividing at the apical surface of the neuroepithelium. These cells show a high proliferation capacity and an adequate control of their growth is very important. The protein c-Fos is known as an AP-1 transcription factor and as a protein that activate phospholipid synthesis. c-fos -/- mice, although viable, die at approximately 7 months of age, are infertile and growth-retarded with respect to their WT littermates. We know that the absence of c-fos in the developing cerebral cortex increases the number of apoptotic cells while reduced the number of differentiated cells. We determined the underlying bases of these differences. Neurospheres cultures show differences in the proliferation kinetics between c-fos -/- and c-fos +/+ NSPCs. The distribution of these cells along the cell cycle indicated an increase in the S phase length and a reduction in the G1 phase. Dual injections of EdU and BrdU in c-fos-/- and c-fos+/+ E14.5 also showed an increased number of cells at the S-phase in the embryonic cortical telencephalon of c-fos-/- mice, without changes in mitosis. These results sugest that the absence of c-fos modified the length of the S and G1 phases of the cell-cycle of NSPCs, which could be the explanation for the differences in apoptosis and differentiation between c-fos +/+ and c-fos -/- mice.

NS-P02
DEVELOPMENT OF A METHOD TO KNOCK DOWN HS3ST2 EXPRESSION IN A CELLULAR MODEL OF TAUOPATHY

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Tau abnormal phosphorylation has been identified as a critical event characterizing tauopathies such as Alzheimer's disease (AD) and frontotemporal dementias. It seems to require polyanions such as heparin. Furthermore, recent findings show that heparan sulfates (HS) can induce Tau abnormal phosphorylation in cellular models of tauopathy, were they colocalize and interact with Tau. Moreover, the enzyme HS3ST2, responsible for the 3-O-sulfation of HS, is overexpressed in AD brains. All this raises the question whether HS3ST2, or its products, are involved in the development of Tau-mediated neurodegeneration. In this work we aim to set up a method to knock down HS3ST2 expression in a mutation-dependent cellular model of tauopathy, consisting of differentiated SH-SY5Y human neuroblastoma cell line expressing mutant P301L Tau. To achieve it, we transducted cells with a lentiviral vector encoding shRNA sequences designed to induce HS3ST2 mRNA degradation. Transduction efficiency was assessed by fluorescence microscopy and flow cytometry. A protocol for qPCR and western blot determination of knock down efficiency was designed. We also characterized Tau and HS cell location in both normal and tauopathy conditions by confocal microscopy. Our results indicate that inhibiting HS3ST2 gene expression is possible with conditions optimization.

NS-P03
SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ASSOCIATED TO ALZHEIMER'S DISEASE IN THE ARGENTINE POPULATION

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Late-Onset Alzheimer's Disease (LOAD) is the most frequent form of dementia in adults. It is a complex disorder most likely caused by multiple genetic and environmental susceptibility factors. Currently, there are no known treatments to prevent, cure or delay the onset of the disease. Besides, only the allele e4 of apolipoprotein E gene (*APOE4*) has been irrefutably recognized as a risk factor, however it is absent in approximately 42% of patients with AD. In order to identify new genetic biomarkers, over the last decade, genome-wide association studies have opened up new avenues in detecting susceptibility SNP factors for LOAD. Our main goal is to identify which of these SNPs present high prevalence in the Argentinian population, and to evaluate their use as possible genetic-risk biomarkers. We recruited 267 patients with cognitive impairments, and 175 individuals without obvious clinical dysfunction. Cases and controls were genotyped for 95 SNPs previously associated to LOAD, and 97 biallelic SNPs associated to ancestry. So far, we observed that the studied group is an admixture of European and Native populations that presents the expected incidence of *APOE4* between cases and controls. We also validated other SNPs as risk/susceptibility factors. We are still analyzing the results to generate a risk-associated pattern for LOAD that will be tested in a second case-control experiment.

NS-P04
BRAIN BIOENERGETICS AND COGNITIVE PERFORMANCE IN A TRANSGENIC RAT MODEL OF EARLY ALZHEIMER'S DISEASE

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Synaptic bioenergetic deficiencies may be associated with early Alzheimer's disease (AD). To explore this concept we assessed pre-synaptic mitochondrial function in hemizygous (+/-) TgMcGill-R-Thy1-APP rats. The low burden of Aβ and the wide array of behavioral and cognitive impairments described in 6 month-old hemizygous TgMcGill-R-Thy1-APP rats (Tg(+/-)) support their use to investigate synaptic bioenergetics deficiencies described in subjects with early AD. Here we show that pre-synaptic mitochondria from Tg(+/-) rats evidence a decreased respiratory control ratio and spare respiratory capacity associated with deficits in complex I enzymatic activity. Cognitive impairment was prevented and bioenergetic deficits partially reversed when Tg(+/-) rats were fed a nutritionally complete diet from weaning to 6 month-old supplemented with pyrroloquinoline quinone, a mitochondrial biogenesis stimulator with antioxidant and neuroprotective effects. These results provide evidence that, as described in AD brain and not proven in Tg mice models with AD-like phenotype, the mitochondrial bioenergetic capacity of synaptosomes is not conserved in the Tg(+/-) rats. This animal model may be suitable for understanding the basic biochemical mechanisms involved in early AD.

NS-P05
NEUROTOXICITY OF PEPTIDE ADAN IN *Drosophila*: A MODEL FOR THE STUDY OF AMYLOID–ASSOCIATED DEMENTIAS

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Familiar Danish Dementia (FDD) is associated with a mutation of the BRI gene. FDD and Alzheimer's disease (AD) share amyloid accumulation (ADan and Aβ, respectively) and neurofibrillary tangles made of hyperphosphorylated protein Tau. We used *Drosophila melanogaster* (Dm) to compare the toxicity of amyloid peptides in the CNS expressed by the GAL4–UAS system. ADan expression impaired negative geotaxis in a dose–dependent manner. Homozygous flies climbed 60% less ($p<0{,}001$) than heterozygous both at 7 and 21 days, and 53% less ($p<0{,}001$) than flies expressing the non–amyloid peptide BRI2–23. Toxicity correlated with the accumulation of oligomeric, non fibrillar ADan species and oxidative stress. To study the role of Tau in ADan toxicity, we generated tau–deficient recombinant flies. The effect of ADan upon geotaxis was significantly lower in a tau–deficient background at 3 days (33%, $p<0{,}05$) and 7 days (39 %, $p<0{,}05$), suggesting that toxicity of ADan may be mediated in part by Tau, as proposed for Aβ in AD. Yet, preliminary data suggest that the phosphorylation pattern of endogenous Tau induced by ADan may be different to that reported for Aβ. This animal model will allow a systematic study of mechanisms of

neurotoxicity aimed at a comprehension of the molecular processes underlying amyloid–associated dementias. This work was supported by the Alzheimer's Association (IIRG 11–205127)

NS-P06
INFLUENCE OF β-AMYLOID ASSEMBLY ON SYNAPTOSOMAL STRUCTURE AND 2-ARACHIDONOYLGLYCEROL METABOLISM

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Among the multiple functions of 2-arachidonoylglycerol (2-AG) in the central nervous system, we can highlight its role as neuroprotective molecule. We have previously demonstrated a deregulation in 2-AG hydrolysis in an *in vitro* model of Alzheimer´s disease (AD), in rat cerebral cortex synaptosomes (Syn). The aim of the present study was to analyze the effect of βA oligomers (mimicking early AD stage) and fibrils (mimicking late AD stage) in Syn, and also to evaluate 2-AG synthesis by diacylglycerol lipase (DAGL) and lysophosphatidate phosphohydrolase (LPAase) in these AD models. Syn were isolated by differential centrifugation, purified in ficoll gradients, and incubated with different βA peptide conformations. LPAase and DAGL activities were assayed using radiolabeled substrates. We observed that βA oligomers disrupted synaptosomal membranes while fibrils not only caused synaptosome aggregation but also showed membrane damage probably exerted by oligomeric like structures. Also, similarly to 2-AG hydrolysis, its synthesis is differentially modulated in early and late AD stages. Whereas oligomers decreased DAGL activity, fibrils increased both LPAase and DAGL activities. Our results show important differences in early and late AD stages in 2-AG metabolism that could be partially responsible for the neurodegeneration observed in this pathology.

NS-P07
PERINATAL STRESS MODIFIES THE EXPRESSION OF CLOCK GENES

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Circadian systems express biological rhythms that display an entrainable oscillation of about 24 h. It is set by the intrinsic transcriptional activity of a group of genes called clock genes which are rhythmically expressed in the brain, at the suprachiasmatic nucleus (SCN) of the hypothalamus and peripheral tissues like lung and liver. At the cellular level, these rhythms are controlled by transcriptional feedback loops that produce oscillations in gene expression. We asked whether adverse experiences like perinatal malnutrition affect the expression of clock genes leading changes in circadian regulation in mice.
CF1 dams were fed a normal protein diet (NP) with 20% of protein or low protein diet (LP) with 8% of protein during pregnancy and lactation, and the male offspring was analyzed. We hypothesize that perinatal malnourished may also modulate the SCN activity in mice. We observed that *Bmal1*, *Per1* and *Per2* expression, oscillating genes in SCN, were phase delayed (NP vs LP). Moreover, *Creb1*, a key gene in cognition and memory was also delayed. Therefore, these results imply that the effects of fetal environment on adult health would include alterations in the function of the systems governing the control and regulation of circadian rhythms in behavior and metabolism. These findings show that perinatal stress like protein restriction has an important role on regulation of gen expression of the clock system.

NS-P08
NEURONAL MODULATION OF STRESS RESPONSE IN *Caenorhabditis elegans*

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In response to environmental challenges isolated cultured cells can autonomously trigger widely conserved molecular mechanisms to minimize cellular damages. However, this intrinsic capacity should be finely regulated in multicellular organisms. The neural coordination of the systemic stress response was first demonstrated in *C.elegans*. However, the identity of the signal that integrates stress perception with the response in non-neuronal cells is unknown. Our analysis of the *C.elegans* neuronal wiring diagram reveals that the circuits activated upon exposure to stressors converge in the tyraminergic neuron, RIM. Tyramine is the invertebrate counterpart for adrenaline. By using genetics, pharmacology, behavioral analysis and microscopic techniques, we found that tyramine-deficient animals are resistant to thermal and oxidative stress as well as to starvation and pathogen infection. Besides, these mutants

exhibit autophagy and lypolisis induction, even under favorable growth conditions. We also determined that TYRA-3 is the receptor involved in the tyraminergic control of stress response. Our results suggest that, independently of the etiology of the stress, inhibition of tyramine release is a neuroendocrine signal required for a coordinated triggering of the response in *C.elegans*. This study contributes to the understanding of the neurohormonal signaling in multicellular organisms.

NS-P09
AUTOPHAGY PARTICIPATION IN A MOUSE MODEL OF OXYGEN-INDUCED RETINOPATHY (OIR)

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Autophagy has been implicated in neurodevelopment and other physiological processes. However, it has also been involved in cell survival or death in some diseases. In retinopathy of prematurity (ROP), the hypoxic insult generates damage in organelles and misfolding of proteins, which could be eliminated by autophagy. Herein, we analyze the role of autophagy in the OIR model, which closely resemble ROP and Diabetic Retinopathy. C57/BL6 mice exposed to 75% O2 from postnatal day (P) 7 to 12 were brought to room air (RA) for additional 9 days (P26). Age-matched mice maintained in RA for 26 days, respectively, were used as control. All mice were injected intraocularly with 3-methyladenine (an autophagy inhibitor) or saline, into one eye of each mouse, at two different times: P12 and P17. Animals were sacrificed at P26. Retinal function and morphology, cell death (TUNEL) and immunofluorescence staining for autophagy (LC3) showed that the injection at P12, resulted in the more profound damage in both conditions, even though structural and functional alterations were more severe in OIR mice. Western blot of retinas allowed us to corroborate changes in stress and detoxifying proteins. In OIR, LC3 staining was observed in GFAP positive cells coinciding with results obtained in Müller cells under hypoxic conditions. The results suggest that autophagy blockade is detrimental for retinal tissue.

NS-P10
SINGLE-CHANNEL ANALYSIS OF NICOTINIC ACETYLCHOLINE RECEPTOR α4β2 ACTIVATION AND MODULATION BY LYPD6

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Nicotinic acetylcholine receptor (nAChR) α4β2 is the most abundant nAChRs in the brain, which can be modulated by endogenous molecules such as Lypd6. Our aim is to characterize its activation kinetic and how Lypd6 affects this process. Analysis of channel activation was carried out on cells transiently transfected with α4β2 subunits by patch-clamp in the cell-attached configuration. Acetylcholine (ACh) elicited single-channel activity at all concentrations whose frequency increases with concentration (0.5-100 µM). Two channel amplitudes were recorded, a high (HC) and low (LC) conductance. LC channel predominated at low ACh concentration, meanwhile HC channel became more frequent at higher concentration. Both channel-conductance exhibited 2 open components although duration and activation patterns were different: LC channel gated mostly as single events meanwhile HC channel showed a cluster behavior. Our data suggests that α4β2 assemble with 2 stoichiometries: one with high affinity but low efficacy (LC channel (α4)2(β2)3) and the other with low affinity and high efficacy (HC channel, (α4)3(β2)2). Co-expression of Lypd6 with nAChR yields a 10-fold increase in channel frequency without significant changes in conductance, mean open time or channel activation patterns. Thus indicating Lypd6 modulation of α4β2 by receptor desensitization, channel trafficking or cell membrane stability.

Plant Biochemistry and Molecular Biology

PL-P01
CHARACTERIZATION OF PHOTOSYSTEM PARAMETERS OF TRANSPLASTOMIC LINES EXPRESSING VACCINE ANTIGENS

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Chloroplasts transformation technology has emerged as an alternative platform for antigen expression in plants. In particular, we demonstrated that the fusion of *Toxoplasma gondii* SAG1 antigen to *Leishmania infantum* Hsp83 (LiHsp83-SAG1) significantly increased the level of SAG1 accumulation in chloroplasts compared to SAG1 alone. However, LiHsp83-SAG1 line showed chlorotic phenotype and growth retardation. In order to understand these unintended pleiotropic effects, we characterized the photosystem parameters of LiHsp83-SAG1 and compared them with SAG1 and GRA4 transplastomic lines versus wild type plants (WT). Completely developed leaves from 42 old-days plants were used to measure foliar area, chlorophyll content by chemical and spectrophotometry (SPAD) methods and chlorophyll fluorescence (OJIP). SAG1 foliar area was 17% bigger than WT, while LiHsp83-SAG1 was 23% smaller than WT. SPAD and chemical methods showed similar results, transplastomic lines had less chlorophyll content than WT. OJIP analysis showed no differences among SAG1, GRA4 and WT plants. On the other hand, LiHsp83-SAG1 revealed low values for most of the parameters measured, such as maximum quantum yield of primary photosystem II photochemistry and electron transport per excited cross section. This first approach suggests that photosystem parameters in LiHsp83-SAG1, especially those related to PSII, are damaged.

PL-P02
CHARACTERIZATION OF TWO MAIZE FLAVONE SYNTHASES CLASS II FROM MAIZE

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Flavones are found extensively in plants and have diverse physiological functions. There are two classes of flavone synthase (FNS) enzymes that catalyze the conversion of flavanones into flavones. The FNS class II comprises oxygen- and NADPH-dependent cytochrome P450 membrane-bound monooxygenases .We identified two genes encoding putative FNSIIs in maize named *Zm*CYP93G6 (G6) and *Zm*CYP93G7 (G7). Relative expression was assessed in different maize tissues. *G6* showed higher relative expression levels than *G7* in roots, hypocotyls and radicles, while *G7* transcripts were predominant in pericarps, silks and anthers. To examine whether these enzymes could participate in flavone biosynthesis, we cloned the open reading frames in a yeast expression vector, transformed the WAT11 yeast strain and cultures of transformed yeast cells were fed with the flavanones substrates. LC-MS/MS analyses showed that yeast expressing G6 or G7 accumulated the corresponding flavones. Interestingly, the analysis did not detect 2-hydroxinaringenin as an intermediate product, indicating that the reaction mechanism is not similar to that of flavanone 2-hydroxilases. Moreover, so as to determine their activities *in planta*, we generated Arabidopsis transgenic plants expressing *G6* or *G7* and the levels of flavones accumulated will be analyzed by HPLC. Together, our results provide evidence that G6 and G7 are FNSII enzymes.

PL-P03
CHARACTERIZATION OF A NOVEL *Ostreococcus tauri* PHOSPHOGLUCAN PHOSPHATASE LIKELY INVOLVED IN STARCH METABOLISM

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Phosphoglucan phosphatases (PP) remove phosphates from complex carbohydrates. In plants, these enzymes are vital components in the remobilization of leaf starch at night. To understand the evolution of catalysis and regulation of these enzymes we decided to investigate their existence in the tiniest eukaryotic green algae *Ostreococcus tauri*. *O. tauri* transcriptome BLAST searches revealed one locus encoding a protein with high sequence similarity to plant PP. Through an exhaustive *in silico* analysis we concluded that the putative protein could be a chloroplastic enzyme which shares several structural features with its plant counterparts. To further characterize this finding the cDNA was cloned to express and purify the recombinant protein in *E. coli* cells. *In vitro*, we verified phosphatase activity of the

recombinant enzyme against the non-specific substrate p-nitrophenyl phosphate as well as its ability to bind polysaccharides. Besides, by native PAGE we could determine that this enzyme could act as a dimer. Finally, glucan-phosphatase activity, on its real substrate, will be assayed in order to strength the idea of its role in starch metabolism.

PL-P04
A PROTEIN KINASE IS REQUIRED FOR PROPER NODULATION IN THE COMMON BEAN-*Rhizobium etli* SYMBIOSIS

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Phaseolus vulgaris is an agronomical important legume that establishes a nitrogen-fixing symbiosis with *Rhizobium etli*. In this interaction, bacteria infect the root to reach the nodule, a post-embryonic organ formed by division of cortical cells. A previous study from our laboratory showed that the C1 subunit of the plant nuclear factor NF-Y is a key regulator of infection and nodule organogenesis. In order to identify other factors involved in this signaling pathway, a yeast two hybrid screening was performed. One of the positives clones encodes a protein kinase with a transmembrane domain at the N-terminus. The interaction has been confirmed by both co-immunoprecipitation and bimolecular fluorescence complementation. The protein is distributed between the plasma membrane and the cytoplasm. Post-transcriptional silencing of the kinase by RNAi led to a reduction in the number and size of nodules and affected the infection thread formation in *P. vulgaris* roots. Interestingly, partner selection between high- and low- quality symbiotic partners was also affected in coinoculation experiments. Taken together, these results suggest that this gene is required for rhizobial infection, nodule organogenesis and the molecular mechanism underlying bacterial strain preference.

PL-P05
CHARACTERIZATION OF THE MECHANISM OF STONE HARDENING DURING THE DEVELOPMENT OF PEACH FRUIT

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Lignification of peach (*Prunus persica*) fruit endocarp involves a strictly coordinated process, which consists of lignin biosynthesis, deposition in secondary cell wall and programmed cell death. Understanding the mechanisms and regulations involved in lignin synthesis and the formation of stone in the development of endocarp in peach fruit, is relevant not only scientifically but also economically by the benefits that could come off in terms of fruit and biofuel production. In the present work, proteomic studies were performed in order to identify differential protein expression during early stages of peach fruit development. By using two-dimensional differential gel electrophoresis, 30 differentially expressed proteins among the E1 (10 DAB), S1 (30 DAB) and S2 (50 DAB) proteome samples were detected. From them, 14 proteins increased along the development, while 16 proteins decreased from E1 to S2 stages. Detected proteins include polyphenoloxidases and ABA/WDS induced proteins, among others. Furthermore, the level of expression of some enzymes involved in the phenylpropanoid pathway, which results in anthocyanin synthesis and precursors of lignin, was analyzed by qRT-PCR in endocarp, mesocarp and exocarp tissues obtained from the three stages. Overall, the present study may contribute to understand the molecular basis for stone formation during early peach fruit development.

PL-P06
PPTRAF IS A HEAT TREATMENT-INDUCED RNA CHAPERONE WITH A ROLE AGAINST CHILLING INJURY IN PEACH FRUIT

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Once harvested, peaches [*Prunus persica* L. Batsch] ripen quickly making refrigeration essential to allow transportation and marketing. However, cold storage can produce chilling injury (CI) symptoms, severely affecting nutritional and organoleptic quality of fruit. Our group has characterized the differential transcriptome, metabolome and proteome of peaches cv. Dixiland subjected to a heat treatment (HT, 3 days at 39 °C), an effective strategy in preventing and/or reducing CI-associated disorders. PpTRAF was identified as one of the HT-induced transcripts and it encodes a TRAF (TNF Receptor-Associated Factor)-type Zn-finger protein with unknown function in plants. We confirmed its HT and cold storage induction in several peach cultivars by RT-qPCR and assessed its molecular

function as RNA chaperone through an *in vivo* bacterial system at low temperatures. The subcellular localization of PpTRAF was predicted *in silico* (along with some of its orthologs) and determined experimentally by transient transformation of *Nicotiana benthamiana* leaves to be both cytosolic and nuclear. In addition, PpTRAF protein architecture was compared with the one of its orthologs and its structure was predicted by ab initio modelling. These findings give support to a putative role of PpTRAF in one or more processes of RNA metabolism during the storage of peach fruits at low temperatures.

PL-P07
STUDY OF THE EFFECT OF UV-B RADIATION ON THE RETINOBLASTOMA PATHWAY IN *Arabidopsis thaliana*

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The development and growth of multicellular organisms depend on the correct spatiotemporal coordination of cell proliferation, cell differentiation, and subsequent cell specialization. Cell division is a tightly regulated process that is influenced by innate genetic cues as well as by external environmental signals. The Retinoblastoma pathway (RBR) has an important role in the control of cell division and proliferation in plants. Because UV-B radiation negatively affects cell proliferation and development, we analyzed the effect of this radiation in different *Arabidopsis thaliana* mutants and transgenic lines, these plants have altered expression of genes encoding members of the RBR pathway, in particular the E2F transcription factors. We are now investigating the role of E2Fc, which is a transcription factor that negatively regulates the expression of genes that participate in cell division. Our results show that plants with decreased levels of E2Fc (E2FcRNAi) that were UV-B irradiated, had decreased inhibition of leaf growth than wild type plants. We also evaluated physiological parameters after UV-B exposure, and analyzed the expression of UV-B regulated genes. Together, our data demonstrate that E2Fc participates in UV-B responses in *Arabidopsis thaliana*.

PL-P08
THE ATPASE ACTIVITY AND ASSEMBLY OF CLPB3/HSP100 FROM *Arabidopsis thaliana* CHLOROPLASTS

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To counteract protein aggregation, bacteria, fungi and plants contain a bi-chaperone system composed of ATP dependent Hsp70 and ClpB/Hsp100 chaperones, which rescue aggregated proteins and provide thermotolerance to cells. The ClpB component of this system is an AAA+ ATPase that forms an hexameric functional ring-like structure of identical protomers, each one containing two nucleotide binding domains (NBD-1 and -2). ClpB oligomerize in response to different stimuli such as nucleotide binding or ionic strength, which in turn regulate the activity of the chaperone. In this work, we provide the first characterization of the ClpB3/Hsp100 from *A. thaliana* chloroplasts. Oligomerization in the presence of ATP was studied by SEC and the innate ATPase activity analyzed under several conditions by the Malachite Green method. We have established that ATP stimulates hexamerization while high ionic strength favors disassembly of the complexes. Furthermore, we have determined the kinetic parameters of the enzyme and its activity under a range of pH values and temperatures. ClpB3 lacks activity at pH values equal to or lower than 4.5 and has an optimal activity at temperatures around 62°C. Since the overall behavior of the enzyme resembles that of bacterial and yeast homolog, these results suggest that ClpB3 from *A. thaliana* could act similarly in the acquisition of plant thermotolerance

PL-P09
GROWTH INHIBITION OF PHYTOPATHOGENIC FUNGI BY CHLOROGENIC ACID

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Chlorogenic acid (CGA), recognized as a strong antioxidant, is a plant secondary metabolite which is the ester of caffeic acid and quinic acid. It is synthesized trough the phenylpropanoid pathway and accumulates in diverse plant tissues. CGA is the major phenolic compound present in sunflower seeds, although its function is currently unknown. It has been suggested that, as other secondary metabolites, it could act as a phytoanticipin being part of the plant's defensive arsenal. The aim of this work was to determine whether CGA could control the growth of the pathogenic

fungi *Sclerotinia sclerotiorum* and *Fusarium solani*, gaining insight into its mechanism of action. Microscopic analysis showed a dose-response effect of CGA on mycelial growth inhibition and spore germination for both fungi. 15 µg/µl CGA completely abolished spore germination and caused cell death. Early membrane permeabilization was observed in treated spores, revealed by the uptake of probes indicative of altered membrane integrity. Lower concentrations of CGA (2-10 µg/µl) do not abolished spore germination but produced a partial inhibition of mycelial growth and severe changes in hyphal morphology.

PL-P10
LINKING SENSORY ATTRIBUTES, CHILLING INJURY AND CHEMICAL COMPOSITION OF PEACH FRUITS

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Fruits from three peach varieties with different susceptibility to chilling injury (CI) were selected to analyze quality parameters and consumer´s acceptance, after storage at 0ºC. The varieties selected were Flordaking (FD, CI-susceptible), Rojo 2 (R2, CI-intermediate susceptibility) and Spring Lady (SL, CI-resistant). The % of expressible juice, soluble solids concentration (SSC), and metabolite content assessed by GC-MS in the mesocarp of peach fruits, were analyzed at harvest and after cold storage. The consumer´s acceptance of cold-stored fruits was performed by a panel of 100 hundred consumers. The variables juiciness and flavor evaluated by the consumers were correlated to the % of expressible juice and SSC, respectively. The varieties selected by consumers as the juiciest were those with the highest % of expressible juice, while the varieties perceived with green flavor showed the lowest SSC. The degree of CI was negatively correlated to consumer's acceptance. Regarding metabolite analysis, it was found a positive correlation between raffinose content and CI resistance, with SL displaying 2- to 3-times higher content than the more susceptible cultivars. Therefore this metabolite may be directly related to such resistance. Since CI greatly impairs consumer´s satisfaction, identification of metabolites involved in CI-resistance would be helpful to assist breeding programs

PL-P11
SYMBIOTIC ASSOCIATIONS IN THE RHIZOSPHERE. METABOLITE EXCHANGE AND GENE EXPRESSION REGULATION

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Trichoderma species are soil borne fungi able to establish a symbiotic association with plant roots or execute biological control of pathogenic fungi. Functional studies using the model strain *Trichoderma virens*, revealed the importance of plant-produced sucrose as a key player in the control of the symbiotic association. Particularly, previous studies showed that a mutant *T. virens* strain, impaired in the expression of an intracellular invertase (*tvinv*), displays five times greater root colonization rate than wild type strain. To further investigate the role of plant metabolites during the symbiotic association, we sought to compare the differential effect of sucrose on wild type and a tvinv knock out strain. When comparing protein patterns (SDS-PAGE) in culture filtrates, we detected five differential bands identified as a bifunctional catalase-peroxidase, transaldolase, 6-phosphogluconate dehydrogenase and two unknown proteins. The differential expression was further confirmed through activity assays and/or RT-qPCR. Importantly, similar correlations were also found when fungal mycelium was inoculated to A. thaliana roots in hydroponic systems. These results confirmed the importance of sucrose metabolism inside fungal cells for gene expression regulation during host colonization, including a peroxidase likely involved in ROS detoxification or cell wall degradation

PL-P12
BIOCHEMICAL CHARACTERIZATION OF NOVEL DNA-BINDING PROTEINS RELATED TO PLANT-PATHOGEN INTERACTIONS

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DNA-binding proteins play crucial roles in genomic organization, cell cycle and gene expression regulation. Genomic surveys in plant-pathogenic fungi identified candidate proteins likely to be targeted to host nuclei. Previous

functional characterization showed that CgEP1 defines a novel class of DNA binding proteins transported to the host nucleus, and is essential for stalk rot development in maize plants. In the present study, we expressed and biochemically characterized the recombinant version of CgEP1. Although, *in silico* studies suggest a molecular weight around 40 kDa, the purified protein was processed into discrete polypeptides that range from 40 to 5 kDa displaying two main forms named: bCgEP1 (40 kDa) and sCgEP1 (5 kDa). This observation suggested that CgEP1 might self-proteolytic activity. Besides, structural characterization of CgEP1 at different pHs with or without DNA reveals that several oligomeric forms of CgEP1 are involved in the different conditions evaluated. To analyze the DNA-CgEP1 interaction, incubations of bCgEP1 and sCgEP1 with DNA in different conditions showed that the interaction is dependent on the number of protein molecules, and that CgEP1 also has the ability to alter DNA structure. These results points to novel mechanisms for protein-DNA interactions and shed some light into the molecular mechanisms underlying plant disease development

PL-P13
FUNCTIONAL CHARACTERIZATION OF DC1 DOMAIN-CONTAINING PROTEINS: THEIR ROLE IN PLANT DEVELOPMENT

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A comprehensive study of genes involved in plant defense responses led us to identify a set of genes coding for DC1domain -containing proteins whose expression is strongly induced by pathogens and elicitors in *Arabidopsis thaliana*. The analysis of insertional mutants of these genes revealed that at least one of them, *At2g17740* is required for the normal development of the female gametophyte. Expression reporter lines *pAt2g17740-GUS, p35S-At2g44370-GFP* and *pAt2g17740-At2g44370-GFP* were generated in order to functionally characterize this gene. The analysis of *pAt2g17740-GUS* reporter lines showed expression in hypocotyls, roots, leaves and apical meristem, depending on the developmental stage. Furthermore, enhanced GUS activity was detected under abiotic stress in the whole rosette. Lines expressing At2g17740 as a translational fusion with GFP allowed us to detect the expression in the embryo sac, in agreement with its requirement to fulfill female gametophyte development. In addition, based on the GFP-reporter lines results we report a subcellular localization of this protein in the plasmatic membrane and endomembrane system. Supported by Conicet, ANPCyT, CIC and UNMdP.

PL-P14
PHOSPHORUS DEFICIENCY IN SOYBEAN PLANTS

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Phosphorus (P) is a macronutrient necessary for energy storage and an integral component of several compounds in plant cells. Limitation of crop productivity by P is widespread and is likely to increase in the coming years. Plants have evolved different mechanisms to cope with low soil P-availability such as modification of root architecture, and exudation of organic acids. Nitric oxide (NO) plays important roles in plants, being involved in physiological processes and stress responses. The hypothesis of this work was that NO participates in early soybean responses to P restriction. Seven-day-old plants were exposed for 24 h to a medium lacking P. Total P concentration decreased significantly in roots (6.6 ± 0.6 and 3.5 ± 0.8 µmol/g FW in P+ and P-, respectively) and leaves (10 ± 1 and 5.8 ± 0.2 µmol/g FW in P+ and P-, respectively). Electron paramagnetic resonance (EPR) and fluorescence microscopy were employed to assess endogenous NO levels. Confocal laser microscopy (employing DAF-FM DA) clearly showed higher NO level in P- leaves. Analysis of nitrotyrosines by two-dimensional PAGE/western blot/Mass spectrometry showed an increase in protein tyrosine nitration in leaves of P- plants. In addition, differential leaf proteins expression was analyzed by nLC-ion trap MS. These results suggest that NO is involved in the early events related to P scarcity. PICT 2012-0429.

PL-P15
STRUCTURE-GUIDED STUDY OF THE ACTIVE SITES OF GLYOXALASE I FROM *Zea mays*

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The glyoxalase system is ubiquitous among all forms of life, due to its central role at relieving the cell from the accumulation of methylglyoxal, a toxic metabolic by-product. In higher plants, this system is up regulated under diverse metabolic stress conditions, such as defense response to infection by pathogenic microorganisms. *Fusarium verticillioides* is the most common pathogen of maize and we have observed that the expression of GLXI is upregulated in moderately resistant maize lines compared with susceptible ones. In this study, glyoxalase I from *Zea mays* has been characterized both biochemically and structurally, reporting the first atomic model of a GLXI available from plants. Our results show that this enzyme comprises a single polypeptide with two structurally similar domains, one of which harbors a functional Ni(II)-binding active site, as confirmed through site-directed mutagenesis. E144Q mutant abolished one of the amino acid residues involved in metal cofactor binding, allowing us to confirm that GLXI is a Ni(II)-dependent metalloenzyme. The putative function of the remaining cryptic active site is being evaluated through regeneration of the ability to bind metal by the V278E mutant.

PL-P16
STUDY OF THE PYRUVATE CARBOXYLASE ACTIVITY OF NADP-MALIC ENZYME IN C_3 PLANTS

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NADP-malic enzyme (NADP-ME) catalyzes the reversible oxidative decarboxylation of malate to pyruvate, CO_2 and NADPH. In *A. thaliana*, cytosolic NADP-ME2 is the isoform that mostly contributes to the forward NADP-ME activity. Studies with recombinant NADP-ME2 showed that it also has a high catalytic efficiency for the reverse reaction, *i.e.*, the reductive carboxylation of pyruvate, and that this activity is differentially regulated by cell metabolites and pH. In this work, Arabidopsis mutant and overexpressing lines in NADP-ME2 were used to assess this possible anaplerotic reaction of NADP-ME. Total pyruvate carboxylation activity was measured in leaf extracts, being lower in plants lacking NADP-ME2 and higher in overexpressing lines, compared to wild type. In addition, the level of substrates and products of the reaction was analyzed. The plants overexpressing NADP-ME2 displayed higher malate and lower pyruvate content relative to wild type throughout day and night. Moreover, NADP/NADPH ratio was increased in these plants during the night. These results suggest that NADP-ME2 may be preferentially catalyzing the reverse over the forward reaction in the overexpressing lines. Thus, the pyruvate carboxylase activity of NADP-ME may take place in plant cells, contributing to the synthesis of C_4 compounds and being regulated by cell metabolic status and environmental changes.

PL-P17
POST TRANSCRIPTIONAL SILENCING OF RNASES DURING PHOSPHATE STARVATION IN *Nicotiana*

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Ribonucleases (RNases) T2 are endonucleolytic enzymes that catalyze the cleavage of single-strand RNA producing mononucleotides with a terminal 3' phosphate (Pi). In plants, different classes of RNases T2 are induced in a variety of stress scenarios, including Pi starvation. The induction of these RNases contributes to Pi mobilization from nucleic acid sources. In Nicotiana alata, class III NnSR1 (Nicotiana non S-RNase1) and class I S-like-RNase NE are, thus far, the only RNases induced for Pi mobilization. NnSR1 is localized in endoplasmic reticulum of root cells while RNase NE is secreted to the rizosphere to scavenge extracellular RNA sources. To discern whether the induction of these two RNases is simultaneous or sequential, as well as the Pi threshold triggering such induction, plants were cultivated under Pi limiting concentrations. Early induction of NnSR1, assayed by western blot and in gel RNase activity, suggested that the intracellular RNA may be the first source of Pi used by the cell under Pi stress. To understand the functional role of these RNases, a post transcriptional silencing strategy was developed. An hypervariable region of both RNase NE and NnSR1was selected to clone into a binary vector. Preliminar PCR, restriction analysis and subsequent plasmid DNA sequencing indicated successful cloning to express the hairpin RNA required for silencing RNase expression.

PL-P18
MALATE ROOT METABOLISM IN PROCESSES ASSOCIATED WITH THE MAINTAINING OF CELLULAR WATER STATUS

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Water scarcity is a major cause of crop losses worldwide and great efforts are being made to achieve drought tolerant crops, especially in countries like Argentina, whose economy is based on the agricultural production. In Arabidopsis cells, the malic enzyme (ME) family is represented by four enzymes which catalyze the malate oxidative decarboxylation in the presence of NADP. NADP-ME1 is a cytosolic isoform which stands out due to its evolutionarily conservation, delimited expression to roots and kinetic characteristics. Here, a more detailed analysis using reporter gene fusions showed the presence of NADP-ME1 in the region of maturation. The treatment with absicic acid (ABA), a drought response mediator, specifically induced the expression of NADP-ME1 in the meristematic cells. In line with this, Arabidopsis mutant plants lacking NADP-ME1 presented an increased resistance to drought and lower response of root growth to ABA. These results suggest that NADP-ME1 plays a very specialized role in roots possibly regulating the concentration of the substrates and/or reaction products to feed metabolic pathways and regulatory functions triggered by the water stress signal.

PL-P19
TRANSIENT EXPRESSION OF *ST*ASP-PSI DOMAIN IN POTATO LEAVES DURING *Phytophthora infestans* INFECTION

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Plant specific insert (PSI) is a domain present in the precursors and mature atypical plant aspartic proteases (APs). Several plant APs have been associated with the plant mechanism of defence against pathogens. However, only two, *St*AP1 and *St*AP3, for *Solanum tuberosum* APs of these proteases, contain the PSI domain into the mature form. We have previously reported the cytotoxic activity of the recombinant *St*AP-PSI towards plant pathogens. However the role of PSI domain of StAPs in the plant mechanism defense is still unknown. The aim of this work was to analyze the effect of transient expression of *St*AP-PSI in potato leaves infected by *P. infestans*. Results obtained show that *St*AP-PSI expression reduces the *P. infestans* affected area in a 60 % compared with the control ones. Analysis by qPCR shows an increase of the transcript level of the hypersensitive response marker (hsr203J) in potato leaves that express *St*AP-PSI. Hsr203J induction was independent of the *P. infestans* infection, however the highest increase of this gene was detected in leaves at 6 h. after infection. Additionally, an increase of the WRKI1 transcript level was detected in potato leaves that express *St*AP-PSI. Results obtained here indicate that, PSI domain of *St*APs could have a direct (as antimicrobial compound) and indirect (as an inductor molecule) role in the plant mechanism to restrict the pathogen spread.

PL-P20
IDENTIFICATION AND CHARACTERIZATION OF KEY CIRCADIAN CLOCK GENES IN TOBACCO HAIRY ROOTS

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Circadian clocks emerged to respond to external signals and to anticipate predictable changes in the environment that occur during the day/night transition strongly suggesting that a key function of the circadian clocks is to confer adaptive advantages. The main goal of this work was to identify and characterize different clock genes (CG) in *Nicotiana tabacum* hairy roots cultures (HRC). To identify tobacco CG, the screening of the publicly available tobacco and Arabidopsis expressed sequence tag (EST) databases was performed using the tBLASTn protocol. The candidate CG, including LHY, TOC1, FKF1, PRR7 and PRR9, were selected on the base of the high identify and the lowest E value. The results showed that not only tobacco seedlings but also the HRC grown *in vitro* keep the circadian expression of CG assessed. Moreover, the expression profiles observed were similar to the oscillatory patterns reported for *A. thaliana* CG. In addition, the synchronization by a photoperiod with light/dark cycles was very important to maintain the oscillation in the CG expression for both seedlings and the HRC. Therefore, this is the first finding showing that the HRC, devoid of aerial part, were subject to synchronization and displayed circadian CG expression, this further supports the in vitro cultures as a model system to study different biological processes involving CG.

PL-P21
RECOMBINANT EXPRESSION OF *Arabidopsis thaliana* SNRK1 SIGNALLING CASCADE

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The SnRK1 signalling cascade is involved in cell energy homeostasis maintenance in plants. The system is activated by starvation conditions and triggers changes in gene expression and enzyme activity by phosphorylation, thus regulating carbon/nitrogen metabolism. In Arabidopsis, SnRK1 is composed of 9 genes and its mode of action and regulation remains inconclusive. We developed a recombinant strategy to express (co-express) in *E. coli* proteins from the Arabidopsis SnRK1 signalling cascade: an SnRK1 α catalytic subunit, regulatory subunits (β and γ) and the Geminivirus Rep-Interacting Kinase 1 (GRIK1, At3g45240). The expression and purification strategy was optimized to obtain a recombinant His6-tagged GRIK1 in (auto)phosphorylated form. Besides when the latter was co-expressed with the SnRK1 α2 both proteins resulted phosphorylated. Using a spectrophotometric kinase assay coupled to PyRKase/LDHase enzymes, we determined that the recombinant His6-tagged GRIK1 was active an able to transphosphorylate and activate the SnRK1 α2 subunit. Results indicate that co-expression strategies would provide adequate molecular tools to produce different SnRK1 kinases. The characterization of the target specificity and the kinetic/regulatory properties of these recombinant SnRK1 kinases would critically contribute to better understand the regulation of carbon and nitrogen metabolism in plant cells.

PL-P22
ON THE ROLE OF PEACH ALDOSE 6 PHOSPHATE REDUCTASE AGAINST OXIDATIVE STRESS

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Glucitol (Gol) metabolism in plants has been studied in the last 20 years, though the mechanisms regulating the enzymes involved are not deeply understood. Gol is a compatible solute of relevance to protect cells exposed to osmotic stress. Gol is produced in leaves by two succeeding steps: i) NADPH dependent reduction of glucose 6 phosphate into glucitol 6 phosphate by aldose 6-phosphate reductase (Ald-6PRase), and ii) the subsequent dephosphorylation of Gol-6P. Here we report that beyond the osmoprotectant role of Gol its metabolism would be regulated in response to oxidative stress. Under stressful conditions, susceptible proteins are oxidized, with consequent detrimental changes. NADPH is the main reducing power source to sustain antioxidant systems involved in maintaining cell functionality. Ald-6PRase activity is diminished by oxidants compounds and the inactivation is not restored by reducing agents. Furthermore, oxidants produce destabilization inducing protein aggregation. Experiments performed with labelled glutathione showed that Ald-6PRase is glutathionylated, a modification that protects the enzyme against oxidative aggregation. Oxidative inactivation of Ald-6PRase would decrease the carbon flux through the polyol pathway, thus preserving NADPH to feed antioxidant systems. The process would be modulated by glutathione to balance oxidative and osmotic stresses.

PL-P23
STUDYING CARBON AND ENERGY METABOLISM IN AUTOTROPHIC AND HETEROTROPHIC *Chlorella* CELLS

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In plants, photosynthetic (or autotrophic) and non photosynthetic (or heterotrophic) cells have different carbon and energy fluxes, being photosynthates produced by autotrophic cells and providing carbon and energy to heterotrophic cells. Instead, some unicellular green algae can growth as photosynthetic or heterotrophic (also mixotrophic) cells according to the culture conditions. *Chlorella* sp, is a particular example, since it is able to produce and accumulate varying proportions of lipids (predominantly heterotrophic) and/or starch (mainly photosynthetic) according to the growing conditions. The metabolic changes produced in each kind of cells are not completely understood. To study the dynamics of carbon partitioning in *Chlorella sp.* we grew the cells in two different media: BOLD3N and M16, auto- and hetero-trophic, respectively. The resulting cells showed different protein profiles by 2D-electrophoresis. Differential expression of specific proteins was also detected by western blot analysis. We determined the activity of enzymes involved in key steps of carbon metabolism and found important differences in cells accumulating starch or

lipids. The ability of *Chlorella* to modify its metabolism and change the type of carbon reserve constitutes a useful system to understand carbon partitioning in cells accumulating lipids and/or starch, a subject that is critical in biotechnology.

PL-P24
CHARACTERIZATION OF THE CHLOROPLAST RETROGRADE SIGNAL THAT REGULATES ALTERNATIVE SPLICING

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We have previously shown that light/dark conditions affect several alternative splicing events in Arabidopsis thaliana. Using drugs that inhibit the photosynthetic electron transport chain, we found that DCMU mimics the effect of darkness, whereas DBMIB mimics the effect of light on alternative splicing. This led us to propose the redox state of the plastoquinone pool as the switch that triggers a retrograde signal that modifies alternative splicing choices in the nucleus. Using high performance liquid chromatography we demonstrated that the redox state of the plastoquinone pool changes with our light/dark protocol. In order to investigate the nature of the retrograde signal, we have established the experimental conditions to obtain Arabidopsis mesophyll protoplasts. Similarly to Arabidopsis seedlings, protoplasts display a light/dark effect on alternative splicing. We incubated protoplast preparations in light or dark for a certain time, and then centrifuged them to collect the conditioned medium. The conditioned medium obtained from light-exposed protoplasts causes changes in alternative splicing in protoplasts kept in the dark, similar to those observed in protoplasts exposed to light. This suggests that, upon light incubation, protoplasts produce a signaling molecule that accumulates in the conditioned medium and then modulates alternative splicing.

PL-P25
CHARACTERIZATION OF CALMODULIN-BINDING PROTEIN IQ67-DOMAIN (IQD) IN *Arabidopsis thaliana*

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Calmodulins (CaM) and CaM-like (CML) proteins are principal sensors of intracellular calcium fluctuation, which are generated by plants in responses to abiotic and biotic stimuli. A novel class of putative plant-specific CaM and CML targets is the IQ67-Domain (IQD) family protein. Surprisingly, little is known about the biological roles of these proteins. We have studied the response to different abiotic stress condition of three members of *Arabidopsis thaliana* IQD family (IQD28, -30 and -32). Their transcripts levels were greatly induced in roots after osmotic treatment and drought, in agreement with the presence of cis-element like ABRE in their promoter regions. The subcellular localization of IQD28 and -30 proteins was also studied. Agrobacterium-mediated transient transformation of *N. benthamiana* leaves with CFP-fusion proteins revealed a cytosolic localization for both proteins. Since a putative nuclear localization is predicted for them, transgenic Arabidopsis plants were generated by Floral dip to evaluate changes in the localization in response to osmotic stress, ABA and drought. Roots of knock out lines are being phenotypically characterized in response to abiotic stress. We have cloned, expressed and purified IQD28 and -30 recombinant proteins and we are currently testing their ability to bind calmodulin as well as looking for their targets.

PL-P26
THE SESQUITERPENE BOTRYDIAL PRODUCED BY *Botrytis cinerea* INDUCES PHOSPHATIDIC ACID PRODUCTION

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Phosphatidic acid is a lipid second messenger involved in the induction of plant defense responses. It is generated via two distinct enzymatic pathways, either via phospholipase D (PLD) or by the sequential action of phospholipase C and diacylglycerol kinase (PLC/DGK). In tomato (*Solanum lycopersicum*), phospholipase C enzymes are encoded by a multigene family named SlPLC1 – SlPLC6. Recently we have shown that SlPLC2 silenced plants are less susceptible to the necrotrophic fungus *Botrytis cinerea*. Botrydial is a phytotoxic sesquiterpene produced by *B. cinerea* that induces diverse plant defense responses such as production of reactive oxygen species (ROS). The aim of

this work was to determine whether botrydial is able to induce PA production using tomato cell suspensions as a model system. We observed that PA production was induced in botrydial-treated tomato cells in a time and dose-dependent manner. Botrydial triggered the formation of PA via PLC/DGK but not via PLD. Responses downstream of botrydial-induced PA were studied. PLC inhibitor U73122 or DGK inhibitor R59022 diminished ROS production induced by botrydial. It can be concluded that PLC/DGK-derived PA is a novel component of the signaling pathway activated by botrydial.

PL-P27
COMPARATIVE ANALYSIS OF ALKALINE/NEUTRAL INVERTASES AMONG PLANTS

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Sucrose (Suc) plays a central role in plant growth and development, and in response to environmental stresses. Alkaline/Neutral-Invertases (A/N-Inv) (localized in the cytosol, mitochondria and/or in plastids) hydrolyze Suc to hexoses for maintenance functions. Recently significant roles in root morphogenesis, germination, photosynthetic apparatus biosynthesis, abiotic and biotic stress tolerance have been revealed. A/N-Inv multigene families have been reported in *A. thaliana* (9 genes), *O. sativa* (8), *P. trichocharpa* (16), *V. vinifera* (9), *L. japonicus* (7) and *M. esculenta* (11). In the present study we identified the A/N-Inv family in the wheat genome, analyzed its structure, phylogeny and expression patterns, which were compared with other plants. Similarly to rice, we identified 4 and 3 putative genes coding for cytosolic (Ta-NIN5 to 8) and organelle isoforms (Ta-NIN1 to 4), respectively, whose sequences were categorized into the two major clades described. Expression analyses showed that genes coding for cytosolic isozymes expressed in roots and leaves, and were differentially regulated in response to cold and drought stress. We conclude that organellar wheat A/N-Invs are conserved and common to distantly related plants, and some of the cytosolic isoforms are induced in response to environmental stresses. Supported by CONICET, ANPCyT (PICT 1288), UNMdP and FIBA.

PL-P28
BIOFILM FORMATION DURING CITRUS CANKER REQUIRES THE *Xanthomonas* OUTER MEMBRANE PROTEIN OPRB

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Citrus canker restricts citrus fruit export, and *Xanthomonas citri* subsp. *citri* (Xcc) is the causal agent of this worldwide distributed disease. Previously, we detected that the expression of the outer membrane porin OprB is increased during bacterial Xcc biofilm formation, OprB belongs to the family of carbohydrates transport proteins. To further study its role during disease establishment, we constructed a Xcc deletion mutant (XccΔoprB) and confirmed that this protein is required for the formation of biofilm structures, as well as for bacterial adherence to the plant cells, and also virulence. Furthermore, disease assays with both wild type and mutant pathogenic strains revealed a decrease of canker lesions by 50% when lacking OprB. Given the known function of OprB in other bacteria, glucose uptake was measured and we demonstrated that OprB is necessary for glucose uptake to the cell. Moreover, metabolite abundance between the two strains was compared and differences of carbon intermediate metabolites were detected revealing that Xcc undergoes a reprogramming of the carbon metabolism when it senses a shortage of glucose input. Overall, our results point to the participation of OprB in the process of biofilm formation and virulence with a main role in bacterial metabolic changes, highlighting that environmental nutrient availability may define pathogen virulence.

PL-P29
PAP-SAL1 CHLOROPLAST RETROGADE PATHWAY IS INVOLVED IN IRON HOMEOSTASIS REGULATION IN *Arabidopsis*

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Iron (Fe) is an essential element for human nutrition and vegetables represent a major dietary source of the nutrient. Plant Fe metabolism is a subject of active research, some of its key components are yet to be deciphered, including signals and sensors regulating Fe nutritional status. New plastid retrograde signals, from the chloroplast and mitochondria to the nucleus, have been recently identified, such as 3′-phosphoadenosine 5′-phosphate (PAP).

Transcriptome analysis of Arabidopsis mutant plants in PAP-SAL1 pathway revealed that the ferritin genes AtFer1, AtFer3, and AtFer4 are upregulated in these genetic backgrounds, thus establishing a link between the PAP retrograde signaling pathway and the regulation of Fe homeostasis. In this work, we focused in the relationship between Fe metabolism and the PAP-SAL1 retrograde signaling pathway. Four mutants in this route -alx8, fry1-6, xrn4, and xrn2/xrn3- showed altered gene expression and modified activities of proteins involved in Fe uptake, transport and storage. In addition, these plants grown in high Fe media exhibited different levels of oxidative stress damage compared with the wild type. These preliminary results suggest that the PAP-SAL1 retrograde signaling pathway could play important roles in Fe homeostasis and bioavailability, possibly communicating the high Fe demand organelles with the nucleus.

PL-P30
EFFECT OF ORAL SECRETION OF *Nezara viridula* ON SOYBEAN SEEDS AT MORPHOLOGICAL AND BIOCHEMICAL LEVEL

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The high level of injure to Soybean seeds caused by *Nezara viridula* is believe to be related to their feeding behaviour, morphology of mouth parts, and saliva, though no information about the specific composition is available yet. On the other hand, plants respond to the attack recognizing cell injury and oral secretions, triggering mitogen activated protein kinases (MAPK) pathway and inducing defenses against herbivores. Soybean MAPK's role in mediating responses to insects attack remains largely unexplored. Field studies were conducted to evaluate the superficial damage produced by herbivory to soybean seeds by scanning electronic microscopy (SEM). We also determined by protein gel analysis that the suite of proteins comprising the stink bug watery saliva are very distinct depending on the diet. We also examined the early MAPKs involvement in defense modulation and late activity response of Phe-ammonia lyase (PAL) and lipoxygenase (LOX) enzymes. Lastly, after insect attack saliva affected the expression of the salicylate inducible pathogenesis related gene PR1. Our results suggest that stink bug saliva may elicit a plant self-protection response not only increasing MAPK activity, but also triggering a tightly regulated induction of SA and JA-mediated defenses.

PL-P31
FEMALE GAMETOGENESIS REQUIRES MITOCHONDRIAL FERREDOXINS IN *Arabidopsis.*

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In Arabidopsis, the female gametophyte (FG) is a polarized structure composed by seven cells: the egg cell, the central cell, two synergids and three antipodal cells. Here, we show the study of Arabidopsis mutants with insertions in nuclear genes encoding for mitochondrial ferredoxins and a ferredoxin reductase (ADX and ADXR respectively). Siliques of +/adxr heterozygous plants presented abortions and reciprocal crossings showed that transmission trough the FG is compromised. DIC studies revealed adxr mutants with abnormal FGs. The study of cell identity by using the expression of specific markers indicated that all cells are specified in those embryo sacs that are able to reach maturity. However, aniline blue staining showed abnormal growing patterns of pollen tubes (PTs), including PTs invading embryo sacs and the attraction of two PTs, which suggests a communication problem between the male and the female gametophytes. The expression pattern of ADXR was only detected inside the FG in the ovule. Furthermore, expression was also detected in leaves, sepals and stomata. Plants carrying artificial miRNAs (amiRs) targeting ADXR, ADX1 and ADX2 are under study to know if these three genes have sporophytic functions.

PL-P32
DYNAMICS OF SOLUBLE CARBOHYDRATES IN *Ilex paraguariensis* PLANTS SUBJECTED TO DROUGHT STRESS

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Soluble sugars are considered as important metabolites in plants under water deficit and carbohydrate storage and it has been traditionally considered a measure of carbon shortage or surplus for growth that reflects the plant carbon source-sink balance. In this sense, the aim of this work was to examine the sugars content in leaves and roots of *Ilex paraguariensis* plants subjected to progressive soil water deficit. Samples were collected at Ψsoil= ~ -0,04 (field capacity) to -3MPa including a re-watering (RW) treatment. Net CO_2 Assimilation (A) was measured with Li-Cor LI-

6400. Sugar extraction was performed according to ISO 2103. Glucose (Glu), fructose (Fru) and sucrose (Su) were separated and quantified by HPLC-RI. After 35-day experiment a decreased from 9.8±0.4 to 5.7±0.2 mol CO_2 $m^{-2}s^{-1}$, when Ψ soil dropped from~ -0.04 to -3MPa, respectively. Therefore, leaf Glu and Fru contents decreased 2 folds respect to the well-watered conditions while Su remained unchanged. At the same time, the content of soluble sugars in roots increased 2-2.5 folds. After 48 hs of irrigation, the levels of Glu and Fru from both organs are similar to the non-stressed condition. Decreasing levels of hexoses concentration in the leaves of stressed-plants could be the result of a lower rate of CO_2 fixation due to stomatal closure and / or export to the roots where would contribute to osmotic adjustment.

PL-P33
EXPRESSION ANALYSIS OF GENES INVOLVED IN C/N BALANCE IN RESPONSE TO STRESS IN *Microcystis* STRAINS

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Cyanobacteria are oxygenic photosynthetic organisms found in a diverse range of habitats that play a key role in the biogeochemical C/N cycle. Their ability to assimilate CO_2 from the environment comes from the Carbon Concentrating Mechanisms, which comprises inorganic carbon (Ci, as CO_2 and HCO_3^-), transporters for Ci uptake and protein microbodies named carboxysomes, where CO_2 concentration and fixation by Rubisco take place. *Microcystis* strains produce blooms and are able to synthesize microcystins, powerful hepatotoxins. It was shown that microcystins bind to carboxysome proteins, giving them stability during oxidative stress, a fairly common condition in a bloom. Also, genes involved in microcystin synthesis are up-regulated under stress. In the present work, we analyzed the expression of genes involved in C and N assimilation, microcystin synthesis, and redox balance in a *Microcystis* model strain (PCC 7806) and in a native toxic strain, under different C/N ratios and stress conditions. In PCC 7806, gene expression was up-regulated at higher C/N ratios. Interestingly, in the native strain that produced toxins constitutively, those genes were expressed at a high level even under control conditions. Our data support that microcystin production is related to stress and that may be involved in the cyanobacterium survival.

PL-P34
NITRIC OXIDE MEDIATES VESICLE TRAFFICKING OF PIN2 AUXIN TRANSPORTER IN *Arabidopsis*

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The plant hormone auxin is transported from cell to cell with strict directionality by influx and efflux carrier proteins. PIN efflux transporters exhibit polar plasma membrane localization. They determine the direction and rate of intracellular auxin flow. The subcellular localization of PIN2 protein is driven by endocytosis and recycling through vesicle trafficking in a process termed constitutive cycling. Auxin signaling trough SCF TIR1/AFBs complex has been also involved in PIN2 endocytosis and plasma membrane localization. Recently, it has been described that TIR1 auxin receptor is regulated by S-nitrosylation. In order to study the TIR1/AFB-mediated auxin signaling pathway and its regulation by S-nitrosylation in the control of PIN2 localization, pharmacological and functional approaches are being carried out. We found that the scavenging of endogenous nitric oxide (NO) by cPTIO impairs the auxin-mediated PIN2 plasma membrane localization. As well, we demonstrated that the physiological NO donor, GSNO, mimics the auxin action on PIN2 localization. In order to present a functional-genomic approach we crossed *Arabidopsis* TIR1 S-nitrosylation mutants with PIN2-GFP reporter plants. Finally, the mechanisms underlying this regulation will be discussed.

PL-P35
COLD STRESS RESPONSE AND THE SMALL HEAT SHOCK PROTEINS IN TOMATO FRUIT

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Ripening is the late developmental phase of tomato (*Solanum lycopersicum* L) fruit that starts when the green fruit reaches the final size and is completed when the fruit is red. Storing fruit at low temperature could cause physiological disorders known as chilling injury. It has been reported that small heat shock proteins (sHSP)

accumulated under cold stress. The objective of this study was to evaluate the role of sHSP under chilling conditions in fruits from two tomato cultivars, cv Micro-Tom and Minitomato (tolerant and sensitive to chilling stress, respectively). Additionally, fruit from transgenic plants with altered level of a mitochondrial sHSP were analysed in their phenotype, antioxidant response, gene expression and sHSP protein levels. Results showed that sHsps were induced during ripening in fruit from both cultivars. However, in Micro-Tom fruit but not in Minitomato fruit sHsps were induced in response to storage at low temperature. Silenced fruits were more susceptible to chilling injury while overexpressing fruits were more tolerant to the cold treatment. These results substantiate the hypothesis that sHSPs may participate in the mechanism of genotype chilling tolerance. Overall these data indicate that sHSPs are involved in the response of tomato fruit to cold stress, which could have implications for developing tomato cultivars tolerant to environmental stress.

PL-P36
FUNCTIONAL ANALYSES OF GAMMA CARBONIC ANHYDRASES IN *Arabidopsis* MITOCHONDRIAL COMPLEX I

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Plant mitochondrial complex I contains an extra domain named Carbonic Anhydrase (CA) domain which is composed by gamma type carbonic anhydrases. This domain was proposed to function in complex I assembly and in photorespiration. In Arabidopsis thaliana there are five of these proteins called CA1, CA2, CA3, CAL1 and CAL2, however only four were found into the CA domain. So far, the exact localization of CA3 subunit remains unknown although is considered a complex I subunit. Here, we show that ca2-ca3 double mutants present a growth retardation phenotype in normal air but are not rescued by cultivating plants at high CO2 atmosphere suggesting strong respiratory rather than photorespiratory phenotype. Moreover, ca1-ca3 double mutants present an embryo lethal phenotype. In order to analyze the interaction between CA and CAL proteins in plant cells and elucidate the CA domain composition, bimolecular fluorescence complementation assays were carried out. Our results show that while CA1 and CA2 proteins are able to interact with CAL proteins, the CA3 protein does not although it is able to form homodimers. Despite we could not find direct interactions between CA3 and bona fide CA domain proteins, our results strongly suggest that CA3 is essential for normal complex I function.

PL-P37
THE CA DOMAIN OF RESPIRATORY COMPLEX I IS INVOLVED IN PHOTORESPIRATION AND IS REQUIRED FOR EMBRYOGENESIS

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In *A. thaliana* there are 5 γ carbonic anhydrases, called CA1-3, CAL1-2. These are subunits of the respiratory complex I and form, with the exception of CA3, the CA domain in the membrane arm. None of the single mutants shows any visible phenotype. To investigate the role of the CA domain, we performed crosses to obtain double mutants. The ca2cal1 and ca2cal2 double mutants show a growth retardation phenotype in normal air which is rescued by cultivating plants in a high CO2 atmosphere. Moreover, carbon assimilation is reduced and glycine accumulates, suggesting a photorespiratory imbalance. These results strongly suggest that the CA domain of plant complex I contributes to sustain an efficient photosynthesis. The ca1ca2 double mutants present an embryo lethal phenotype due to strong respiratory problems triggering ROS accumulation. Furthermore, ca1ca2 embryos accumulate abnormal oil bodies during embryogenesis. Dry seeds are collapsed, however are able to germinate later than normal. Indeed, seedlings die most likely due to oxidative stress. The ca2 single mutants contain 80% reduction of complex I. Silencing both, CA1 and CA2 genes, causes an even stronger reduction in complex I, however seeds are viable. These results suggest that ca1ca2 double mutants might contain undetectable amounts of complex I triggering the lethal phenotype. A model of the CA domain composition is presented.

PL-P38
CONTROL OF CHARCOAL ROT DISEASE BY PGPR STRAINS: THE ROLE OF ANTIOXIDANT SYSTEM IN SOYBEAN PLANTS

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Charcoal rot is a plant disease caused by the soil fungus *Macrophomina phaseolina*. The biocontrol agents 9 and 54 were tested for their effectiveness against root rot of soybean caused by *Macrophomina phaseolina*. Both PGPR strains were isolated from disease suppressive soils in different locations of Santa Fe Province. The fungal strain was isolated from infected soybean plants showing root rot symptoms. The control of disease was determined according to the ability to avoid fungal colonization. Inoculation with strain 9 was the most effective treatment to control infection (54%).In situ production of ROS was evaluated by Schiff, DAB and NBT staining. Rootlets infected showed a 2.3-fold increase on HO levels. Strains 9 and 54 improved partially HO content. None of the applied treatments was able to reverse the suppressive effect of the pathogen on Oproduction, while gene expression antioxidant enzyme was downregulated in infected radicles. However, as a result of strain 9-fungus interaction, SOD, CAT, APOX and HO genes were overexpressed. In conclusion,fungal colonization was efficiently prevented by inoculation with strain 9. Its ability to improve ROS production and gene expression of antioxidant enzymes could be related to its capacity to control disease.

PL-P39
POSTTRANSLATIONAL PHOSPHORYLATION OF ADP-GLUCOSE PYROPHOSPHORYLASE IN WHEAT SEEDS

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Many signals modify carbon metabolism during stages of seeds development. Phosphorylation is a mechanism regulating metabolism, but the knowledge about target enzymes in plants is poor and no report is available concerning a comparative study of the regulation taking place in seeds accumulating different carbon reserves. We found that ADP-glucose pyrophosphorylase (ADPGlcPPase, the key enzyme for starch synthesis) is differentially regulated by phosphorylation in wheat seeds (forming starch) respect to castor beans (storing tri-acyl-glycerides). Experiments of iron phosphopeptide enrichment showed that ADPGlcPPase becomes phosphorylated when wheat seeds reach advanced developmental stages, which is associated with an increase of its activity. To further study the effect of phosphorylation, we developed an expression system in *Escherichia coli* to produce small (S) and large (L) subunits of wheat ADPGlcPPase with specific activities of 0.5 U/mg (L+S) and 0.007 U/mg (S), while L showed no activity when produced alone. This system is also useful for the design of mutant (including phospho-mimic) proteins in specific residues selected from bioinformatic and from phospho-proteomic studies. Results will be critical to understand the role played by phosphorylation of the enzyme as well as to explore the distribution of the regulatory mechanism between ADPGlcPPases from different sources.

Structural Biology

SB-P01
EXPLORATORY SEARCH STRATEGIES AND PHYLOGENETIC ANALYSIS OF FUSOGENIC PROTEINS IN EUKARYA

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Fusogenic proteins are required for cell-cell fusion events to occur. In processes such as gamete fusion or the formation of polykaryotic cells, the merger of two lipid bilayers requires an input of energy to bring them into contact and distort them into high energy states before the two cells can fuse. The best characterized examples of these proteins belong to enveloped viruses which rely on fusogens to invade their cellular targets. Their structures fall into three distinct classes. All of the known eukaryotic fusogens have shown a high degree of homology with viral fusogens. In the case of syncytin for example, its molecular structure has been shown to be homologous to a class I viral fusogens, it has been shown to rescue fusogenic function in viruses and the multiple transfer events that lead to

its presence in mammals have been studied. By using remote homology detection techniques to search for other viral fusogen homologues in eukariotic proteomes it may be possible to find the unknown fusogens and get a better picture of where these proteins came from. By combining these techniques with model generation and phylogenetic analysis it is possible to reconstruct the complex evolution of these proteins in the context of their structural homologues, the viral fusogens, all the way to their present state as the final gatekeepers of gamete merger during sexual reproduction.

SB-P02
THREE-DIMENSIONAL STRUCTURE AND LIGAND-BINDING SITE OF CARP FISHELECTIN (FEL)

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Carp FEL (fishelectin or fish egg lectin) is a 238 amino acid lectin that can be purified from the fish eggs exploiting its selective binding to Sepharose followed by elution with N-acetyl glucosamine. We previously reported its amino acid sequence and other biochemical properties. The glycoprotein has four disulphide bridges and the structure of the oligosaccharides linked to Asn 27 was described.
The poster will describe the three-dimensional structure of apo carp FEL (cFEL) and of its complex with N-acetyl glucosamine determined by X-ray crystallography to resolutions of 1.35 and 1.70 Å respectively. The molecule folds as a six-blade beta-propeller and internal short consensus amino acid sequences have been identified in all the blades. A calcium atom binds at the bottom of the funnel shaped tunnel located in the centre of the propeller.
Two ligand binding sites are present in each of the two protomers in the dimer. The first site, alpha, is closer to the N terminus of the chain and is located in the crevice between the second and the third blade while the second site, beta, is located between the fourth and the fifth blade. The amino acids that participate in the contacts have been identified as well as the conserved water molecules in all the sites. Both sites can bind the two anomers of N-acetyl glucosamine, clearly recognizable in the electron density maps.

SB-P03
NO-INDUCED GAPDH AGGREGATION IS INCREASED BY GOSPEL PROTEIN AND INHIBITED BY NAD

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GOSPEL is the protein reported to compete with Siah1 for binding to GAPDH under S-nitrosylation-induced stress conditions, thus preventing GAPDH-bound Siah1 nuclear translocation and subsequent apoptosis. It is not known whether GOSPEL is able to affect the formation of amyloid-like GAPDH aggregates, which also occurs under NO oxidative stress conditions promoting cell death. Here we report the in vitro enhancement by GOSPEL of the GAPDH aggregation produced by the NO donor NOR3. The unique cysteine residue of GOSPEL was partially required for co-aggregation, since the cysteine-free GOSPEL mutant C47S produced a minor aggregation increase. GOSPEL did not affect the lag time preceding GAPDH aggregation but did accelerate this process once it has begun. Such acceleration is proposed to occur through its binding to disulfide-bond dimers and low molecular weight disulfide-crosslinked GAPDH oligomers which act as seed for further protein disulfide-interlink and insoluble aggregate formation. Both GAPDH aggregation and co-aggregation with GOSPEL were inhibited by NAD, a heretofore not described protective effect of this coenzyme against the damaging consequences of oxidative stress. This is the first report on GOSPEL favoring the oxidized GAPDH aggregation involved in cell death, in opposite way to the helpful effect against the apoptotic nuclear translocation of the glycolytic enzyme.

SB-P04
PRODUCTION AND FUNCTIONAL CHARACTERIZATION OF COLLECTIN-11 MUTANTS ASSOCIATED WITH 3MC SYNDROME

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C-type lectins are proteins that bind carbohydrates in a Ca2+-dependent manner. Collectins are C-type lectins that contain a collagen-like domain. Their basic functional unit is a trimer whose monomeric subunits have, besides the collagen-like region, a coiled-coil neck domain and a C-type lectin domain, also referred to as carbohydrate recognition domain (CRD). Collectin-11 (CL-11) was initially identified in a library of human kidney, so it is also known as collectin kidney or CL-K1. It binds fucose and more weakly mannose, as well as polysaccharides isolated from certain microorganisms. CL-11 would also bind plasmid DNA and bacterial and mammalian genomic DNA.

Besides, it has been defined as a new molecule in the lectin pathway of the complement associated MASPs system. Mutations in the genes that encode MASP-3 and CL-11 cause a rare genetic disorder named 3MC syndrome, that exhibits a characteristic facial dysmorphism, growth deficiency, learning disability, genital, limb and renal anomalies and, in some cases, heart abnormalities and other skeletal malformations. Here we describe the method we used to obtain the fragment comprising the neck and the CRD of wild type CL-11 and of three mutants associated to 3MC syndrome, after refolding from inclusion bodies, and present an analysis of their ability to bind ligands.

SB-P05
NOVEL ACTIVE SITE STRUCTURE CONFORMATION OF AY-WB PHYTOPLASMA MALIC ENZYME

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Phytoplasmas are wall-less phytopathogenic bacteria that produce devastating effects on a wide variety of plants. Phytoplasmas genomes have lost many metabolic genes as a result of reductive evolution, it seems that malate is probably a major energy source for phytoplasmas as these bacteria are limited in the processing of carbohydrates. Considering this, we have performed a detailed characterization of the Candidatus Phytoplasma AYWB malic enzyme (AYWB-ME). All the results obtained indicate that this enzyme is involved in energy generation. The differential properties of this enzyme, in combination with the fact that AYWB-ME is one of the smallest among all MEs characterized, made us wonder how this small protein has retained the enzymatic activity and gained its particular modulation. To answer this issue, we have recently obtained the first structure of a AYWB-ME at 2.6 Å resolution. The structure shows a dimer conformation based on a scaffold with unique characteristics. Among these, it is observed that the active site of each monomer is complemented by essential residues (e.g. Tyr 36) from the other monomer. Besides, a complete comparison with the human ME showed different catalytic and structural residues. AYWB-ME structure has a novel strategy for a ME to build its active site, making this enzyme an interesting starting point to make a rational genetic design of ME

SB-P06
NOVEL INSIGHTS INTO *Arabidopsis thaliana* NADP-ME2 REGULATION BY FUMARATE

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Arabidopsis thaliana is a plant species that accumulates high levels of organic acids and uses them as carbon, energy and reducing power sources. NADP-ME2 is the only cytosolic malic enzyme (ME) present in all Arabidopsis organs providing most of the total NADP-ME activity. NADP-ME2 has a complex regulation by fumarate that depends on pH and substrates/fumarate concentrations. Kinetics experiments show activation of NADP-ME2 possibly caused by the binding to a putative allosteric binding site. However, at high fumarate concentrations seems to compete with the substrate malate at the active site and causes inhibition. Until now there is not any plant ME structure solved yet. In this work, we performed homology modeling, molecular docking and dynamics studies trying to discover the putative residues and/or motives involved in this complex fumarate behavior. The results confirmed the presence of two fumarate binding sites: an allosteric site (activation) and the active site (inhibition). Besides, they show a novel fumarate interaction with the backbone of the residue Leu62, present in the activating site not shown before in any structure of MEs. In order to study the participation of Leu62 in the activation of ME2 we expressed and characterized the mutant L62W-ME2. By kinetics assays we found out that, as predicted by molecular dynamics, this mutant is no longer activated by fumarate.

SB-P07
SELF-AGGREGATION OF HUMAN APOLIPOPROTEIN A-I. STUDIES WITH PYRENYL-LABELED CYSTEINE MUTANTS

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The apolipoprotein A-I (apo A-I) is the major protein of high density lipoproteins (HDL), to which antiatherogenic properties are attributed for its role in the transport of cholesterol excess from peripheral tissues to the liver for

catabolism and disposal.Apo A-I is composed of several amphipathic alpha-helices. In water solution, they form a bundle with poorly defined tertiary structure. Depending on the concentration, apo A-I is self-aggregated to form dimers and oligomers of different size.The aim was to obtain information on the apo A-I self-aggregation in solution, as well as its interaction with membrane, since it is important to understand the mechanisms of HDL generation. Six cysteine mutants (K107C, K133C F104C, L137C, K226C and F225C) were specifically labeled with pyrenyl maleimide in these six positions corresponding to the hydrophilic and hydrophobic faces of helices 4, 5 and 10. Fluorescence emission spectra in function of the protein concentration showed that pyrene excimer formation occurs only in the case of labeled F225C and K133C mutants, indicating the participation of helices 5 and 10 in the contact regions for oligomerization.

SB-P08
ISOLATION AND CHARACTERIZATION OF A NEW PROTEINACEOUS NEUROTOXIN FROM SNAIL EGGS

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Pomacea is a group of freshwater snails characterized by an unusual reproductive strategy where adult females lay egg masses above the waterline. Aerial oviposition exposes the eggs to several environmental stressors and terrestrial predators. An adaptation associated with this strategy is the development of a perivitelline fluid (PVF) that includes multifunctional proteins with defensive properties. Among them is the neurotoxin PcPV2 present in*Pomacea canaliculata* eggs. Previous analysis showed that the PVF of related species, P. maculata, is also toxic to mice and contains a putative toxin similar to PcPV2, named PmPV2. The aim of the present work was to isolate and characterize PmPV2. A purification protocol was developed. Structural analyses included electrophoretic and spectroscopic analyses. Structural stability at different pH and temperatures was followed by small angle X-ray diffraction and fluorescence. Functional analysis included toxicity in mice and haemaglutinating capacity. Native PmPV2 is a globular 403-kDa protein, with a Rg= 44 Å and a Dmax= 130 Å. It consists in an octamer of 4 identical 98 kDa heterodimers assembled by non-covalent forces, each composed of 68 and 30 kDa subunits linked by disulfide bonds. PmPV2 is stable up to 70 °C and between pH 4-10. PmPV2 is a strong neurotoxin (LD50=0.25 mg/kg) with haemaglutinating activity displaying high structural stability.

SB-P09
A STRUCTURALLY STABLE SNAIL EGG CAROTENOPROTEIN PROVIDES ANTINUTRITIVE DEFENSES AGAINST PREDATION

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Eggs of the freshwater snail Pomacea maculata have virtually no reported predators. Its major protein is the oligomeric carotenoprotein PmPV1, which provides egg coloration and would protect embryos against environmental factors. In this study we evaluated the structure-function relationships of PmPV1 and its role in the egg defense system. PmPV1 showed a globular and anisometric shape and a remarkable stability under a wide range of temperatures (25-85°C) and pH (2.0–12.0), as revealed by SAXS, fluorescence and absorption spectroscopy. High GndHCl concentration (4 M) is needed to completely unfold PmPV1. Also, resistance to SDS denaturation was observed. This high stability was consistent with the capacity to withstand proteolytic digestion, evaluated by in vitro treatment with gastrointestinal and fungal proteases. In vivo capacity to withstand the gastrointestinal tract was assayed by oral administration of PmPV1 to mice and feces protein analysis. PmPV1 was able to pass unchanged through the gut and was recovered in feces in its native conformation.
As a whole the structural stability and the antinutritive role described for PmPV1 would explain, at least in part, the near absence snail eggs predators. The selective pressure of the harsh gastrointestinal environment would have resulted in the modulation of PmPV1 conformational stability to enhance its resistance to degradation.

SB-P10
THIORREDOXIN FROM *Anticarsia gemmatalis* AGAINST SOYBEAN (*Glycine max*) PROTEASE INHIBITORS

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Anticarsia gemmatalis is a major pest of soybean (*Glycine max*). However, soybean has digestive protease inhibitors (PI) that act as defense against insects attack. Although these PIs are an effective defense against many herbivores the mortality of A. gemmatalis is very low. Since thioredoxins proteins (Trx) are able to reduce disulfide bonds in some proteins, we believe Trx from the gut of A. gemmatalis could be a key component in the resistance to the soybean PI defense. Given that AgTrx has not been previously characterized, the aim of this study was to analyze Trx expression from guts of larvae that fed on diets with different levels of PIs. We found that the expression of insect Trx was stimulated by the presence of inhibitors in the diet. Full length AgTrx was cloned, sequenced and expressed in E. coli in order to accurately measure its specific activity. Additionally, we solved its three-dimensional structure by X-ray crystallography at 1.95 Å resolution. These results present a first glimpse into the mechanism of AgTrx against plant defenses.

Signal Transduction

ST-P01
REQUIREMENT OF HIF-1 AND INSULIN PATHWAY TO CONFER THERMOTOLERANCE BY CHLOROGENIC ACID IN *Caenorhabditis elegans*

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In previous studies we demonstrated that chlorogenic acid (CGA) confers thermal stress resistance in *C.elegans* exposed to lethal temperature (37ºC). Its role in the stress resistance is not fully understood. The objective of this study was to investigate the molecular mechanism of CGA-induced thermal stress resistance in *C. elegans*. Here we have studied how DAF-2 insulin receptor pathway (IRP) (that regulates aging and stress) and HIF-1 pathway are involved in this CGA activity. Worms preincubated for 18h with Wortmannin or LY294002 (both PI3K inhibitors) showed a higher thermal resistance in the wild type N2 strain. DAF-16 is a forkhead transcription factor that transduces the effects of the IRP. The PI3K inhibition leads to induction of DAF16, which control several antioxidant and chaperone genes. PI3K inhibitors did not show activity in DAF16 mutant. However, CGA was active in DAF16 and DAF2 (insulin receptor) mutant. We have previously demonstrated that CGA preincubation increases the protein levels of HIF-1 and their activity in *C. elegans*. Herein we observed the HIF-1 induction by heat acclimation (25ºC) or cobalt ion preincubation conferred thermal stress resistance in N2 strain. Moreover, CGA was inactive in HIF-1 mutants whereas PI3K inhibitors kept their activities. These results indicate that CGA activity required HIF-1 pathway but no IGF pathway.

ST-P02
CHARACTERIZATION OF THE UNFOLDED PROTEIN RESPONSE BY FLUORESCENT REPORTERS IN SINGLE CELLS

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The Unfolded Protein Response (UPR) is a cellular stress signaling cascade activated by different signals such as accumulation of misfolded proteins in the Endoplasmic Reticulum (ER). This homeostatic response involves the activation of three parallel pathways: IRE1, PERK and ATF6, and promotes cell survival in the short term but stimulates apoptosis if misfolded protein levels remain high. The integration of the information derived from these sensors is essential for cell-fate decision-making. In order to characterize UPR dynamics in human single cells, we developed a set of fluorescent reporters to monitor the activation of UPR pathways in real time, allowing us to analyze the dynamic shuttling of UPR components between different subcellular compartments and to measure cell-to-cell variation in the activation of UPR. We generated stable Hela Kyoto cell lines expressing these reporters and designed a protocol for automated imaging, segmentation and quantitative analysis of cells and UPR activation. Our

goal is to find patterns that provide us with information about the state of the cell and which can be associated to a specific cell fate. Since UPR deregulation plays an important role in malignant neoplasms as myeloma, breast and prostate cancer, we hope that these patterns can help us understand why certain cells develop tolerance to stress conditions or escape antitumor drugs.

ST-P03
SYNTHETIC *Caenorhabditis elegans* DAF-12 LIGAND REGULATE ROOT-KNOT NEMATODE INFECTION AND *C. capitata* LIFE CYCLE

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The root-knot nematode Meloidogyne is able to infect almost all cultivated plant and its life cycle starts when the preparasitic juveniles J2 invade the root, develop into J3, J4 juvenile stages and induce the development of galls. Meloidogyne genome has homologues to almost all the components of the dafachronic acids biosynthetic and signaling pathways. In particular there is a homologue to CeDAF-12 which is a ligand dependent transcription factor that mediates the choice between arrest or reproductive development in *C. elegans*. In this study, we evaluated the potential use of a synthetic DAF-12 antagonist (DAF4) in controlling the infection of tomato plants. We treated the J2 with 100uM DAF4, infected tomato roots and determined the number of galls. Our results showed that treatment of Meloidogyne with DAF4 decreased the number of galls found probably by interfering with its development. In addition, we analyzed if DAF4 (5 and 10 mM) added to food at the beginning of the larval stage III had the ability to interfere with: normal development of the third larval stage, the metamorphosis and the emergence of the medfly in *C. capitata*. Larval development span, size and weight of the pupae and the % of flying adults and pupae was determined. DAF4 did not delayed the onset of metamorphosis, but caused a 25-28% reduction in the weight and the size of the pupae.

ST-P04
HEAT SHOCK ACTIVATION OF P38-BASED MAPK PATHWAY IN THE YEAST *Saccharomyces cerevisiae*

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Survival and growth depends on the way cells respond to multiple, often conflicting, external signals. We study signal integration in the model organism *S. cerevisiae*. Here we focused on how cells respond to heat shock in a high osmolarity environment using S. cerevisiae as a model system. When exposed to a change in temperature, yeast orchestrate a system-wide response. Initially, there is an accumulation of trehalose, a disaccharide with chaperone-like activity. However, the resulting high internal osmolarity increases the turgor pressure, activating the Cell Wall Integrity (CWI) MAPK pathway, which strengthens the cell wall, and alleviates the internal pressure by releasing glycerol through the Fps1 channel. Perhaps conflictingly, heat shock also activates transiently the High osmolarity glycerol (HOG) MAPK pathway, which increases the accumulation of glycerol. Here, we show that heat activates HOG in a sustained manner when cells are pre-adapted to high osmolarity. In these conditions, the mechanism of HOG activation involves the aperture of Fps1 by the MAPK of the CWI pathway, the consequent loss of glycerol and decrease in turgor pressure, which eventually leads to the activation of HOG. Our results highlight the complexity of signal integration by intracellular networks, and suggest that p38 based signaling might play central roles in this process.

ST-P05
FAR FROM STEADY-STATE CELL RECEPTORS EXHIBIT IMPROVED SIGNALING PROPERTIES: A NUMERICAL STUDY

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Sensing extracellular information often involves binding of a ligand to a receptor. We have shown that cells able respond to ligands before they reach binding equilibrium are able to discriminate ligand concentrations otherwise indistinguishable. We call this effect Pre-equilibrium Sensing and Signaling (PRESS). Here we study PRESS using a more realistic receptor model that considers a reversible isomerization between active and inactive states, each of which may bind the ligand. We found that this model can do PRESS, except for the case in which binding mostly occurs with the active state of the receptor. In this case, fast binding would reduce the availability of pre-equilibrium

information even when preceded by slow isomerization step. On the other hand, if the reaction rates are such that the activation pathway undergoes a fast binding process followed by a slower activation of the bound receptor, PRESS occurs but in a limited range of doses. Our computational study allows for identifiability of conditions (relationships between kinetic rates) necessary for this system to improve its sensing capabilities around a given dose range and using a certain time window.

ST-P06
DESIGN AND IMPLEMENTATION OF A BIOLOGICAL CIRCUIT ABLE TO RESPOND TO PRE-EQUILIBRIUM INFORMATION

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Many cellular processes rely on the ability of cells to sense and respond to chemical information in their immediate surroundings. Cells can achieve this by binding chemical signals (ligand) to specific receptor proteins, that convert the chemical information into intracellular signals to which the cells can respond. Furthermore, different concentrations of ligand may be distinguished by the fraction of bound to unbound receptor. Although this is generally only possible for concentrations that do not saturate the receptors, we recently showed that cells' ability to distinguish between ligand concentrations could in theory be expanded into the saturating range, by the utilization of pre-equilibrium information resulting from the ligand–receptor binding kinetics. We termed this mechanism pre-equilibrium sensing and signaling (PRESS). Here we explore the possibility of biologically implementing this idea as a simple, controllable genetic circuit. Via computational modeling and experiments we show that repression-based sensing, aided by slow influx of ligand, generates pre-equilibrium dynamics that may enable cells to distinguish between different saturating concentrations of ligand. The work may improve our understanding for how cells process information they receive before signaling reaches steady state, and could be applied to the design of better biological sensors.

ST-P07
A NOVEL ROLE OF THE PHYSICAL INTERACTION BETWEEN A G-PROTEIN COUPLED RECEPTOR AND THE ASSOCIATED RGS

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Receptor theory postulates that cells transduce a stimulus proportional to the amount of ligand-receptor complex to downstream effectors. Therefore, modifications in the abundance of receptors should modify the dose response curve (DoR). Increasing the amount of total receptors should render the system more sensitive and, if the downstream response is not saturated, increase the amplitude of the response. The G-protein coupled receptors (GPCR)-based pheromone response pathway of *Saccharomyces cerevisiae* does not present this behaviour. Its DoR curve is robust to changes in the amount of receptor. We made a detailed mathematical model of this system which allowed us to postulate a mechanism that explains this behaviour. Here, using mutant strains, we tested a key prediction of our model experimentally: the requirement of a physical interaction between the receptor Ste2 and the regulator of G protein signaling (RGS) Sst2. Our results show that this modified pathway loses its original robustness to changes in receptor abundance, consistent with our hypothesis. Therefore, we present a novel mechanism for the GPCR-based response that can be extended to other studies.

ST-P08
COORDINATED REGULATION OF GENES OF DIFFERENT AMINO ACID UTILIZATION PATHWAYS IN YEASTS

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Yeast cells can use a variety of compounds as nitrogen source through different specific pathways. Synthesis of proteins involved in each of these pathways is tightly regulated. The availability of readily transported and metabolized nitrogen sources, which are known as good nitrogen sources, results in the strong repression of genes involved in transport and metabolism of poor ones. The utilization of poor sources such as leucine, gamma-aminobutyric acid (GABA) and allantoin requires the synthesis of pathway-specific catabolic enzymes and permeases. In this work we showed that the expression of the proteins responsible for the uptake and catabolism of different poor nitrogen sources occurs sequentially following a certain order determined by a tight regulation. This order could promote the utilization of a given nitrogen source whereas the utilization of others, which may be less

useful, could be down-regulated in some growth media. We demonstrated here that the transcription factor Dal81 is central in the regulation that leads to the hierarchical expression of the genes studied and, consequently, in the utilization of leucine, allantoin and GABA. The mechanisms that lead to the temporal order in the expression of genes involved in the use of poor nitrogen sources also involve another transcription factors such as Leu3 and Stp1.

ST-P09
PROGESTERONE TRIGGERS INTRACELLULAR CALCIUM WAVE BY A CATSPER-INDEPENDENT MECHANISM IN HUMAN SPERM

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Previous works have shown that progesterone acts on CatSper calcium channels. These ion channels are found exclusively in the membrane of the sperm tail. Progesterone opens CatSper channels and triggers a fast increase in intracellular Ca2+. This ion is crucial for sperm functions, such as the regulation of capacitation, hyperactivation and acrosome reaction. The aim of this study was to investigate whether progesterone can trigger an intracellular calcium increase in absence of extracellular calcium. We used fluorescent calcium sensors in real time dynamic assays in single cell -with high speed and spatial resolution- and population measurements in a spectrofluorimeter. Capacitated human sperm were loaded with Fluo3-AM, then incubated in a calcium-free medium and treated with progesterone. We found that in the absence of extracellular Ca2+, progesterone induces a calcium increase that starts in the midpiece/neck region and propagates to the acrosome region. These findings indicate that progesterone can trigger the release of calcium from intracellular calcium stores by a CatSper independent mechanism.

ST-P10
IDENTIFICATION OF POTENTIAL SUBSTRATES OF YARROWIA LIPOLYTICA PKA BY COMPARATIVE PROTEOMIC ANALYSIS

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The cAMP-dependent protein kinase (PKA) is an enzyme that engages the transference of a phosphate group of ATP to its target protein; its activity is regulated by intracellular cAMP concentration. In *Yarrowia lipolytica*, a dimorphic fungus of biotechnological interest, we found that PKA is involved in morphogenesis, cell metabolism and adaptation to stress conditions. The regulatory and catalytic subunits are encoded by sole genes, RKA1 and TPK1, respectively. In this work, proteomics and molecular biology techniques were adapted to identify potential target proteins of *Y. lipolytica* PKA. The strategy used was based on comparison of immunoenriched proteins with an anti-phosphoPKA substrate antibody from a wild type strain and a mutant strain without PKA activity (Δtpk1). The protocol performed involved the linkage of protein A-Sepharose to a monoclonal anti-phosphoPKA substrate antibody. Phosphoproteins from the wild type and mutant strains were enriched, and were resolved by 2D-PAGE electrophoresis. Finally, spots only found in the wild type strain and absent in the PKA mutant were selected and the proteins were identified by MALDI-TOF. It was possible to identify three putative PKA substrate proteins: the self regulatory subunit of PKA, Rka1; a protein with homology to Saccharomyces cerevisiae ubiquinone biosynthesis monooxygenase CoQ6 and the translational elongation factor EF1-α.

ST-P11
MKP-3 IS UPREGULATED IN APOPTOSIS INDUCED BY BORTEZOMIB IN ENDOTHELIAL TUMOR CELLS

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The Kaposi's Sarcoma-associated Herpes virus G Protein-Coupled Receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi Sarcoma. Persistent expression and activity of vGPCR is required for NF-kB pathway activation and tumor maintenance in endothelial cells. We have previously demonstrated that bortezomib decreases nuclear activity of NF-kB and induces apoptosis in endothelial cells expressing vGPCR. In this work, we investigated whether bortezomib regulated an ERK specific MAPK phosphatase-3 (MKP-3) expression as part of its antiproliferative effects in vGPCR cells. The results showed that bortezomib decreased vGPCR cell number and induced cell morphology changes in a dose-dependent manner. In addition, Bortezomib increased MKP-3 protein expression followed by a reduction of FOXO1 and ERK1/2 phosphorylation. These changes were accompanied by a

reduction of nuclear ERK1/2 phosphorylation and actin cytoskeleton reorganization. In line with FOXO1 dephosphorylation/activation, p21 mRNA levels were found increased upon bortezomib treatment. Taken together, we propose that MKP-3 decreases ERK1/2 and FOXO1 phosphorylation, which turns into FOXO1 activation, and increases p21 mRNA as part of bortezomib actions in vGPCR cells.

ST-P12
CROSSROADS TO SPERM ACROSOMAL RESPONSIVENESS: PKA, ACTIN DYNAMICS AND MEMBRANE HYPERPOLARIZATION

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Capacitation is the process by which mammalian sperm gain fertilizing capacity. It is characterized by acquisition of acrosomal-responsiveness (AR), which in turn is related to actin polymerization, membrane hyperpolarization, and activation of PKA. The mechanisms underlying these events and the interplay between them remain unknown. We have shown that Src modulates hyperpolarization through regulation of SLO3, and that Src activation depends on PKA. Our aim is to deepen in the pathways underlying AR, in which the role of Src could interplay with actin dynamics to regulate SLO3. We studied the kinetics of actin dynamics by determining the G-actin/F-actin ratio, and observed a polymerization process towards the end of capacitation. However, we also detected a transient actin depolymerization that precedes the polymerization step. Our results indicate that Src is linked to these processes and that PKA is also necessary for actin polymerization. PKA is activated almost immediately after exposure to capacitating condition. Although Src depends on PKA activation, its activated state is only observed after 15 min after the beginning of capacitation. Our data show that PKA directly phosphorylates Src at Ser17, which might impact on its later activation. Most importantly, PKA sustained activity is mandatory for the maintenance of Src activity throughout capacitation, that will later ensure AR.

ST-P13
TOR SIGNALING IN PLANTS

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Target of rapamycin (TOR) acts as a master regulator to control growth and metabolism by integrating nutrient, energy, and growth factors in all eukaryotic species. TOR kinase forms two different complexes, TORC1 and TORC2, which mainly regulate the balance between anabolic and catabolic processes and cytoskeleton structure, respectively. TORC1 is conserved in plants but not TORC2. To gain insights in TOR signaling in photosynthetic eukaryotes we studied the TOR complex in two model organisms: *Ostreococcus tauri*, unicellular algae located in the base green lineage, and *Arabidopsis thaliana*. We investigated the TOR complex conservation in *Ostreococcus* by using TOR inhibitors and analyzing the expression of tor gene in diverse C/N availability. Regarding *Arabidopsis*, we evaluated the effect of sucrose (Suc), the main product of photosynthesis and a signal molecule, on the expression of TORC1 components. RT-PCRs were performed on wild-type (wt) or a TOR conditional mutant young seedlings treated with different concentrations of the sugar. Suc down-regulated the transcription of TOR complex in wt tissues; however, this regulation was likely lost in the TOR mutant. Our results indicate that TOR signaling is conserved in the plant lineage with specific features, particularly regarding the upstream effectors, occupying Suc a central role. Supported by CONICET, ANPCyT, UNMdP and FIBA.

ST-P14
ROLE OF TPK2 CATALYTIC SUBUNIT OF PKA IN MRNA AGGREGATION IN *Saccharomyces cerevisiae.*

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We previously showed that catalytic subunit of cAMP dependent Protein Kinase, Tpk2, is involved in processing bodies (PBs) and stress granules (SGs) assembly under stress conditions through its Q-rich N-terminal domain and its kinase activity. It has been demonstrated that several mRNAs are localized in granules in actively growing cells and these granules serve as platforms for PBs formation during glucose starvation. Here, we analyze the role of Tpk2 in the formation of PDC1 mRNA granules in quiescent cells, after severe heat shock or glucose starvation. To assess this, we used strains expressing m-Tagged PDC1 mRNA in Tpk2, tpk2QΔ, or tpk2 kinase dead backgrounds. Analyses of polysome profiles during exponential growth, heat stress or quiescence were similar in all strains. During

stationary phase, PDC1 mRNA is localized in granules, which mostly do not co-localize with SGs or PBs. The kinase activity or Q-rich domain of Tpk2 does not affect these granules. Upon re-feeding the total number of PDC1 mRNA granules increases, showing a similar proportion of co-localization with PBs and SGs. Tpk2 Q-rich domain does not have a role in this process, but tpk2 kinase dead strain presented no increase in PDC1 mRNA granules upon refeeding and a higher association with SGs. Reduction of PDC1 mRNA granules after severe heat stress and glucose starvation was dependent on Tpk2 catalytic activity.

ST-P15
PKA CONTROLS MRNA GRANULES DURING QUIESCENCE AND CELL CYCLE RESUMPTION IN *Saccharomyces cerevisiae*

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Localization of an mRNA to a specific site is a highly regulated process that modulates its translation. In order to analyze the localization of different mRNAs in quiescent cells, we performed confocal microscopy over strains carrying the m-TAG system for ENO2, PDC1, MFA2, TIF1, TDP1 and VNX1 mRNAs. These mRNAs are accumulated in 1-2 foci during quiescence. When cells are re-fed by addition of fresh medium for 30 minutes, TDP1 and VNX1 mRNAs granules are disassembled; but ENO2, PDC1, MFA2, and TIF1 mRNA granules increase to 2-6 granules per cell. The co-localization of mRNA granules and stress granules or P-bodies presents differences between the mRNAs. Analysis of mRNA localization in strains harboring each PKA catalytic subunit deletion, indicated that each PKA isoform controls specific mRNA granule formation. To assess the presence of active translation sites we used immunofluorescence over cells treated with puromycin. A proportion of the puromycin-marked sites of protein synthesis overlap with both ENO2 and TIF1 mRNA granules during quiescence. After fresh medium addition, translationally active mRNA granules increase in an mRNA specific manner. Therefore, our results suggest that PKA controls mRNA storage into cytoplasmic foci during quiescence; and that these mRNA granules could regulate localized protein translation.

ST-P16
ABSCISIC ACID CONTENT VARIES DURING *Apis mellifera* DEVELOPMENT: IN THE SEARCH FOR A FUNCTIONAL SENSE

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The honeybee (*Apis mellifera)* obtains all the nutritional needs from nectar and pollen from a wide diversity of plant species. Nectar is the source of carbohydrates and pollen provides proteins, lipids and other nutrients required for growth and immunity. Abscisic acid (ABA) is a fundamental hormone involved in diverse physiological processes in plants. It has been also demonstrated that ABA is functionally active in many animal species where its specific receptor LANCL2 was characterized. In a recent publication (Negri et al., 2015), we demonstrated that honeybee colonies supplemented with ABA improve their fitness and enhance individual immune response. In this presentation, we report that ABA is a natural compound present in honey, royal jelly, larvae and adult honeybees. We found that ABA content and the transcript levels of its putative receptor, AmLANCL2, are higher in adult honeybees compared to larvae. Our results show that ABA supplementation would not influence the development of *A. mellifera* growing in the lab under standardized conditions (34 °C). However, the delay of the larvae development exposed to low temperature (25 °C) was reversed by supplementation with ABA. Furthermore, ABA treatment increased the number of surviving individuals exposed to 25 °C and also shortened the recovery time of individuals after exposition to short periods of low temperatures.

ST-P17
EXPRESSION REGULATION OF YEAST PKA SUBUNITS, TPK1 AND BCY1, BY UORF

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Upstream open reading frames (uORFs) are regulatory elements located in 5' untranslated regions, which can repress the translation of downstream coding sequences. In *S. cerevisiae*, the catalytic subunit of protein kinase A (PKA) is encoded by three genes TPK1, TPK2 and TPK3; while the regulatory subunit is encoded by the BCY1 gene. In TPK1 a uORF was described in the 5' UTR, containing five codons length, and in BCY1 5´UTR we defined one uORF of

50 codons length. In order to study the role of TPK1 and BCY1 uORFs, full-length 5´ sequence (WT-UTR) or a version with the mutated uORF start codon (mut-UTR) of each subunit were cloned into a β-galactosidase (β-Gal) reporter vector. β-Gal activity and mRNA levels were measured. We found that TPK1 and BCY1 uORFs negatively regulate translation and stabilize the mRNAs. Under heat shock stress, β-Gal activity was upregulated for both Tpk1 WT-UTR and mut-UTR while mRNA levels were upregulated only for Tpk1 WT-UTR. Therefore the translational efficiency resulted higher for the mut-UTR construct. In the case of BCY1, heat shock stress resulted in a downregulation of β-Gal activity and mRNA levels for both WT-UTR and mut-UTR. Thus, uORF could contribute to regulate the expression of PKA Tpk1 and Bcy1 subunits at the post-transcriptional level

ST-P18
YEAST PKA SUBUNIT PROMOTERS ARE DIFFERENTIALLY REGULATED IN FERMENTATIVE AND OXIDATIVE GROWTH

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In *Saccharomyces cerevisiae* three signaling pathways are required to correctly respond to glucose availability: the Rgt2/Snf3, the Snf1/Mig1 and the cAMP/PKA pathways. In glucose depletion conditions, genes involved in the metabolism of alternative carbon sources are induced by Snf1. Three genes encode the PKA catalytic subunit, TPK1, TPK2, TPK3; while the regulatory subunit is encoded by one gene, BCY1. Employing reporter gene methodology we studied the activity regulation of PKA subunits promoters in fermentative (glucose) or non fermentative (glycerol) growth. Our results indicate that the activity of all promoters is stronger in glycerol than in glucose medium and that the kinase activity of PKA inhibits its own promoters, in glycerol growing cells. Mig1-Hxk2 regulatory complex inhibits TPK1 promoter while Snf1 kinase activates TPKs and BCY1 promoters, possibly acting through Cat8 transcription factor. In addition, Mig3 transcription factor differentially modulates TPK3 and BCY1 promoters, and Mig2 only activates TPK2 promoter. The results were further confirmed by mRNAs quantification by qRT-PCR, and measurements of Tpk1 and Bcy1 proteins levels by Western blot. Results of ChIP assays show the presence of Cat8 and Mig1 in the TPK1 promoter transcriptionally active

ST-P19
CELL WALL INTEGRITY (CWI), MAPK AND PKA SIGNALING CROSS-TALK IN *Saccharomyces cerevisiae*

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The signal transduction cascade CWI pathway in *S. cerevisiae* responds to cell wall stress signals like heat shock, through a family of cell surface sensors (WSC1-3). These sensors activate MAPK cascade that serve to amplify a small signal initiated at the cell surface. CWI pathway cross talks with other signaling pathways as the protein kinase A pathway. We analyzed the regulation of TPK1 (one of the catalytic subunit of PKA) expression during heat stress by CWI pathway. Using β-galactosidase reporter and quantification of mRNA by qRT-PCR we demonstrate that Wsc1, Wsc2 and Wsc3 receptors are not redundant for heat shock response. The involvement of MAPK kinase pathway in TPK1 expression regulation was demonstrated using different deletion strains. The results also show that other parallel pathway should be involved. PKA phosphorylates Slt2/Mpk1 kinase, the last component in the MAPK cascade, producing an inhibition in the signaling pathway. Slt2/Mpk1 is negatively regulated by Sdp1 phosphatase, whose transcription is regulated by Msn2/4 transcription factors during heat shock. The localization of Msn2-GFP under this stress was analyzed in WT and wsc1Δ, wsc3Δ strains, and showed to be different in the mutant strains in comparison with the wt strain. The results indicate that exists a crosstalk among MAPK and PKA pathway during heat shock.

ST-P20
PROMOTER OF TPK1 CATALYTIC SUBUNIT OF PKA FROM *Saccharomyces cerevisiae*: CHROMATIN REMODELING DURING HEAT SHOCK

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Chromatin remodeling at gene promoters plays a critical role in initiation of transcription. This process is performed by a large class of ATP-dependent chromatin remodeling complexes as SWI/SNF, ISWI, CHD and INO80. Some have been suggested to play redundant roles and/or functionally interact with each other. During heat shock stress, the promoter of PKA catalytic subunit, TPK1 from *S. cerevisiae* is upregulated, chromatin remodeled and the two well-

positioned nucleosomes are evicted. In order to understand the mechanism by which the promoter is activated and the nucleosomes remodeled, the involvement of different remodelers was studied as a first approach using a β-galactosidase reporter assay in null strains for each remodeler. The results indicate that SWI/SNF, and INO80 null strains lose the heat shock response. The remodeling of nucleosomes was further studied by MNase protection assay. The results show that a strain lacking the SWI/SNF activity, loses its ability to remove nucleosomes from TPK1 promoter during heat shock. Thus our results indicate that SWI/SNF and INO80 family are involved in remodeling of nucleosomes on TPK1 promoter in response to heat shock. Finally a strain with a deficient PKA activity (tpk1w-1) in which TPK1 promoter is fully activated nucleosomes are also evicted even in the absence of heat shock.

ST-P21
INVOLVEMENT OF IMPORTINS IN THE TRANSLOCATION OF ARGININE DEIMINASE TO THE NUCLEI IN *Giardia lamblia*.

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In the protozoan parasite *Giardia lamblia*, the enzyme Arginine Deiminase (ADI) plays a key role during the proliferative and the differentiation process. Particularly, during encystation ADI translocates from the cytoplasm to the nuclei. In eukaryotic cells, proteins larger than 50 kDa with nuclear localization signals (NLS) are transported across the nuclear envelope by a family of transport proteins called karyopherins or importins. This process first involves the recognition of NLSs by the adaptor importin α and binding by its N-terminal importin-β-binding (IBB) domain to importin β. We found in the Giardia database two genes (GL50803_16202 and GL50803_15106) which by in-silico analysis match importin α and β, respectively. GL50803_16202 (importin α) shows the classical armadillo repeats composed of a pair of alpha helices that form a hairpin structure. GL50803_15106 (importin β) shows 24 HEAT repeats that form rod-like helical structures. An inhibitor of nuclear transport like importazol (importin β inhibitor) reduced the growth of Giardia trophozoites and the number of cyst produced. Also, there is an accumulation of ADI over the nuclear envelope. These results suggest that in Giardia the classical nuclear transport is functional being this mechanism employs by ADI to translocate from the cytoplasm to the nuclei.

ST-P22
SONIC HEDGEHOG PATHWAY REGULATES THE POST-TRANSCRIPTIONAL REGULATOR SMAUG1

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Mammalian mSmaug1 is an mRNA repressor involved in translational regulation in hippocampal synapses and forms cytoplasmic silencing foci (S-foci) similar to Stress Granules and Processing Bodies (Baez and Boccaccio, JBC 2005; Baez et al., JCB 2011). Both Drosophila and mammalian Smaug repress reporter mRNAs carrying specific motifs termed Smaug-Recognition-Elements (SREs), which consist of a stem-loop with the sequence CNGGN(0-8). Using an ad hoc MATLAB algorithm, we carried out an in silico search to identify mammalian transcripts carrying SREs. We found that several human transcripts linked to glycolysis, lipid metabolism and mitochondrial function are potential Smaug targets, and similar results were reported in Drosophila. Mice carrying a mutation in Smaug1 present a phenotype similar to that of Sonic Hedgehog (Shh) mutants. The Shh pathway is a major signaling pathway and we found that pharmacological manipulation of Shh affects S- foci formation. These results suggest that Smaug repression is regulated by Shh, likely affecting the expression of key cellular transcripts.

ST-P23
PERSISTENTLY ACTIVE RAB3A AND A CHIMAERIC PROTEIN PREVENT THE LATE STAGES OF SPERM EXOCYTOSIS

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The acrosome reaction is a type of regulated exocytosis that releases the acrosomal granule content in the vicinity of the egg during fertilization. Minutes after the arrival of the triggering signal, the acrosomal and plasma membranes dock at multiple sites and fusion pores open at the contact points. Immediately afterwards fusion pores dilated

spontaneously, originating tubules and vesicles that were shed, together with the acrosomal contents in the vicinity of the egg. Full length, geranylgeranylated and active Rab3A elicits human sperm exocytosis per se. The carboxy-terminus domain of Rab3A is necessary and sufficient to promote exocytosis whereas its amino-terminus prevents calcium-triggered secretion when added after docking of the acrosome to the plasma membrane. This effect depends on Rab3A's inability to hydrolyze GTP. A fluorescent dye applied to the medium entered the acrosome of sperm with their plasma membrane permeabilized with streptolysin O and incubated with Rab3A/Rab22A followed by calcium as exocytosis trigger. These findings suggest that there was a connection between the intravesicular and extracellular compartments, most likely through fusion pores opened between the plasma and acrosomal membranes. Thus, we propose that inhibitory Rabs interfere with the vesiculation of membranes and release of the acrosomal contents after the opening of fusion pores.

ST-P24
MULTIPLE PATHWAYS FOR CHOLESTEROL SIGNALING IN *Tetrahymena thermophyla*

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The free-living protozoon *Tetrahymena thermophila* does not require neither synthesize sterols. When sterols are present in the media they are incorporated and modified by desaturations at several positions. Desaturating enzymes are induced whereas the synthesis of the sterol surrogate "tetrahymanol" is repressed. Tetrahymanol is completely displaced from the cell membranes by sterol derivatives. By RT-qPCR we evaluated the effect of cholesterol and known effectors of canonical signaling pathways on the transcription of two reporter genes: C7-desaturase (Des7) and squalene synthase (SqS), a key enzyme in tetrahymanol synthesis. Des7 and SqS transcripts were respectively, 2-fold induced and 10-fold repressed after one hour of cholesterol addition, in agreement with our previous RNAseq results. 8-Br-cAMP, wortmannin, PMA, BAPTA-AM and U73122 did not show significant effect on Des7 transcription, whereas Gö6976 slightly induced Des7. Interestingly, PMA alone repressed 10 fold SqS, suggesting the gene is downregulated by cholesterol via PKC signaling. It was confirmed by using the PKC inhibitor Gö6976, which abrogated the effect of cholesterol. Notably, BAPTA-AM and the PI-PLC inhibitor U73122 (1 and 10 uM) did not show significant effect on SqS. These results and the proposed model suggest that Des7 and SqS are transcriptionally regulated by two independent non-canonical pathways.

ST-P25
MDR METHOD REVEALS INTERACTION AMONG BAX AND BCL2 POLYMORPHISMS IN BREAST CANCER

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BCL-2 family is a group of evolutionarily conserved proteins related to apoptosis which is subdivided into two main classes: proapoptotic like Bax and Bad, and anti-apoptotic like Bcl-2 and Bcl-xL. Association studies between Bcl-2 and Bax SNPs and cancer have provided new insights into the mechanisms of carcinogenic development. The diversity of physiological functions in which Bcl-2 and Bax are involved, suggest a high level regulation, therefore the analysis of its promoters is important to understand the function of proteins. The aim of this study was to estimate the combined effect of Bax and Bcl2 promoters SNPs (-248G>A and -398C>A, respectively) in the phenotype of breast cancer. A total of 82 breast carcinomas and 66 control samples from Posadas-Misiones were genotyped by RFLP-PCR. We used the multifactor dimensionality reduction (MDR) method to improve the identification of polymorphism combinations associated with breast cancer. The MDR method detected interaction between Bax and Bcl2 promoters SNPs and the risk of breast cancer (OR=8,47; IC 95% = 1,56 - 45,76; p=0,0089). For model interaction between Bax and Bcl2 promoters SNPs (-248G>A and -398C>A, respectively); the Testing Accuracy (TA) was 0.7415. Genotypes combinations for Bax and Bcl2 associated with breast cancer risk were: GG-CA y GA-CA (Bax-Bcl2).

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