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- SAIB -

45th Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

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

November 10-13, 2009

San Miguel de Tucumán, Tucumán
República Argentina

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-Plants-
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ACKNOWLEDGMENTS

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Consejo Nacional de Investigaciones Científicas y Técnicas
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(ANPCyT)

European Molecular Biology Organization
(EMBO)

International Union of Biochemistry and Molecular Biology
(IUBMB)

American Society for Microbiology
(ASM)

SAIB 2009 CONGRESS OVERVIEW

Tuesday, November 10 th	Wednesday, November 11th	Thursday, November 12th	Friday, November 13th
	8:30-10:30 **Symposia** Room A: Membrane Biogenesis and Trafficking in Health and Disease.- Room B: Microbiology	8:30-10:30 **Symposia** Room A: Plants Room B: Lipids	8:30-10:30 **Symposia** Room A: Membrane Biogenesis and Trafficking in Health and Disease Room B: Signal transduction
	10:30-11:00 Coffee break	10:30-11:00 Coffee break	10:30-11:00 Coffee break
	11:00-13:00 **Oral Communications** Room A: ST (C01/08) Room B: PL (C01/05) Room C: LI (C01/08)	11:00-13:00 **Oral Communications** Room A: PL (C06/10) Room B: MI (C01/09) Room C: CB (C01/08)	11:00-13:00 **Oral Communications** Room A: CB (C09/14) and SB (C01/02) Room B: MI (C10/18) Room C: LI (C09/11) and BT (C01/05)
	13:15-15:30 Lunch	13:15-15:00 Lunch	13:15-15:30 Lunch
14:00-18:00 Registration	15:30-16:30 Room A **Lecture** Richard Lehner	15:00-16:30 Room A **Junior Faculty Lectures** Gabriela Pagnussat Rodolfo Rasia Viviana Rapisarda	15:30-16:30 Room A **Lecture** Vassilios Papadopoulos
18:00-18:15 Room A **Opening Ceremony** 18:15-18:30 **Tribute to Dr. Rodolfo Ugalde** Juan José Cazzulo 18:30-19:30 **Lecture** Silvio Gutkind	16:45-18:45 Posters - coffee MI (P01/31) CB (P01/27) PL (P01/32) LI (P01/12) BT (P01/11) EN (P01/07) ST(P01/07)	16:45-18:45 Posters - coffee MI (P32/63) CB (P28/56) PL (P33/67) BT (P12/24 EN (P08/14) ST(P08/17)	16:45-18:45 Posters - coffee MI (P64/94) CB (P57/86) PL (P68/97) LI (P13/23) SB (P01/12) NS (P01/11)
19:30-20:30 **Sols Lecture** Manuel Guzman	19:00- 20:00 Room A **Lecture** Marcio Alves-Ferreira	19:00- 20:30 Room A **Tribute to Darwin** Pablo Goloboff Enrique Caviedes-Vidal Marcelo Rubinstein	19:00- 20:00 Room B **EMBO Lecture** Philippe Sansonetti
21:00 Cocktail	19:00- 20:00 Room B **Theatre Play** Personally, Einstein	20:30 Room A SAIB General Assembly	21:00 Room A Closing dinner

BT: Biotechnology; CB: Cell Biology; EN: Enzymology; LI: Lipids; MI: Microbiology; NS: Neuroscience; PL: Plant Biochemistry and Molecular Biology; SB Structural Biology; ST: Signal Transduction

PROGRAM

TUESDAY, November 10th, 2009

14:00-18:00 **REGISTRATION**

18:00-18:15 Room A

OPENING CEREMONY

Beatriz Caputto

SAIB president

CIQUIBIC-CONICET. Facultad de Ciencias Químicas

Universidad Nacional de Córdoba, Córdoba, Argentina

18:15-18:30 Room A

TRIBUTE TO Dr. RODOLFO UGALDE

Juan José Cazzulo.

IIB-INTECH, CONICET. Universidad Nacional de San Martín

18:30-19:30 Room A

OPENING LECTURE

IUBMB Symposium: Membrane Biogenesis and Trafficking in Health and Disease

J. Silvio Gutkind

Oral and Pharyngeal Cancer Branch, NIDCR,

National Institutes of Health, Bethesda, MD, USA

"G Protein-Regulated Signaling Networks and Cancer"

Chairperson: Beatriz Caputto, CIQUIBIC-CONICET. Universidad Nacional de Córdoba

19:30-20:30 Room A

"**ALBERTO SOLS" LECTURE**

Manuel Guzman

Departamento de Bioquímica y Biología Molecular.

Facultad de Biología. Universidad Complutense.

Madrid, España.

"Signaling by the Endocannabinoid System"

Chairperson: Diego de Mendoza, IBR-CONICET. Universidad Nacional de Rosario

21:00 **COCKTAIL**

WEDNESDAY, November 11th, 2009

08:30-10:15 Room A

IUBMB SYMPOSIUM

"Membrane Biogenesis and Trafficking in Health and Disease I"

Chairpersons: Nora Calcaterra, IBR-CONICET. Universidad Nacional de Rosario

José Luis Daniotti, CIQUIBIC-CONICET. Universidad Nacional de Córdoba

08:30 - 09:05 ***Luis S. Mayorga***
IHEM-CONICET. Universidad Nacional de Cuyo, Mendoza, Argentina
"Biochemical and topological membrane alterations during the sperm acrosomal exocytosis"

09:05-09:40 ***Santiago Quiroga***
CIQUIBIC-CONICET. Universidad Nacional de Córdoba, Córdoba, Argentina
"Regulation of membrane expansion at the nerve growth cone: Axonal specification and elongation"

09:40-10:15 ***Claudio G. Giraudo***
Department of Cell Biology, School of Medicine, Yale University. New Haven, CT, USA
"A clamping mechanism for Calcium-regulated exocytosis"

08:30-10:30 Room B
SIMPOSIUM
"Microbiology"
Chairpersons: Eleonora García Véscovi, IBR-CONICET. Universidad Nacional de Rosario
Andrea Smania, CIQUIBIC-CONICET. Universidad Nacional de Córdoba

08:30-09:00 ***Rasika Harshey***
(Sponsored by American Society for Microbiology)
Molecular Genetics and Microbiology, University of Texas at Austin, Texas, USA
"Cell density and motility protect swarming bacteria against antibiotics"

09:00-09:30 ***José Luis Puente***
Departamento Microbiología Molecular. Instituto de Biotecnología-UNAM
Cuernavaca, México
"Mechanisms controlling virulence gene expression in attaching and effacing bacterial pathogens"

09:30-10:00 ***Mario F. Feldman***
Alberta Ingenuity Centre for Carbohydrate Science, University of Alberta, Edmonton, Alberta, Canada.
"Glycoproteins and membrane vesicles: Bacteria can do that too"

10:00-10:30 ***Hebe Dionisi***
Centro Nacional Patagónico (CENPAT-CONICET), Puerto Madryn, Chubut, Argentina
"Novel molecular approaches for studying microbial communities"

10:30-11:00 **Coffee break**

11:00-13:00 **ORAL COMMUNICATIONS**
Room A
Signal Transduction (ST-C01/ST-C08)
Chairpersons: Gustavo Chiabrando CIBICI-CONICET. Universidad Nacional de Córdoba
Estela Machado, Universidad Nacional de Rio Cuarto

11:00-11:15
ST-C01
THE HYDRATION LEVEL OF A TRANSMEMBRANE HYDROPHILIC MOTIF MODULATES DesK ACTIVITY
Cybulski LE; Martin M; Mansilla MC; Fernandez A; De Mendoza D
Instituto de Biología Molecular y Celular de Rosario

11:15-11:30
ST-C02
ROLE OF LIPID BIOSYNTHESIS IN THE ACTIVATION OF THE YycFG SIGNAL TRANSDUCTION PROTEINS
Porrini L; Schujman GE; De Mendoza D
IBR - CONICET, Facultad de Cs. Bioq. y Farm., UNR. Rosario, Argentina

11:30-11:45
ST-C03
CELL SIGNALING IN *Trypansoma cruzi*: CLONING AND EXPRESSING A CLASS I PI-3 KINASE (TCPI3K)
Gimenez AM[1]; Schoijet AC[2]; Torres HN[2]; Flawia MM[2]; Machado EE[1]; Alonso GD[2]
[1]Depto. de Biología Molecular, FCEFQyN, U.N.R.C. [2]INGEBI (UBA CONICET)

11:45-12:00
ST-C04
ALPHA-2M/LRP-1 SYSTEM INDUCES Shc PHOSPHORYLATION AND NFkB ACTIVATION IN MACROPHAGE CELLS LINES
Cáceres LC; Lorenc VE; Sánchez MC; Chiabrando GA
CIBICI (CONICET). Dpto. Bioq. Clín. F.C.Q., U.N.C.

12:00-12:15
ST-C05
CHARACTERIZATION OF ANG II AT2 SIGNALING PATHWAY IN PND15 RAT HINDBRAIN
Seguin LR; Ciuffo GM
IMIBIO-SL,CONICET. UNSL. San Luis

12:15-12:30
ST-C06
DOPAMINE ACTIVATES ERK1/2 IN PROXIMAL TUBULE CELLS: ROLE OF PKC, PKA AND REACTIVE OXYGEN SPECIES
Acquier A; Mori Sequeiros García MM; Paz C; Mendez CF
IIMHNO, Department of Biochemistry, School of Medicine, University of Buenos Aires

12:30-12:45
ST-C07
HORMONE-DEPENDENT PHOSPHORYLATION OF ACYL-COA SYNTHETASE 4 (Acs14) IN STEROIDOGENIC CELLS
Castilla R; Smith E; Castillo AF; Poderoso C; Podestá EJ
IIMHNO and Department of Biochemistry, School of Medicine, University of Buenos Aires

12:45-13:00
ST-C08
THE ACTION OF TYROSINE PHOSPHATASES ON STAR PROMOTER ACTIVITY IS MEDIATED BY ARACHIDONIC ACID
Cooke M; Di Cónsoli H; Mele P; Gorostizaga A; Paz C; Podesta EJ; Cornejo Maciel F
IIHMNO - Dept. Bioquímica, Facultad de Medicina, UBA

11:00-12:15 **ORAL COMMUNICATIONS**

Room B

Plant Biochemistry and Molecular Biology (PL-C01/PL-C05)

Chairpersons:Fernando Carrari. Instituto de Biotecnología, INTA-Castelar
Mariana del Vas. Instituto de Biotecnología, INTA-Castelar

11:00-11:15
PL-C01
ANALYSIS OF RPL10 GENES IN *Arabidopsis thaliana*: ROLES IN DEVELOPMENT, TRANSLATION, AND UV-B RESPONSE
Falcone Ferreyra ML; Pezza A; Casati P
CEFOBI. Fac. Cs. Bioq. y Farm. UNR. Suipacha 531. 2000 Rosario. Argentina

11:15-11:30
PL-C02
REDOX REGULATION OF GLYCOLYTIC TRIOSE-P BRANCH POINT IN THE CYTOSOL OF WHEAT CELLS
Piattoni CV; Guerrero SA; Iglesias AA
IAL (UNL-CONICET), Lab. de Enzimología Molecular y Lab. de Bioquímica Microbiana, FBCB, Santa Fe.

11:30-11:45
PL-C03
MUTATIONS IN TWO PUTATIVE PHOSPHORYLATION MOTIFS IN LePRK2 SHOW ANTAGONISTIC EFFECTS ON TUBE LENGTH
Salem T[1]; Mazzella MA[1]; Wengier DL[1]; Motillo V[2]; Parisi G[2]; Muschietti J[1,3]
[1]INGEBI-CONICET, BA., Arg. [2]Ctro. Est. Inv., UNQ, Bernal, Arg. [3]Dto. FBMC, FCEN-UBA, BA., Arg.

11:45-12:00
PL-C04
NON-CANONICAL PROCESSING OF miRNAs IN PLANTS
Bologna NG; Mateos JL; Bresso EG; Palatnik JF
Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET), Rosario, Argentina.

12:00-12:15
PL-C05
HAHB10 SUNFLOWER TRANSCRIPTION FACTOR IS INVOLVED IN PHYTOHORMONE MEDIATED RESPONSE TO BIOTIC STRESS
Dezar CA; Giacomelli JI; Ré DA;Manavella PA; Alves-Ferreira M; Baldwin IT; Bonaventure G; Chan RL
Lab. de Biotecnología Vegetal, IAL-CONICET, Ciudad Universitaria, Santa Fe

11:00-13:00 **ORAL COMMUNICATIONS**

Room C

Lipids (LI-C01 / LI-C08)

Chairpersons: María Corvi. IIB-INTECH, CONICET. Universidad Nacional de San Martín
Ana Ves Losada. INIBIOLP-CONICET. Universidad Nacional de la Plata

11:00-11:15
LI-C01
DIFFERENTIAL REGULATION OF PHOSPHATIDYLCHOLINE-DERIVED SIGNALING PATHWAYS IN SYNAPTIC MEMBRANE RAFTS
Mateos MV; Salvador GA; Giusto NM
Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). UNS-CONICET

11:15-11:30
LI-C02
PGD2 INCREASES PTDCHO SYNTHESIS BY INTRANUCLEAR CCTα MOBILIZATION - PARTICIPATION OF ERK 1/2
Favale NO; Sterin-Speziale NB
Cátedra Biología Celular y Molecular, FFYB-UBA, IQUIFIB-CONICET, Buenos Aires, Argentina.

11:30-11:45
LI-C03
LIVER FATTY ACID BINDING PROTEIN (L-FABP) PARTICIPATES IN DIFFERENT CELL PROCESSES IN THE ENTEROCYTE
Falomir Lockhart LJ; Rodriguez Sawicki L; Franchini GR; Storch J; Córsico B
Instituto de Investigaciones Bioquímicas de La Plata (CONICET-UNLP), Fac. de Cs. Médicas, La Plata.

11.45-12:00
LI-C04
EFFECT OF THE HYDROPHOBIC POCKET STRUCTURE ON CATALYTIC PROPERTIES OF THE β-KETOACYL-ACP SYNTHASES
Trajtenberg F[1]; Altabe S[2]; Ficarra A[2]; Buschiazzo A[1]; De Mendoza D[2]; Schujman G[2]
[1]Instituto Pasteur Montevideo, Uruguay. [2]IBR - CONICET, UNR. Rosario, Argentina

12:00-12:15
LI-C05
E2F TOGETHER WITH SP1 REGULATES THE MURINE CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE ALPHA (CCTα) EXPRESSION
Elena C; Banchio C
Instituto de Biologia Molecular y Celular de Rosario, IBR-CONICET. Rosario, Argentina

12:15-12:30
LI-C06
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND NEURONAL DIFFERENTIATION
Marcucci H; Paoletti L; Banchio C
Instituto de Biologia Molecular y Celular de Rosario, IBR-CONICET. Rosario, Argentina

12:30-12:45
LI-C07

DIFFERENTIATION-RELATED CHANGES IN LIPIDS WITH VERY LONG CHAIN POLYENOIC FATTY ACIDS IN GERM CELLS

Oresti GM[1]; Reyes JG[2]; Furland NE[1]; Aveldaño M[1]
[1]INIBIBB, CONICET-UNS, Bahía Blanca, Argentina and [2]Instituto de Química, PUCV, Valparaíso, Chile.

12:45-13:00
LI-C08
NUCLEAR RECEPTORS, HEPATIC LIPID DROPLETS IN SUCROSE INDUCED LIPIDOGENESIS: REVERTION BY N-3 PUFA

Brenner RR; Bernasconi AM; Hein G; Montanar MA; Pellon-Maison M; Chicco A; Lombardo YB
[1]INIBIOLP (UNLP-CONICET) Fac Cs. Médicas, La Plata [2]Depart. Cs Biológicas, UNL, Sta Fe, Argentina

13:00-15:30 **Lunch**

15:30-16:30 Room A
Richard Lehner
Department of Pediatrics, Canadian Institutes of Health Research Group on the Molecular and Cell Biology of Lipids.
University of Alberta. Edmonton, Alberta, Canada
"Role of Carboxylesterase3/Triacylglycerol Hydrolase in Lipid Metabolism"
Chairperson: Claudia Banchio, IBR-CONICET. Universidad Nacional de Rosario

16:30-19:00 **POSTERS with coffee**
Microbiology (MI-P01/MI-P31)
Cell Biology (CB-P01/CB-P27)
Plant Biochemistry and Molecular Biology (PL-P01/PL-P32)
Lipids (LI-P01/LI-P12)
Biotechnology (BT-P01/BT-P11)
Enzymology (EN-P01/EN-P07)
Signal Transduction (ST-P01/ST-P07)

19:00-20:00 Room A
LECTURE
Marcio Alves-Ferreira
Laboratório de Genética Molecular Vegetal, Universidade Federal do Rio de Janeiro, Brasil.
"Stamen development: Discovery and characterization of novel genes"
Chairperson: Eduardo Zabaleta. IIB-CONICET. Universidad de Mar del Plata

20:00-21:00 Room B
Theatre Play
"Personalmente, Einstein"
Performed by Juan Tríbulo.
Directed by Leonardo Goloboff

THURSDAY, November 12th, 2009

08:30-10:30 Room A

SYMPOSIUM
"Plant Biochemistry and Molecular Biology"
Chairpersons: Paula Casatti, CEFOBI-CONICET. Universidad Nacional de Rosario
Carlos García-Mata, IIB-CONICET. Universidad Nacional de Mar del Plata

08:30-09:00 ***Carla Schommer***
IBR-CONICET, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina
"Biological functions of microRNA regulated TCP transcription factors in Arabidopsis thaliana"

09:00-09:30 ***Francesco Paolocci***
National Research Council, Plant Genetics Institute-Perugia, Italia
"Strategies to manipulate the biosynthesis of Proanthocyanidins"

09:30-10:00 ***Fabiana Drincovich***
CEFOBI-CONICET. Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina
"Redundancy or specificity of function? Multiple isoforms for plant malate oxidative decarboxylation"

10:00-10:30 ***Jorge Muschietti***
INGEBI-CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina
"Molecular mechanisms of pollen-pistil interactions in tomato and arabidopsis plants"

08:30-10:30 Room B
SIMPOSIUM
"Lipids"
Chairpersons: Silvia Altabe, IBR-CONICET. Universidad Nacional de Rosario
Marta Aveldaño, INIBIBB-CONICET. Universidad Nacional de Sur

08:30-09:00 ***Muriel Mazet***
Research Unit for Tropical Diseases (TROP), de Duve Institute, and Laboratory of Biochemistry, Université Catholique de Louvain. Brussels, Belgium
"Trypanosoma brucei glycosomal ABC transporters potentially involved in fatty acid transport"

09:00-09:30 ***Betina Córsico***
INIBIOLP-CONICET, Universidad Nacional de la Plata, La Plata, Buenos Aires, Argentina.
"Functional analysis of soluble lipid binding proteins"

09:30-10:00 ***Antonio Uttaro***
IBR-CONICET, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina
"About fatty acids, Trypanosomes and drug targets"

10:00-10:30 ***Elena Posse de Chaves***
Division of Physical Medicine and Rehabilitation, Centre for Neuroscience, University of Alberta, Edmonton, Alberta, Canada
"Cholesterol and sphingolipids in amyloid beta neurotoxicity"

10:30-11:00 **Coffee break**

11:00-12:15 **ORAL COMMUNICATIONS**

Room A

Plant Biochemistry and Molecular Biology (PL-C06/PL-C10)

Chairpersons: Juan Diaz Ricci, INSIBIO-CONICET. Universidad Nacional de Tucumán
Eduardo Zabaleta, IIB-CONICET. Universidad de Mar del Plata

11:00-11:15
PL-C06
PHYLOGENETIC AND EXPRESSION ANALYSES OF WRKY TRANSCRIPTION FACTORS PROVIDE INSIGHTS INTO SUNFLOWER
Giacomelli JI; Ribichich KF; Dezar CA; Chan RL
Laboratorio de Biotecnología Vegetal, Instituto de Agrobiotecnología del Litoral, CONICET/FBCB-UNL. E-mail: giacomelli@fbcb.unl.edu.ar

11:15-11:30
PL-C07
CHARACTERIZATION AND REGULATION OF A MAIZE FLAVONOL SYNTHASE
Rius SP[1]; Falcone Ferreyra ML[1]; Emiliani J[1]; Pourci L[2]; Feller A[2]; Morohashi K[2]; Casati P[1]; Grotewold E[2]
[1]CEFOBI-Univ.Nac.Rosario-Argentina. [2]Department Plant Cellular Molecular Biology. OSU-USA.

11:30-11:45
PL-C08
EFFECTS OF gammaCA2 OVEREXPRESSION ON MITOCHONDRIAL FUNCTION IN ARABIDOPSIS CELL SUSPENSIONS AND LEAVES
Villarreal F; Martin MV; Zabaleta E
Instituto de Investigaciones Biológicas, CONICET-UNMdP, Mar del Plata

11:45-12:00
PL-C09
ENDOCYTOSIS DURING SEED GERMINATION
Pagnussat LA; De La Canal L
Instituto de Investigaciones Biológicas, CONICET-UNMDP, Mar del Plata.

12:00-12:15
PL-C10
SPERMINE ACCUMULATION AND OXIDATION INCREASES *A. thaliana* TOLERANCE TO *P. viridiflava*
González ME[1]; Bortolotti C[2]; Blázquez MA[3]; Carrasco P[4]; Tiburcio AF[2]; Pieckenstain FL[5]; Ruiz OA[1]
[1]IIB-INTECh, Argentina. [2]LFB FFUB, España. [3]IVMCP UPV-CSIC, España. [4]DBBM UV, España. [5]CIC, Argentina.

11:00-13:15 **ORAL COMMUNICATIONS**

Room B

Microbiology (MI-C01/MI-C09)

Chairpersons: Mónica Delgado, INSIBIO-CONICET. Universidad Nacional de Tucumán
Alejandro Viale, IBR-CONICET. Universidad Nacional de Rosario

11:00-11:15
MI-C01
A B-CELL MITOGEN INVOLVED IN IMMUNOMODULATION AND CHRONICITY IN BRUCELLOSIS
Spera JM; Comerci DJ; Ugalde JE; Ugalde RA
Instituto de Investigaciones Biotecnologicas (IIB-UNSAM-CONICET)

11:15-11:30
MI-C02
THE LIGHT ACTIVATED PATHWAY TO VIRULENCE IN *Brucella abortus*
Paris G[1]; Carrica MC[1]; Tseng TS[1]; Frederickson M[3]; Bogomolni R[3]; Briggs W[2]; Goldbaum FA[1]
[1]Instituto Leloir IIBBA-CONICET. [2]Carnegie Institution, Stanford Univ. [3]Dept of Biochemistry, UCSC

11:30-11:45
MI-C03
BLUE-LIGHT DRIVEN ENZYMATIC ACTIVITIES IN EXTREMOPHILIC BACTERIA FROM HIGH-ALTITUDE ANDEAN LAKES
Albarracín VH; Pathak G; Marranzino G; Borsarelli CD; Gärtner W; Farías ME
Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas- PROIMI-CONICET

11.45-12:00
MI-C04
THE LOV HISTIDINE KINASE OF *R. leguminosarum* REGULATES THE SECRETION OF EPS AND FLAGELLAR PROTEINS
Bonomi HR[1]; Posadas D[1]; Paris G[1]; Carrica MC[1]; Bogomolni R[2]; Zorreguieta A[1]; Goldbaum FA[1]
[1]Fundación Instituto Leloir IIBBA-CONICET. [2]Dept of Biochemistry, Univ of California, Santa Cruz

12:00-12:15
MI-C05
INTRACELLULAR PROTEOME OF *Brucella abortus*
Roset MS[1]; Alefantis TG[2]; Grewal P[2]; DelVecchio VG[2]; Ugalde RA[1]
[1]Instituto de Investigaciones Biotecnológicas IIB-UNSAM-CONICET, Argentina. [2]Vital Probes Inc, USA

12:15-12:30
MI-C06
***Serratia marcescens* INVADES EPITHELIAL CELLS AND FINDS A SUITABLE NICHE TO SURVIVE AND REPLICATE**
Fedrigo GV[1]; Campoy EM[2]; Colombo MI[2]; García Véscovi E[1]
[1]IBR-CONICET, U.N.R., Rosario. [2]IHEM-CONICET U.N. Cuyo, Mendoza

12:30-12:45
MI-C07
VIRULENCE GENE REGULATION IN *Shigella sonnei* SPONTANEUS MUTANTS
Robles Luna G; Maldonado L; De Urraza PJ
Laboratorio de Microbiología General, Depto. de Ciencias Biológicas, Fac. de Ciencias Exactas, UNLP

12:45-13:00
MI-C08

REGULATION OF THE SECRETED PHOSPHOLIPASE PhlA AND FLAGELLAR EXPRESSION IN *S. marcescens*
Castelli ME; García Véscovi E
Instituto de Biolgía Molecular y Celular de Rosario, IBR-CONICET, Universidad Nacional de Rosario.

13:00-13:15
MI-C09
MabR, A NOVEL TRANSCRIPTIONAL REGULATOR OF MYCOLIC ACID BIOSYNTHESIS IN MYCOBACTERIA
Salzman V; Mondino S; Peirú S; Gago G; Gramajo H
Instituto de Biología Molecular y Celular de Rosario. IBR-CONICET, Rosario-Argentina

11:00-13:00 **ORAL COMMUNICATIONS**
Room C
Cell Biology (CBC01/CB-C08)
Chairpersons: Mario Guido, CIQUIBIC-CONICET. Universidad Nacional de Córdoba
Walter Beron, IHEM-CONICET. Universidad Nacional de Cuyo

11:00-11:15
CB-C01
***Trypanosoma cruzi* EMPLOYS THE SNARE VAMP-7 TO INVADE HOST CELLS**
Arboit MA; Colombo MI; Romano PS
Instituto de Histología y Embriología (IHEM)-CCT Mendoza-CONICET.

11:15-11:30
CB-C02
***Mycobacterium marinum* INTERACTS WITH LC3 AND BENEFITS FROM AUTOPHAGY INDUCTION**
Lerena MC; Colombo MI
Lab. de Biol. Celular y Molecular, IHEM-CONICET, F.C.M. U.N. Cuyo, Mendoza, Argentina.

11:30-11:45
CB-C03
DISRUPTION OF THE SECRETORY PATHWAY ALTERS THE DEVELOPMENT OF THE *Coxiella* REPLICATIVE VACUOLE
Campoy EM; Zoppino FCM; Colombo MI
Laboratorio de Biología Celular y Molecular. (IHEM-CONICET). F.C.M. U.N.Cuyo. Mendoza.

11:45-12:00
CB-C04
MODELING RAB-DEPENDENT INTRACELLULAR TRANSPORT
Mayorga LS; Campoy E
IHEM (UNCuyo-CONICET), Fac. Cs. Médicas, UNCuyo, Mendoza.

12:00-12:15
CB-C05
GLUCOSIDASE II BETA SUBUNIT MODULATES N-GLYCAN TRIMMING IN FISSION YEASTS AND MAMMALS
Stigliano Id[1]; Caramelo J1[2]; Cabriola CA[1]; Parodi AJ[1]; D'Alessio C[1,2]

[1]Fundación Instituto Leloir and IIBBA, CONICET; [2]Facultad de Ciencias Exactas y Naturales, UBA

12:15-12:30
CB-C06
A ROLE FOR EIF4E ON mRNA TURNOVER IN PROCESSING BODIES
Ferrero PV; Layana C; Rivera Pomar R
Centro Regional de Estudios Genómicos, UNLP y Departamento de Cs Básicas y Experimentales, UNNOBA.

12:30-12:45
CB-C07
SEARCH FOR ENDOGENOUS SMALL RNAs AFFECTING ALTERNATIVE SPLICING BY TGS
Alló M; Bertucci P; Agirre E; Eyras E; Valcarcel J; Kornblihtt AR
Laboratorio de Fisiología y Biología Molecular, FCEyN-UBA e IFIByNE-CONICET

12:45-13:00
CB-C08
ROLE OF KRÜPPEL-LIKE FACTOR 6 IN PSG GENE EXPRESSION DURING TROPHOBLAST DIFFERENTIATION
Racca A; Camolotto S; Bocco JL; Genti-Raimondi S; Panzetta-Dutari GM
CIBICI-CONICET. Bioquímica Clínica. Fac Cs Químicas. UNC. Córdoba, Argentina.

13:15-15:00 **Lunch**

15:00-16:30 Room A

SHORT LECTURES "JUNIOR FACULTY"

Chairperson: Luis Quesada Allué. IIBBA-CONICET, Fundación Instituto Leloir

Gabriela Pagnussat
IIB-CONICET, Universidad de Mar del Plata, Mar del Plata, Buenos Aires, Argentina
"Auxin directs patterning and gamete specification in the Arabidopsis female gametophyte"

Rodolfo Rasia
IBR-CONICET. Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina
"Is NMR slow? From gene to fold in only two weeks"

Viviana Rapisarda
Instituto Superior de Investigaciones Biológicas
INSIBIO-CONICET, Tucumán, Argentina
"*Genes expression regulation and new insights on functions of the aerobic respiratory chain components in Escherichia coli"*

16:30-19:00 **POSTERS with coffee**
Microbiology (MI-P32/MI-P63)

Cell Biology (CB-P28/CB-P56)
Plant Biochemistry and Molecular Biology (PL-P33/PL-P67)
Biotechnology (BT-P12/BT-P24)
Enzymology (EN-P08/EN-P14)
Signal Transduction (ST-P08/ST-P17)

19:00-20:30 Room A

TRIBUTE TO DARWIN

Chairperson: Néstor Carrillo, IBR-CONICET. Universidad Nacional de Rosario

19:00-19:30 ***Pablo Goloboff***
Facultad de Ciencias Naturales e Instituto Miguel Lillo.
Universidad Nacional de Tucumán, Tucumán, Argentina
"Darwin and the tree of life"

19:30-20:00 ***Enrique Caviedes-Vidal***
Instituto Multidisciplinario de Investigaciones
Biológicas de San Luis (IMIBIO-SL), San Luis, Argentina
"Evolutionary design of energy acquisition in flying vertebrates"

20:00-20:30 ***Marcelo Rubinstein***
Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI)
Buenos Aires, Argentina
"Molecular evolution as a driving force to understand gene function"

20:30 **SAIB General Assembly**

FRIDAY, November 13th, 2009

08:30-10:30 Room A

IUBMB SYMPOSIUM

"Membrane Biogenesis and Trafficking in Health and Disease II"

Chairpersons: Cecilia Alvarez, CIBICI-CONICET. Universidad Nacional de Córdoba.
Marisa Colombo, IHEM-CONICET. Universidad Nacional de Cuyo

08:30-09:00 ***Hugo D. Luján***
Laboratorio de Bioquímica y Biología Molecular, Facultad de Medicina,
Universidad Católica de Córdoba, Córdoba, Argentina
"Molecular mechanisms of protein trafficking during cyst wall formation in Giardia"

09:00-09:30 ***Francisco J. Barrantes***
INIBIBB-CONICET, Universidad Nacional del Sur, Bahía Blanca, Argentina
"Social" domain organization and dynamics of nicotinic acetylcholine receptor at the cell membrane"

09:30-10:00 ***Hugo J.F. Maccioni***
CIQUIBIC-CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina
"Golgi COG complex deficiencies: Just a congenital disorder of glycosylation?"

10:00-10:30 ***Esteban C. Dell´Angelica***

Department of Human Genetics, University of California, Los Angeles, CA, USA

"Hermansky-Pudlak syndrome and the biogenesis of lysosome-related organelles"

08:30-10:30 Room B

SYMPOSIUM

"Signal Transduction"

Chairpersons: Ernesto Podestá. IIMHNO, Facultad de Medicina. Universidad de Buenos Aires
Anabella Srebrow. IFIBYNE-CONICET. Universidad de Buenos Aires

08:30-09:00 ***José Luis Bocco***

CIBICI-CONICET. Dpto. Bioquímica Clínica Facultad de Ciencias Químicas
Universidad Nacional de Córdoba. Córdoba, Argentina

"Regulation of KLF6 transcription factor function by JNK and p38 signaling pathways"

09:00-09:30 ***Martine Culty***

The Research Institute of the McGill University Health Centre, McGill University.
Montreal, Quebec, Canada

"Signaling pathways regulating testicular gonocyte development"

09:30-10:00 ***Cristina Paz***

IIMHNO, Departamento de Bioquímica Humana. Facultad de Medicina.
Universidad de Buenos Aires. Buenos Aires, Argentina.

"Mitochondrial and nuclear kinase complex: role of MAPKs and MKPs in the cyclic AMP-mediated response"

10:00-10:30 ***Guillermo Daniel Alonso***

INGEBI-CONICET. Universidad de Buenos Aires. Buenos Aires, Argentina

"Signal transduction under stress conditions in Trypanosoma cruzi"

10:30-11:00 **Coffee break**

11:00-13:00 **ORAL COMMUNICATIONS**

Room A

Cell Biology (CB-C09/CB-C14) and Structural Biology (SB-C01/SB-C02)

Susana Genti-Raimondi, CIBICI-CONICET. Universidad Nacional de Córdoba
María Carolina Touz, INIMEC-CONICET, Córdoba

11:00-11:15

CB-C09

CCN6-DEPENDENT REGULATION OF ZEB1 IN HUMAN MAMMARY EPITHELIAL CELLS

Lorenzatti G[1]; Pal A[2]; Cabanillas AM[1]; Kleer CG[2]
[1]CIBICI-CONICET, Dpto Bioq Clínica, Fac Cs Qs, UNC; [2]Cancer Center, University of Michigan, USA.

11:15-11:30
CB-C10
THE SERINE-ARGININE RICH PROTEIN SF2/ASF REGULATES PROTEIN SUMOYLATION
Srebrow A; Pelisch F
Instituto de Fisiología, Biología Molecular y Neurociencias-CONICET, FBMC, FCEyN UBA.

11:30-11:45
CB-C11
DNA DAMAGE AND CELL DEATH PATHWAYS: ROLE OF POLY-ADP-RIBOSYLATION IN *Trypanosoma cruzi*
Vilchez Larrea SC; Caicedo SP; Alonso GD; Torres HN; Flawiá MM; Fernández Villamil SH
Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-UBA-CONICET).

11:45-12:00
CB-C12
PHOSPHOLIPID BIOSYNTHESIS IS DISPENSABLE FOR BACTERIAL CELL DIFFERENTIATION
Diez V; Schujman GE; De Mendoza D
IBR - CONICET, Facultad de Cs. Bioq. y Farm., UNR.

12:00-12:15
CB-C13
SPHINGOSINE KINASE 1 IS REQUIRED FOR MIGRATION AND GROWTH OF MELANOMA
Alvarez S[1,2]; Maceyka M[2]; Kordula T[2]; Milstien S[2]; Spiegel S[2]
[1]Universidad Nacional de San Luis Argentina; [2]Virginia Commonwealth University Richmond USA.

12:15-12:30
CB-C14
INVOLVEMENT OF P19INK4D IN REPLICATIVE SENESCENCE
Sonzogni SV; Byk LA; Canepa ET
Laboratorio de Biología Molecular-Depto Química Biológica-FCEN-UBA. Buenos Aires.

12:30-12:45
SB-C01
CYCLODEXTRIN GLUCANOTRANSFERASE FROM *Bacillus circulans* DF 9R: PRODUCT SPECIFICITY AT THE -6 SUBSITE
Costa H[1]; Hidalgo A[2]; Ferrarotti SA[1]; Berenguer J[2]; Biscoglio de Jiménez Bonino MJ[3]
[1]Dpto. Ciencias Básicas, UNLu. [2]CBMSO, UAM. [3]IQUIFIB (UBA-CONICET), FFyB, UBA.

12:45-13:00
SB-C02
FITNESS LANDSCAPE OF METALLO-β-LACTAMASE-MEDIATED ANTIBIOTIC RESISTANCE
Tomatis PE; Meini MR; Vila AJ
Instituto de Biología Molecular y Celular de Rosario (IBR- CONICET) Rosario.

11:00-13:15 **ORAL COMMUNICATIONS**

Room B

Microbiology (MI-C10/MI-C18)

Chairpersons: Hugo Gramajo, IBR-CONICET. Universidad Nacional de Rosario
María Julia Amoroso, INSIBIO-CONICET. Universidad Nacional de Tucumán

11:00-11:15
MI-C10
BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF PHOSPHOTRANS-ACETYLASE FROM *E. coli*
Campos Bermúdez VA; Bologna FP; Drincovich MF; Andreo CS
CEFOBI Facultad Cs. Bioq. y Farm. (UNR). Rosario. Argentina.

11:15-11:30
MI-C11
CLONAL ANALYSIS OF *S. aureus* ISOLATES CARRYING THE *cap5* COMPARED WITH THOSE CARRYING THE *cap8* ALLELE
Lattar SM; Tuchscherr LPN; Centron D; Cerquetti C; Buzzola FR; Sordelli D
Department of Microbiology, University of Buenos Aires School of Medicine, Buenos Aires, Argentina

11:30-11:45
MI-C12
PHOSPHATE-ENHANCED STATIONARY PHASE FITNESS OF *E. coli* IS RELATED TO POLYPHOSPHATE LEVEL
Schurig-Briccio LA; Farías RN; Rintoul MR; Rapisarda VA
INSIBIO e Inst. de Química Biológica "Dr. B. Bloj"(CONICET-UNT) Tucumán, Argentina

11.45-12:00
MI-C13
***Pseudomonas aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE CONTAINS TWO SITES FOR QUATERNAY AMMONIUM**
Otero LH; Beassoni PR; Boetsch C; Domenech CE
Dpto. Biologia Molecular, FCEFQyN, Universidad Nacional de Rio Cuarto

12:00-12:15
MI-C14
EXPRESSION OF AN ALCOHOL DEHYDROGENASE FROM *L. mesenteroides* IN *E. coli* REDOX MUTANTS
Nikel PI[1]; Ramirez MC[1]; Pettinari MJ[2]; Méndez BS[2]; Galvagno MA[1]
[1]IIB, UNSAM; [2]Dep. Qca. Biológica, FCEyN, UBA

12:15-12:30
MI-C15
ARCHAEA IN EXTREME HIGH ALTITUDE ANDEAN LAKES (HAAL)
Maldonado MJ; Ferrero M; Lara JA; Farías ME
CCT-Planta Piloto de Procesos Industriales Microbiológicos. Tucumán.

12:30-12:45
MI-C16
RAPID AND SENSITIVE *Xanthomomas citri* DETECTION BY LAMP COMBINED WITH NAKED EYE EVALUATION METHODS
Rigano LA[1]; Marano MR[2]; Castagnaro AP[3]; Do Amaral AM[4]; Vojnov AA[1]
[1]Centro Milstein-FPC, Argentina. [2]IBR, Argentina. [3]EEAOC, Argentina. [4]Centro ApTa Citros, Brazil

12:45-13:00
MI-C17
NEW GENERIC PRIMERS LEAD TO THE CHARACTERIZATION OF HPV 115, A NOVEL Beta-Papillomavirus species 3
Chouhy D[1]; Gorosito M[2]; Sanchez A[2]; Cavatorta AL[1]; Serra EC[1]; Bergero A[2]; Fernandez Bussy R[2]; Giri AA[1]
[1]Facultad de Cs. Bioquímicas y Farmacéuticas (UNR)/IBR-CONICET. [2]Facultad de Cs. Médicas (UNR)

13:00-13:15
MI-C18
CHARACTERIZATION OF Fijivirus P9-1 VIROPLASM PROTEIN: SIMILARITIES TO ANIMAL REOVIRUS COUNTERPARTS
Maroniche GA; Mongelli VC; Peralta AV; Distéfano AJ; Taboga OA; Hopp EH; Del Vas M
Instituto de Biotecnología, CICVyA, INTA-Castelar

11:00-13:00 **ORAL COMMUNICATIONS**
Room C

Lipids (LI-C09/LI-C11) and Biotechnology (BTC01/BT-C05)
Chairpersons: Mario Baigori, PROIMI-CONICET, Tucumán
José Luis Barra, CIQUIBIC-CONICET. Universidad Nacional de Córdoba

11:00-11:15
LI-C09
GLUCOSYLCERAMIDE SYNTHASE IS ESSENTIAL IN MDCK CELL DIFFERENTIATION
Pescio LG; Iribarren PA; Sterin-Speziale NB
Biología Celular y Molecular. FFyB, UBA. IQUIFIB-CONICET. Buenos Aires, Argentina

11:15-11:30
LI-C10
NUCLEAR NEUTRAL LIPIDS ARE ORGANIZED IN DISCRETE FUNCTIONAL DOMAINS
Layerenza JP[1]; González P[2]; García de Bravo M[1]; Polo M[1]; Sisti MS[1]; Ves-Losada A[1,3]
[1]INIBIOLP (CCT-LP-CONICET-UNLP); [2]Cát Pat-FacCsMéd UNLP & CIC-BsAs; [3]Dpt Cs Biol-FacCsExact UNLP

11:30-11:45
LI-C11
MELATONIN EFFECT ON FE^{2+} INITIATED PEROXIDATION OF SONICATED LIPOSOMES MADE WITH RETINAL LIPIDS
Fagali NS; Catala A
INIFTA-CCT-La Plata-CONICET, Facultad de Ciencias Exactas, UNLP, Argentina

11.45-12:00
BT-C01

EFFECT OF POLY(3-HYDROXYBUTYRATE) PRODUCTION IN RECOMBINANT *E. coli*
De Almeida A[1]; Giordano A[1]; RhodiusV[2]; Gross CA[2]; Nikel P[1,3]; Pettinari MJ[1]
[1]FCEyN, UBA, Argentina. [2]University of California, San Francisco, USA. [3]IIB-UNSAM, Argentina.

12:00-12:15
BT-C02
INTER-SPECIFIC HYBRIDATION IMPROVES FORAGE QUALITY, SALT TOLERANCE AND TANNINS LEVELS IN *Lotus* spp
EscarayFJ[1]; Paolocci F[2]; Carrasco P[3]; Del Valle S[3]; Pieckenstain FL[1]; Ruiz OA[1]
[1]IIB-INTECh. Argentina. [2]IGV-CNR Italia. [3]Universidad de Valencia. España.

12:15-12:30
BT-C03
COMPARATIVE SAFETY ASSESSMENT OF GENETICALLY ENGINEERED ANTI-BROWNING POTATOES
Llorente BE[1]; Rodríguez V[1]; Carrari F[2]; López MG[2]; Bravo-Almonacid FF[1]; Torres HN1; Flawiá MM[1]; Alonso GD[1]
[1]INGEBI (UBA-CONICET) Bs As, Argentina. [2]Instituto de Biotecnologia INTA-Castelar, Argentina.

12:30-12:45
BT-C04
IMMUNOLOGICAL CHARACTERIZATION OF A HYPOALLERGENIC VARIANT OF ANTIGEN 5
VinzonS[1]; Massari N[2]; Rivera E[2]; Biscoglio de Jiménez Bonino MJ[1]
[1]IQUIFIB (UBA-CONICET); FFyB, UBA. [2]FFyB, UBA.

12:45-13:00
BT-C05
CHYMOTRYPSIN-SUSCEPTIBLE MICROCIN J25 DERIVATIVES: POTENTIAL APPLICABILITY IN FOOD PRESERVATION
Pomares MF; Salomón RA; Zenoff AM; Farías RN; Vincent PA
Dep. de Bqca. de la Nutrición. INSIBIO (UNT-CONICET). Tucumán

13:15-15:30 **Lunch**

15:30-16:30 Room A

LECTURE

Vassilios Papadopoulos

Department of Medicine, McGill University, Montreal, Quebec, Canada.

"Translocator protein (18-kDa): a conserved mechanism of cholesterol transport in health and disease"

Chairperson: Ernesto Podestá, IIMHNO, Facultad de Medicina. Universidad de Buenos Aires

16:30 19:00 **POSTERS with coffee**

Microbiology (MI-P64/MI-P94)
Cell Biology (CB-P57/CB-P86)
Plant Biochemistry and Molecular Biology (PL-P68/PL-P97)
Lipids (LI-P13/LI-P23)

Structural Biology (SB-P01/SB-P12)
Neuroscience (NS-P01/NS-P11)

19:00-20:00 Room B

“EMBO” LECTURE

Philippe Sansonetti

Head of the Unité de Pathogénie Microbienne Moléculaire
Institut Pasteur, Paris, France

"Rupture, invasion and inflammatory destruction of the intestinal epithelium by Shigella: the Yin and the Yang of innate immunity"

Chairperson: Alberto Kornblihtt. IFIBYNE-CONICET. Universidad de Buenos Aires.

21:00 **Closing Dinner**

IUBMB-Conference

G PROTEIN-REGULATED SIGNALING NETWORKS AND CANCER

Gutkind JS
Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health, Bethesda, MD 20892, USA.

The G protein-coupled receptor (GPCR) family constitutes the largest group of cell surface proteins involved in signal transduction. GPCRs participate in a wide variety of physiological functions and human disease conditions, as reflected by the fact that GPCRs are the target of 50-60% of all current therapeutic agents. The role of GPCRs in cancer is an area of active investigation, as although there are infrequent mutations in GPCRs, these receptors contribute to tumor progression when persistently stimulated by tumor or stromal-released agonists. Emerging information on how cancer cells co-opt chemokine networks and their G protein-initiated signaling circuitry to promote angiogenesis and to invade their surrounding and target tissues will be discussed. GPCRs also play a central role in viral infection and viral tumorigenesis. The Kaposi's sarcoma (KS) associated herpesvirus (KSHV), which causes KS, the most common AIDS-associated neoplasm, expresses a constitutively active GPCR, *vGPCR*. Surprisingly, while using an *in vivo* endothelial-specific retroviral system to express KSHV oncogenes in mice, we observed that only *vGPCR* was able to promote KS-like vascular lesions. Current studies exploring the molecular mechanisms underlying the transforming activity of *vGPCR* will be also presented. Ultimately, this knowledge may provide unique opportunities for cancer prevention and treatment.

LI-Conference

ROLE OF CARBOXYLESTERASE 3 / TRIACYLGLYCEROL HYDROLASE IN LIPID METABOLISM

Lehner R
University of Alberta. Edmonton, Canada. E-mail: richard.lehner@ualberta.ca

Excessive accumulation of triacylglycerol in peripheral tissues is tightly associated with obesity, and has been identified as an independent risk factor for insulin resistance, type 2 diabetes and cardiovascular complications. Carboxylesterase3/Triacylglycerol Hydrolase (Ces3/TGH), a lipase residing in the endoplasmic reticulum, mobilizes triacylglycerols stored in lipid droplets (LDs) for the secretion of very-low density lipoproteins. Ablation of Ces3/TGH expression in mice (Tgh-/-) results in decreased plasma triacylglycerol, apolipoprotein B and fatty acid levels. Despite the attenuation of very-low density lipoprotein secretion, TGH deficiency does not increase hepatic triacylglycerol levels. Tgh-/- mice exhibit increased food intake, respiratory quotient and energy expenditure without change in body weight. These metabolic changes are accompanied by improved insulin sensitivity and glucose tolerance. Tgh-/- mice have smaller sized pancreatic islets but maintain normal glucose stimulated insulin secretion. These studies demonstrate that TGH represents an ideal therapeutic target for lowering blood lipid levels.

PL-Conference

STAMEN DEVELOPMENT: DISCOVERY AND CHARACTERIZATION OF NOVEL GENES

Romanel E; Banhara A; Alves-Ferreira M
Laboratório de Genética Molecular Vegetal, Universidade Federal do Rio de Janeiro, Brasil. E-mail: alvesfer@biologia.ufrj.br

Despite rapid advances in understanding the principles of plant development, much remains to be learned about the molecular mechanisms underlying organ and cell-type specification, as well as about the genes that execute these fundamental biological processes. To obtain detailed information about gene expression during stamen development in Arabidopsis (*Arabidopsis thaliana*), we compared, by microarray analysis, the gene expression profile of wild-type inflorescences to those of the floral mutants *apetala3 (ap3)*, *sporocyteless/nozzle (spl)*, and *male sterile1* (*ms1*), in which different aspects of stamen formation are disrupted. These experiments led to the identification of groups of genes with predicted expression at early, intermediate, and late stages of stamen development. We applied reverse genetics to characterize several of the genes identified in the microarray experiments and uncovered novel regulators of stamen development and microsporogenesis, including members of B3 family of transcription factors and a putative phosphatidylinositol 4-kinase.

ST-Conference

TRANSLOCATOR PROTEIN (18-KDA): A CONSERVED MECHANISM OF CHOLESTEROL TRANSPORT IN HEALTH AND DISEASE

Papadopoulos V
The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada.

Steroid hormones play essential role in the maintenance of eukaryotic homeostasis, adaptability to the environment, developmental and reproductive functions. Gonads, adrenal, brain and placenta are the main sites of steroid synthesis. Under the control of pituitary hormones, the adrenals and gonads synthesize steroid hormones from cholesterol. Hormone-induced cholesterol transport from the cytosol to the inner mitochondrial membrane (IMM) is the rate-determining step in steroid biosynthesis. Translocator protein (18-kDa; TSPO) is an outer mitochondrial membrane (OMM) high affinity drug- and cholesterol-binding protein that takes up free cholesterol from a cytosolic donor and transfers it to IMM through the mitochondrial contact sites. Using biochemicals, molecular and pharmacological tools the function of TSPO in the physiology of steroid formation but also at the pathophysiology of a number of human diseases was demonstrated. Such diseases include hormone-independent hypercortisolism, adrenal and gonadal cell tumorigenesis associated with constitutive steroid formation, stress- and disease-induced hypecortisolism and neurobehavioral disorders. In parallel, TSPO was found to be conserved from bacteria to humans where its drug- and cholesterol-binding domains and functions were conserved. While surveying for *Tspo*-like genes, we identified a paralogous gene, *Tspo2*, encoding an evolutionary conserved family of proteins that arose by gene duplications before the divergence of avians and mammals. TSPO2 retained the cholesterol, but not drug, binding domain and was shown to mediate cholesterol redistribution-dependent erythroblast maturation during mammalian erythropoiesis.

IUBMB I-S01.
BIOCHEMICAL AND TOPOLOGICAL MEMBRANE ALTERATIONS DURING THE SPERM ACROSOMAL EXOCYTOSIS

Mayorga LS, Castillo Bennett J, Zanetti N, Roggero CM, Tomes CN
IHEM (UNCuyo-CONICET), Fac. Cs. Médicas, UNCuyo, Mendoza, Argentina. E-mail: lmayorga@fcm.uncu.edu.ar

Sperm have a single opportunity to fertilize an egg; hence they must release the content of their secretory granule (the acrosome) at the right time and place. In resting sperm, the factors that participate in exocytosis are maintained in an inactive state. SNAREs are assembled in cis complexes whereas NSF and synaptotagmin are inactivated by phosphorylation. The sperm/egg interaction triggers a calcium influx from the extracellular medium that initiates several processes, including the activation of PTP1B and calcineurin, two phosphatases required for the desphosphorylation of NSF and synaptotagmin, respectively. At the same time, the acrosome swells and forms deep invaginations in the outer acrosomal membrane. The protruding edges of these invaginations contact the plasma membrane and these appositions are stabilized by the assembly of trans SNARE complexes. For the opening of fusion pores, calcium must be released from inside the acrosome to activate a synaptotagmin/complexin interplay that is necessary for the final steps of the exocytosis. Fusion pore opening and expansion in the ring of apposed membranes generates hybrid vesicles that together with the acrosomal contents, are released during the acrosomal exocytosis. This complex process is absolutely necessary for fertilization in human and animals.

IUBMB I-S02.
REGULATION OF MEMBRANE EXPANSION AT THE NERVE GROWTH CONE: AXONAL SPECIFICATION AND ELONGATION

Quiroga S.
Dpto. de Química Biológica y CIQUIBIC, Fac. de Ciencias Químicas, Univ. Nacional de Córdoba-CONICET. E-mail: squiroga@mail.fcq.unc.edu.ar

Axonal growth is one of the hallmarks of neuronal polarization, and requires axonal membrane expnasion by exocytosis of plasmalemmal precursor vesicles (PPVs) at the nerve growth cone. We have demonstrated that IGF-1 stimulates the regulated exocytosis of PPVs via activation of the Pi3k pathway. Few details are known, however, about the PPVs targeting mechanisms. Results from our laboratory show that the cascade critical for the regulation of membrane expansion in neurons includes Tc10 and the exocyst complex. We have also examined the role of growth factors in polarization and established that IGF-1 is the growth factor initiating axonal specification and that a particularly early event, in neurons that do not yet exhibit a discernible axon, is the segregation of activatable IGF-1 receptors in one neurite. Activation of the IGF-1 receptor requires its insertion into the plasmalemma, controlled by Tc10 activity and the exocyst complex. Because IGF-1 activates Tc10 and triggers exocyst assembly it may regulate the insertion of its own receptor. This is a positive-feedback mechanism that could rapidly amplify the membrane expansion response to IGF-1. We propose, therefore, that the process of *IGF-1 receptor mobilization to neurite plasmalemma / receptor activation / and further membrane expansion* may be (one of) the self-reinforcing mechanism(s) necessary for axonal specification.

IUBMB I-S03.
A CLAMPING MECHANISM FOR CALCIUM-REGULATED EXOCYTOSIS

Giraudo CG
Department of Cell Biology; School of Medicine, Yale University. New Haven, CT, USA. E-mail: claudio.giraudo@yale.edu

In regulated exocytosis, the core membrane fusion machinery proteins, the SNARE proteins, are assisted by a group of regulatory factors in order to couple membrane fusion to an increase of intracellular calcium ion (Ca^{2+}) concentration. In neurons, complexin-I and synaptotagmin-I have been shown to be key elements for this tightly regulated process. Many studies suggest that complexin-I can arrest the fusion reaction and that synaptotagmin-I can release the complexin-I blockage in a calcium-dependent manner. Although the actual molecular mechanism by which they exert their function is still unknown, results using the *in vitro* fusion assay "flipped-SNARE cell fusion assay" allow us to propose a simple model in which the accessory helix of complexin clamps fusion by locally forming an alternative four-helix bundle near the membrane. When complexin`s accessory helix is bound to the t-SNARE complex fusion is clamped "off"; alternatively, when the membrane-proximal helix of the v-SNARE is bound, fusion is completed.

IUBMB II-S01.
MOLECULAR MECHANISMS OF PROTEIN TRAFFICKING DURING CYST WALL FORMATION IN *Giardia*

Lujan HD
School of Medicine. Catholic University of Cordoba. Argentina. E-mail: hlujan@ucc.edu.ar

Giardia is a common cause of diarrheal disease. Trophozoites undergo fundamental changes to survive outside the intestine by differentiating into infective cysts. Encystation entails the synthesis, transport, secretion, and assembly of cyst wall materials into a protective cyst wall. We study the molecular events leading to cyst formation, with emphasis on vesicular transport in this parasite reported to lack a Golgi apparatus. In *Giardia* there are three closely related proteins that localize within encystation-specific secretory vesicles (ESVs) in encysting trophozoites and in the cyst wall of cysts. The cyst wall protein genes predict proteins of 26 (CWP1), 39 (CWP2), and 27 (CWP3) kDa. CWP2 differs from CWP1 and CWP3 by a C-terminal extension rich in basic amino acids. Recently, we (a) determined the role of the C-terminal extension of CWP2 during ESV formation, (b) identified and characterized all SNARE proteins, and (c) identified the complete set of ARFs, ARF-GAP, GEFs, RABs and components of COPI and COPII. Our results show that the CWP2 basic tail is necessary to induce the biogenesis of ESVs at the endoplasmic reticulum and found that *Giardia* requires a small number of SNAREs, ARFs, and RABs to accomplish all secretory events that are critical for growth and differentiation, confirming the absence of a typical Golgi apparatus in this eukaryotic cell.

IUBMB II-S02.
"SOCIAL" DOMAIN ORGANIZATION AND DYNAMICS OF NICOTINIC ACETYLCHOLINE RECEPTOR AT THE CELL MEMBRANE

Barrantes FJ
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A combination of experimental techniques (patch-clamp, confocal FRAP and FCS, TIRF single-particle tracking, high-resolution fluorescence microscopy) has been used to analyze the supramolecular organization of the acetylcholine receptor (AChR), the dynamics of the receptor at the cell surface, and the kinetics of receptor internalization. Changes in cholesterol (Chol) content affected muscle and neuronal-type AChR organization and dynamics at the cell surface. Chol depletion produced gain-of-function of single-channel dwell time. Submicron-sized (~250 nm) domains, stable over a period of hours at the cell membrane, could be resolved into AChR "nano-clusters" with a peak size distribution of ~55 nm by STED microscopy. Chol depletion reduced the number of nanoclusters, increasing their size, and changed their supramolecular "social" organization on larger scales (0.5-3.5 microns). FRAP, FCS and TIRF SPT experiments provided information on the dynamics of AChR nanoclusters, disclosing the dependence of their mobility on Chol content and cortical cytoskeleton. Chol content at the plasmalemma may thus modulate cell-surface organization and dynamics of receptor domains, and fine-tune receptor channel function to temporarily compensate for acute AChR losses from the cell surface.

IUBMB II-S03.
GOLGI COG COMPLEX DEFICIENCIES: JUST A CONGENITAL DISORDER OF GLYCOSYLATION?

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The Conserved Oligomeric Golgi complex (COG) is a Golgi-associated tethering heteromer involved in retrograde vesicular trafficking of multiple Golgi glycan processing enzymes. COG deficiencies lead to abnormal organization of the Golgi complex and defective trafficking of these enzymes, leading to glycosylation defects in N-, O- and ceramide linked oligosaccharides. While studying the lipid synthesis status in COG2 null mutant CHO cell (ldlC cells) it was found that in addition to defective sialylation of glycolipids, these cells also show reduced synthesis of a lipid identified as sphingomyelin (SM). Sphingomyelin synthase activity (SMS) and metabolic conversion of NBD-Ceramide into GlcCer and SM were essentially normal in mutant cells, but SMS1 of ldlC cells localized in fragmented Golgi membranes, lacking the compact, perinuclear Golgi localization in wt cells. Transfection of ldlC cells with COG2 cDNA restored the SM synthesis capability and SMS1 localization phenotype to a wt phenotype. Results indicate that Golgi misorganization caused by COG deficiency, in addition to affect glycosylation, also affects the synthesis of SM. Since SM is a conspicuous component of membranes, this finding is worth considering in relation to the clinical phenotype of patients suffering congenital disorders of glycosylation type II.

IUBMB II-S04.
HERMANSKY-PUDLAK SYNDROME AND THE BIOGENESIS OF LYSOSOME-RELATED ORGANELLES

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Lysosome-related organelles are cell-type-specific, specialized compartments of the endosomal-lysosomal system. Examples of these organelles include: the melanosomes, where melanin pigments are synthesized, and the platelet dense granules, where serotonin and other signaling molecules are stored for secretion upon platelet activation. Both melanosomes and platelet dense granules are defective in Hermansky-Pudlak syndrome, a rare Mendelian disorder caused by mutations in any of at least eight genes. Notably, despite the clinical manifestations originating from a few cell types, all of these genes are expressed ubiquitously. One such gene encodes a subunit of Adaptor Protein (AP)-3, a protein complex associated with endosomes and involved in the sorting of integral membrane proteins. My laboratory has characterized the product of the other seven genes and found that they assemble into three stable complexes, named Biogenesis of Lysosome-related Organelles Complex (BLOC)-1, -2 and -3. Like AP-3, BLOC-1 and -2 can be found associated with endosomes and mediate the intracellular trafficking of a subset of integral membrane proteins. However, they seem to act, at least in part, independently of AP-3, and their molecular functions remain unclear. We have established two models of BLOC deficiency in the fly, *Drosophila melanogaster*, and begun a forward genetic screening for modifier genes.

LI-S01.
***Trypanosoma brucei* GLYCOSOMAL ABC TRANSPORTERS POTENTIALLY INVOLVED IN FATTY ACID TRANSPORT**
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Trypanosoma brucei is a flagellated protozoan of the order Kinetoplastida which causes sleeping sickness in humans. *Trypanosomes* contain unique peroxisome-like organelles designated glycosomes which contain enzymes involved in a variety of metabolic processes. Despite good knowledge of the metabolic processes occurring within glycosomes, there is very little knowledge about the transport of metabolites through the membrane of theses organelles. Previously, we identified three homologues of peroxisomal ABC transporters in *T. brucei*. These proteins were shown to be associated with the glycosomal membrane and were designated GAT1-3 for Glycosomal ABC Transporters. We have first determined the topology of the transporters in the membrane by protease protection experiments which showed that the ATP-binding domain is at the cytosolic face. Second, cell lines have been constructed in which the depletion of two transporters by RNAi can be induced. Expression knockdown of these two transporters resulted in a growth phenotype that is dependent on the nutritional conditions of the trypanosomes. These experiments suggest a possible role of this transporter in the energy metabolism under conditions of low or zero glucose levels. Maybe the -oxidation is an alternative pathway for free-energy provision and could gain importance when the presence of glucose is very low. Preliminary lipid and fatty-acid analysis suggest strongly that at least one of the proteins transports the fatty-acid linoleate across the membrane. Finally, we have started to develop expression systems for the transporters using the yeast Pichia pastoris.

LI-S02.
FUNCTIONAL ANALYSIS OF SOLUBLE LIPID BINDING PROTEINS
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The evolution of different families of intracellular soluble lipid binding proteins (SLBPs) may be connected to the wide range of functions attributed to lipids as well as their low solubility in the cellular media.

Among these SLBPs, the mammalian fatty acid binding proteins (FABPs) are ubiquitously expressed, with distinct expression patterns for each of them. The large number of FABP types suggests distinct functions in specific tissues. Individual FABPs possess both unique and overlapping putative functions, some of which are based on specific elements of the protein structure. Structure-function studies indicate that subtle three-dimensional changes that occur upon ligand binding may promote distinct protein-protein or protein-membrane interactions that could determine the specificity of each FABP. Our in vitro studies highlighted the importance of the FABP helical/portal domain, a region that would be vital for the fatty acid transport and membrane binding properties of FABPs. Furthermore, modification of their expression in culture cells had an impact on several cellular processes. It is worth to notice the FABPs' participation in uptake and intracellular distribution of lipids by regulating fatty acid transport; a key component in intracellular lipid homeostasis, which ultimately may impact on systemic metabolism homeostasis.

LI-S03.
ABOUT FATTY ACIDS, TRYPANOSOMES AND DRUG TARGETS
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Needs for new drugs to treat diseases caused by protozoa has impelled the identification of metabolic pathways, absent in the mammalian host or different enough, to consider them as putative targets. It has produced a paramount improvement in our knowledge on parasite physiology, particularly in the last few years after completion of several genome projects, by mean of in silico comparative biochemistry. Fatty acid biosynthesis was considered during decades a promissory target. It was revisited in the last years after the finding that protozoa like *Plasmodium* synthesize fatty acids via a prokaryotic FAS system, whereas trypanosomatids lack a conventional FAS but synthesize saturated fatty acids by an unprecedented elongating complex located at the endoplasmic reticulum membrane. In addition, we found that trypanosomes are highly dependent on de novo synthesis of unsaturated fatty acids. Such pathway presents peculiarities that could be exploded for chemical intervention. Actually, we have validated it by knock down of key enzymes, as Δ9 and Δ12 desaturases in *T. brucei* and by using specific inhibitors. Particularly, Isoxyl acts on Δ9 desaturases, inhibiting growth of *T. brucei* bloodstream form at sub-micromolar concentrations, and cured experimental *T. cruzi* acute infection in mice. This drug was successfully used in the 60´s to treat tuberculosis, so it was already tested in human.

LI-S04.
CHOLESTEROL AND SPHINGOLIPIDS IN AMYLOID BETA NEUROTOXICITY
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Lipids are emerging as key players in Alzheimer's disease (AD). The cholesterol transport protein apoE4 is the most important risk factor consistently associated with non-familial AD; and recently-begun clinical trials are testing whether lowering plasma and/or neuronal cholesterol levels is a viable strategy for treating and preventing AD. Yet, very little is known about neuronal cholesterol homeostasis and several aspects need to be elucidated before affirming that a decrease in brain cholesterol levels will benefit AD patients. We study the role of cholesterol and sphingolipids in several aspects of A biology. Our work indicates that Aß uptake is decreased if the levels of cholesterol and sphingolipids are simultaneously reduced. Under these conditions neurotoxicity is also reduced. Interestingly, A itself inhibits cholesterol synthesis but contrary to our predictions, cholesterol content at the plasma membrane not only is not reduced but it is increased. Indeed, we found that neuronal internalization of Aß causes cholesterol sequestration in intracellular vesicles and at the plasma membrane. Our data indicate that A impairs intracellular trafficking and causes an abnormal distribution of cholesterol.

Our studies provide new evidence of the relevance of cholesterol in AD. Because therapeutic strategies of cholesterol reduction are already being tested in humans for the treatment of AD, fully understanding cholesterol homeostasis in neurons becomes urgent.

MI-S01.
CELL DENSITY AND MOTILITY PROTECT SWARMING BACTERIA AGAINST ANTIBIOTICS
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Swarming bacteria move in multi-cellular groups and exhibit adaptive resistance to multiple antibiotics. Analysis of this phenomenon has revealed the protective power of high cell densities to withstand exposure to otherwise lethal antibiotic concentrations. We find that high densities promote bacterial survival even in a non-swarming state, but that the ability to move, as well as the speed of movement, confers an added advantage, making swarming an effective strategy for prevailing against antimicrobials. We find no evidence of induced resistance pathways or quorum-sensing mechanisms controlling this group resistance, which occurs at a cost to cells directly exposed to the antibiotic. This work has relevance to the adaptive antibiotic resistance of bacterial biofilms.

MI-S02.
MECHANISMS CONTROLLING VIRULENCE GENE EXPRESSION IN ATTACHING AND EFFACING BACTERIAL PATHOGENS
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Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) are important human bacterial pathogens that produce an intestinal histopathology known as the Attaching and Effacing (A/E) lesion. Genes required for A/E lesion formation are located within the Locus of Enterocyte Effacement (LEE). Ler, an H-NS-like protein encoded within the LEE, induces expression of virulence genes located within and outside this pathogenicity island by counteracting the repression exerted by the global regulator H-NS. Ler expression is modulated by a complex mechanism involving several global regulators and A/E-specific positive and negative regulatory proteins such as GrlA and GrlR, encoded by the LEE *grlRA* operon. GrlA is required for the expression of Ler, which in turn further activates the *grlRA* operon establishing a positive regulatory loop. In addition, GrlA also traps GrlR by direct protein-protein interactions to keep it from repressing LEE gene expression. Moreover, typical EPEC strains carrying the EAF plasmid also produce PerC, an alternative *ler* specific activator that links, in a cascade fashion, the expression of a type IV pilus (BFP) with the expression of the LEE. The study of these regulatory networks has illustrated the diverse evolutionary paths that were undertaken to develop converging mechanisms to specifically control virulence genes in A/E pathogens.

MI-S03.
GLYCOPROTEINS AND MEMBRANE VESICLES: BACTERIA CAN DO THAT TOO
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Protein glycosylation has been for long time thought to be restricted to eukaryotes. However, it is now firmly established that bacteria can synthesize both, N- and O-glycoproteins. My lab works on the *Campylobacter jejuni* and *Desulfovibrio desulfuricans* N-glycosylation, and on the *Neisseria meningitidis* O-glycosylation systems. We and others have shown that the bacteria can tolerate the manipulation of the glycosylation systems, and therefore they constitute perfect toolboxes for glycoengineering novel therapeutics and vaccines. Our recent advances in the characterization of these glycosylation systems and the exploitation of these systems for vaccine development will be presented.
Vesicle formation and trafficking has also been considered an attribute of eukaryotic cells. This happened despite the fact that bacterial vesicles have been discovered several decades ago. These vesicles, often called outer membrane vesicles (OMVs) are generated by all Gram negative bacteria studied so far, but were thought to be just cell debris or microscopy artifacts. However, there is growing evidence showing that OMVs are used for the secretion of proteins, including virulence factors that can be transported into eukaryotic cells when the OMVs fuse to the host membranes. We use the dental pathogen *Porphyromonas gingivalis* as a model to study the biogenesis of OMVs. I will discuss our recent results demonstrating the existence of a mechanism that allow bacteria to select the OMV cargo proteins and our experimental evidence that proper OMV cargo selection requires intact LPS.

MI-S04.
NOVEL MOLECULAR APPROACHES FOR STUDYING MICROBIAL COMMUNITIES
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Due to their immense diversity, cumulative mass and activity, microorganisms play vital roles in all ecosystems on Earth. For these same reasons, they are currently exploited to perform several beneficial functions for society. Fundamental understanding of the underlying mechanisms that shape microbial communities and the processes they drive is still fragmentary. The development of ecosystem predictive models remains a long-term objective, but will undoubtedly have a large impact on fields such as biotechnology, agriculture or medicine. The recent advent of high-throughput molecular approaches in microbial ecology, such as the screening of genes, transcripts, proteins and metabolites in environmental samples ("omic" approaches) makes this goal attainable. Examples of current molecular strategies used in our laboratory to analyze microbial communities from marine environments impacted by anthropogenic pollution will be discussed. These include the use of massive parallel 16S rRNA tag sequencing to study microbial community structure, and the analysis of the diversity, relative abundance and biogeography of key pollutant-degrading bacteria and their catabolic pathways, by means of qPCR and metagenomic analysis.

PL-S01.
BIOLOGICAL FUNCTIONS OF microRNA REGULATED TCP TRANSCRIPTION FACTORS IN *A. thaliana*
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TCPs (TB1, CYC, PCF1/2) are a plant-specific group of transcription factor genes. They comprise 24 family members in Arabidopsis. Their common TCP domain does not share similarity with other described DNA binding domains but is predicted to adopt an helix-loop-helix structure, similar to bHLH transcription factors.
The current knowledge about TCP action points towards functions in plant development, more specific plant growth and cell division. Class I TCPs are thought to be positive regulators of growth, whereas class II members regulate growth negatively.
Our work focuses on a subgroup of five class II TCPs that are targeted by microRNA319. These TCPs (TCP2, 3, 4, 10 and 24) are closely related and probably have overlapping functions. They are expressed in developing leaves where they work as inhibitors of cell division. Mutants with decreased TCP levels have bigger leaves and plants with increased TCP activity have smaller than wild-type leaves.
We have been able to discover functions of these TCPs in later stages of leaf life by identifying target genes. We observed that they can bind to the promoters of several enzymes in the jasmonic acid (JA) biosynthetic pathway and indeed plants with reduced levels of TCP activity also have reduced levels of JA. A perspective on the broad biological role of these TCP transcription factors will be presented.

PL-S02.
STRATEGIES TO MANIPULATE THE BIOSYNTHESIS OF PROANTHOCYANIDINS
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Proanthocyanidins (PAs) are flavonoids that share part of their biosynthetic pathway with anthocyanins. PAs play a central role in plant defense against different stresses. As food components, they act as antioxidants and anticarcinogenic compounds. When present in moderate amount (2-5 mg/g of DM) in the forage of legumes, they increase protein assimilation by animals while preventing ruminal bloating. Unfortunately, the major forage legumes accumulate these products in seed coat and not in leaves. Conversely, some tropical species produce too much leaf PAs to prevent the free intake by animals. Thus, research in this field aims to finely tune leaf PA biosynthesis in legumes chiefly to produce bloat-safe varieties. To gain insights into the PA-limiting locus, our undertaking was to ectopically express in model and crop species genes coding for MYB and bHLH transcription factors. Indeed, it is well consolidated that the biosynthesis of anthocyanins and PAs is controlled by a ternary transcriptional complex made up of WD-bHLH-MYB proteins. We report studies where genes coding for activator or repressor MYBs from different plant species were overexpressed in tobacco, Arabidopsis and *Lotus spp*.
We show that *MYB* genes can divert the flavonoid flux from anthocyanins to PAs. We propose that the relative balance between MYB activators/repressors might specify leaf cell commitment to synthesise PAs.

PL-S03.
REDUNDANCY OR SPECIFICITY OF FUNCTION? MULTIPLE ISOFORMS FOR PLANT MALATE OXIDATIVE DECARBOXYLATION
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Malate is an important plant metabolite proposed to exhibit multiplicity of functions, both in C3 and C4 species. Malic enzyme (ME) is implicated in its oxidative decarboxylation, generating CO_2, NAD(P)H and pyruvate. However, it is surprising the expression, even in the same sub-cellular compartment, of several MEs in both types of plants. Thus, the open question is if these MEs are redundant or if they display an specific role in plant metabolism. For example, *Arabidopsis thaliana* contains six genes encoding active ME. But, are all of them just involved in L-malate degradation? The results obtained, after characterizing the recombinant MEs and knock-out mutants, indicate that each isoenzyme displays an specific role *in vivo*, due to its expression pattern and distinct biochemical properties. Moreover, the expression of a C4-ME in Arabidopsis, revealed the importance of malate as respiratory substrate in this C3 species. On the other hand, in the C4 species *Zea mays*, several MEs are also expressed, apart from the photosynthetic NADP-ME exclusively found in bundle sheath chloroplast, which display specific expression pattern with different biochemical properties. As a whole, the divergent properties of the ME isoenzymes suggest that each one fulfils an exclusive metabolic function *in vivo* and that ME co-expression does not imply redundancy but represents specificity of function.

PL-S04.
MOLECULAR MECHANISMS OF POLLEN-PISTIL INTERACTIONS IN TOMATO AND ARABIDOPSIS PLANTS
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After pollen grains germinate on the stigma, pollen tubes elongate within the pistil to transport sperms to female gametophytes for fertilization. During pollination, pollen tubes perceive style signals triggering cytoplasmic events required for tip growth.
We characterized two pollen-specific receptor-like kinases, LePRK1 and LePRK2, from tomato pollen involved in transducing style signals. We showed over-expression of LePRK2-eGFP significantly decreased pollen tube length and increased tube tip width, relative to eGFP tubes. Site-directed mutagenesis in two putative phosphorylation motifs in the cytoplasmic juxtamembrane (JM) domain and overexpression in tobacco pollen showed that both motifs have opposite effects in regulating pollen tube length.
We also used pollen tubes as a single cell system to study the mechanistic of polarized and oscillatory growth. Tomato pollen tubes showed a non-continuous growth rate and Ca^{2+} oscillated with the same period but out of phase.
We also study the participation of aquaporins mediating water and solutes transport during pollen tube elongation. We found AtTIP5;1 and AtTIP1;3 are the only aquaporins selectively and highly expressed in *Arabidopsis thaliana* pollen. They both showed urea and water transport. We found that in *tip1;3* and *tip5;1* homozygous double mutant plants, pollen tube length was significantly reduced under nitrogen starvation.

ST-S01.
REGULATION OF KLF6 TRANSCRIPTION FACTOR FUNCTION BY JNK AND P38 SIGNALING PATHWAYS
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Krüppel-like factor 6 (KLF6) is a transcription factor identified in our group, belonging to a family of gene regulators that shear a conserved zinc finger DNA-binding domain and are implicated in diverse steps of cell growth and differentiation. Independent studies reported mutations within the *klf6* gene in different human neoplasms, suggesting a potential tumor suppressor function for KLF6. We have demonstrated the ability of KLF6 to promote degradation of the c-Jun proto-oncoprotein by the proteasome pathway leading to inhibition of cell proliferation, which is one of the mechanisms contributing to KLF6 tumor suppressor activity. Current experimental evidence suggests that KLF6 function on c-Jun oncoprotein have consequences on apoptotic cell death triggered by UV-radiation. This function is dependent on both JNK and p38 MAP kinases activities. Moreover, a differential KLF6 effect was observed to activate c-Jun phosphorylation by JNK2 in a transient manner which is essential to switch the death programme of otherwise apoptotic-resistant cells due to JNK1 deficiency. Integrity of p38 kinase activity is critical for both KLF6 stability and apoptosis. In conclusion, c-Jun oncoprotein activity can also be modulated by KLF6 in the context of UV-induced cell death in a JNK and p38 dependent pathways. This property can also be exploited by bacterial toxins and during viral infections.

ST-S02.
SIGNALING PATHWAYS REGULATING TESTICULAR GONOCYTE DEVELOPMENT
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Disruption of gonocyte development, the precursor cells of spermatogonial stem cells, may lead to infertility or testicular tumor formation. Our laboratory studies the regulation of gonocyte proliferation and differentiation and how endocrine disruptors may alter these processes. We found that PDGF and 17â-estradiol work in concert to stimulate *in vitro* neonatal rat gonocyte proliferation and this crosstalk involved MAPK pathway activation. Moreover, the estrogenic compounds genistein, bisphenol A and diethylstilbestrol stimulated *in vitro* gonocyte proliferation in a manner similar to 17â-estradiol, suggesting that exposure to environmental estrogens might affect early germ cell development. I*n utero* exposure to these compounds led to increased PDGF receptors (PDGFRs) and MAPK expression in neonatal testis and gonocytes, as well as transient changes in germ cell numbers. Gonocyte differentiation was induced i*n vitro* by retinoic acid (RA), as indicated by increases in the spermatogonial markers c-kit and Stra8. RA also increased the expression of variant PDGFR and â forms that may act in a dominant negative manner against PDGF pathway. These data suggest that PDGFR and nuclear receptor pathway interactions are required for the temporal regulation of neonatal gonocyte development by the Sertoli cell, sites of estrogen, PDGF and RA synthesis.

ST-S03.
MITOCHONDRIAL AND NUCLEAR KINASE COMPLEX: ROLE OF MAPKS AND MKPS IN THE CYCLIC AMP-MEDIATED RESPONSE
Poderoso C; Duarte A; Brion L; Gómez NV; Mori Sequeiros Garcia MM; Podestá EJ; Paz C
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Protein phosphorylation mediated by the concerted action of kinases and phosphatases regulates key biological events such as cell growth, differentiation and apoptosis. The simultaneous stimulation of different signal pathways to produce several cellular responses involves a finite number of kinases and phosphatases. Thus, the spatial and temporal control of these enzymes is crucial for the selectivity and effectiveness of phospho/dephosphorylation events. The regulation of MAP kinases (MAPKs) and MAP kinase phosphatases (MKPs), which provide a negative feedback mechanism for MAPK activity, explains how this control is achieved. Phosphorylation induced by a specific stimulus promotes conformational changes, creates docking sites and causes intracellular relocation of proteins that finally results in the activation of a particular set of MAPKs in a specific compartment. The same stimulus also induces MKPs of different induction kinetics and subcellular localization. Thus, these coordinated events and the existence of a complex containing a specific substrate with kinases, phosphatases, docking and scaffolding proteins leads to the compartment-specific regulation of MAPKs to produce the right response in the right place. Here, the participation of nuclear and mitochondrial kinases and phosphatases complex in the induction and activation of key steroigenic proteins will be presented.

ST-S04.
SIGNAL TRANSDUCTION UNDER STRESS CONDITIONS IN *Trypanosoma cruzi*
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As *Trypanosoma cruzi*, the etiological agent of Chagas disease, passes through its complex life cycle, it comes across with different environmental stressors. One of the most relevant are the extreme fluctuations in osmolarity that occur within the gut of the vector, and also as the infective form passes out of the vector excreta (600-700 mOsm) and suddenly encounters the interstitial fluid of the mammalian host (300 mOsm). We have described a cAMP-specific phosphodiesterase, TcrPDEC2, which showed to be involved in osmoregulation. This enzyme depends on the presence of its FYVE domain, which is known to bind to membranes enriched in phosphatidylinositol 3-phosphate (PI 3-P), to be active. Moreover, TcrPDEC2 showed to be localized in the contractile vacuole complex (CVC), a key organelle involved in the release of water, and treatment with TcrPDEC2 inhibitors affects their capability to respond to hyposmotic stress. To determine if PI 3-P production might have a role in osmoregulation, we characterized TcVps34, the first phosphatidylinositol 3-kinase (PI3K) described in *T. cruzi*. TcVps34 specifically phosphorylates phosphatidylinositol to produce PI 3-P confirming that it belongs to class III PI3K family. Furthermore, in TcVps34-overexpressing parasites the localization of TcrPDEC2 showed to be altered, suggesting a possible role of TcVps34 determining TcrPDEC2 localization.

BT-C01.

EFFECT OF POLY(3-HYDROXYBUTYRATE) PRODUCTION IN RECOMBINANT *E. Coli*

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Poly(3-hydroxybutyrate) (PHB) is a biodegradable plastic, accumulated by many bacteria under unfavorable growth conditions. PHB granules in natural producers are surrounded mainly by a protein called PhaP, which affects the size of PHB granules and the amount of polymer accumulated. In previous work we have cloned and expressed the PHB biosynthetic genes and the *phaP* gene from *Azotobacter* sp. FA8 in *E. coli*. In this work we analyze the effect of PHB production on the expression of several genes in recombinant *E. coli* by qRT-PCR. The results obtained show an increase in the expression of stress related genes in the strain that accumulates PHB when compared to the isogenic strain which does not accumulate the polymer. We also observed that in the strain which produces PHB and also expresses *phaP*, the transcription of stress related genes was lower than in the PHB producing strain. Surprisingly, it was also lower than in the *E. coli* strain which does not accumulate PHB. These results suggest that the PhaP protein has a protective effect on *E. coli*, both in the presence and absence of PHB. Further studies will be needed to better understand the role of PhaP in the physiology and metabolism of *E. Coli*.

BT-C02.

INTER-SPECIFIC HYBRIDATION IMPROVES FORAGE QUALITY, SALT TOLERANCE AND TANNINS LEVELS IN *Lotus spp*

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Lotus tenuis is a keystone species in cattle production of the Salado River Basin (Argentine). Its tolerance to marginal edaphic regions is well known, but its condensed tannins (CT) levels should be improved. By contrast, *L. corniculatus* has adequate CT levels. The CT are polyphenolic oligomers that, in adequate concentration, increase the forage value diminishing internal parasites, avoiding rumen bloat and increasing the "bypass protein".

With the aim to improve the forage quality, we obtained a hybrid between diploid population of *L. corniculatus* collected in the Albufera de Valencia (Spain) and selected genotypes of *L. tenuis* (Argentina). Hybrid status was confirmed by PCR amplification and sequencing of ITS. The lines obtained showed an average content of CT (6,2 mg CT/g MS) between parental lines. The analyses of the relative expression of nine genes of the biosynthetic pathway of CT permit their correlation with foliar CT content. In addition, hybrid plants had a better photosynthetic performance than *L. tenuis* under saline stress. Fresh and dry weights and the Na^+, K^+, Cl^-, chlorophyll, anthocyanins and CT, levels were also assessed.

In resume, the hybrid obtained has a high agronomical potential and constituts a good material to analyze the biochemically and molecular regulation of CT biosynthetic pathway and abiotic tolerance traits in *Lotus* crops species

BT-C03.

COMPARATIVE SAFETY ASSESSMENT OF GENETICALLY ENGINEERED ANTI-BROWNING POTATOES

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Even though genetically engineered (GE) crops have been increasingly adopted worldwide since 1996 and promising approaches to improve quality traits in food crops have been extensively reported in the scientific literature, concerns between scientists, consumers and regulatory authorities on the safety of transgenic crops have led to the development of the "comparative safety assessment" concept. It comprises the analytical nature of the first step of the entire genetically modified food safety assessment in combination with consecutive toxicological and nutritional evaluations.

In this work we attempted to explore the comparative safety concept in GE potato lines with reduced polyphenol oxidase (PPO) activity and diminished browning in tubers. To accomplish this goal, three GE potato lines (-PPO) were characterized at both the physiological and molecular level. The photosynthetic performance was surveyed by means of measuring gas exchange CO_2 assimilation, stomatal conductance and transpiration. Primary metabolism was studied by gas chromatography–mass spectrometry (GC-MS) and intake safety was evaluated using bioinformatic approaches and assayed in mice.

We conclude that apart from small unintended differences in the primary metabolism and the intended trait improvement, "anti-browning potatoes" are comparable to wild-type potatoes and pose no risk to human health.

BT-C04.

IMMUNOLOGICAL CHARACTERIZATION OF A HYPOALLERGENIC VARIANT OF ANTIGEN 5

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Allergic reactions to stings cause significant morbidity and sometimes are fatal. Immunotherapy reduces the mortality and morbidity of insect sting allergy though effective doses may be impaired by potential systemic reactions that allergens can cause. Antigen 5 (Ag5) is the main allergenic protein found in vespid venoms. However, structural and immunological studies already attained in our laboratory suggest that Ag5 from *Polybia scutellaris (Poly s5)*, a South American wasp, is a hypoallergenic variant. The aim of this work is to further characterize this molecule in order to assess its usefulness as a tool in immunotherapy. *Poly s5* and an allergenic homolog from *Polistes annularis (Pol a5)*, were expressed as recombinant proteins in *Pichia pastoris*. They were injected to BALB/c mice and cross-reactivity was determined at the IgG and IgE levels. Results indicate a significant cross-reactivity between both antigens at the IgG level, whereas IgE cross-reactivity is almost negligible. Furthermore, a significant difference in the specific IgE response elicited in mice was observed. All in all, our results lead to the conclusion that *Poly s5* is a suitable hypoallergenic candidate for immunotherapy.

BT-C05.
CHYMOTRYPSIN-SUSCEPTIBLE MICROCIN J25 DERIVATIVES: POTENTIAL APPLICABILITY IN FOOD PRESERVATION

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Microcin J25 (MccJ25) is a 21-residue ribosomally-synthesized lariat-peptide antibiotic. It is active against food-borne diseases causing pathogens such as *Salmonella, Shigella* and *E. coli*, including *E. coli* O157:H7 and non-O157. MccJ25 is highly resistant to digestion by proteolytic enzymes present in the stomach and intestinal contents. MccJ25 would therefore remain active in the gastrointestinal tract, affecting the normal intestinal microbiota, and limiting the potential use of MccJ25 as a food preservative. In the present work we describe a chymotrypsin-susceptible MccJ25 derivative carrying a Gly^{12} substitution to Tyr that retained almost full antibiotic activity and efficiently inhibited the growth of pathogenic *Salmonella newport* and *Escherichia coli* O157: H7 in skim milk and egg yolk. However, unlike the wild-type MccJ25, the MccJ25(G12Y) variant was inactivated by digestive enzymes both *in vitro* and *in vivo*. Further, by means of preliminary experiments, we demonstrated that MccJ25(G12Y) did not affect the rat intestinal microbiota. To our knowledge, our results represent the first example of a rational modification of a microcin aimed at increasing its potential use in food preservation.

CB-C01.
Trypanosoma cruzi EMPLOYS THE SNARE VAMP-7 TO INVADE HOST CELLS
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The etiologic agent of Chagas disease, *Trypanosoma cruzi,* infects a wide variety of mammalian cells. Previous works have demonstrated that the recruitment of acidic compartments to the plasma membrane is necessary to allow the invasion of the parasite into the host cell. In order to study the machinery of fusion involved in this invasion process, we have used CHO cells overexpressing NSF-wt-GFP and NSF-D1EQ-GFP (negative mutant: NM) and we have observed, by confocal microscopy, an increase of infection in NSF-wt cells; whereas there is a significant lower level of infection in the NM cells. We have also studied the role of VAMP-7, a v-SNARE protein. CHO cells overexpressing VAMP-7-wt showed a high level of association between this protein and the *T. cruzi* parasitophorous vacuole (TcPV). Besides, the non-functional protein VAMP-7-NT does not colocalize with the TcPV and decreases the number of parasites which invade the cell. We have also analyzed the relation between the autophagic pathway and VAMP-7. In this case, we have observed that cells overexpressing both, LC3 and VAMP-7 proteins, present a higher percentage of infection than the control cells. These results show that the invasion of T. cruzi is a NSF dependent fusion process, where VAMP-7 plays a key role in both the lysosomal and the autophagic models of *T. cruzi* entry.

CB-C02.
Mycobacterium marinum INTERACTS WITH LC3 AND BENEFITS FROM AUTOPHAGY INDUCTION
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Mycobacterium marinum; a natural pathogen of frogs and fishes; can eventually infect humans, and has been extensively used as a model to study pathogenic mycobacteria. We have studied the interplay of *M. marinum* and the autophagic pathway. For this purpose we have used macrophages overexpressing GFP-LC3, a marker for double membrane autophagosomes. We have previously observed that *M. marinum* resides within an LC3 positive compartment, and this association depends on the bacterial viability. We have also characterized the LC3-positive compartment containing *M. marinum*, which shows features of late endosomes such as Rab7 and LAMP2. However, this phagosome does not acquire the lysosomal marker cathepsin D, nor DQ-BSA labeling, suggesting it is not a degradative compartment. By electron microscopy, we have shown that early after infection (1 to 2 hours) *M. marinum* resides mostly in single membrane compartments, in spite of the presence of LC3 on their membranes; and also that a subset of the infecting bacteria are free in the cytosol at 2 hours post infection. Finally, *M. marinum* survival within the cell appears to benefit upon autophagy induction with starvation medium. All these data suggest a complex interplay of *M. marinum* with autophagy, where *M.marinum* modulates its LC3 positive compartment, and benefits from autophagy induction.

CB-C03.
DISRUPTION OF THE SECRETORY PATHWAY ALTERS THE DEVELOPMENT OF THE _Coxiella_ REPLICATIVE VACUOLE
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Coxiella burnetii, the causative agent of Q fever, is an obligate intracellular bacterium, which replicates within large vacuoles with features of both, phagolysosomes and autolysosomes. We have previously demonstrated that the Coxiella replicative vacuole (CRV) interacts with the secretory pathway since Rab1b Q67L (GTPase defective mutant of Rab1b) was present in the vacuole membrane at later infection times (48 h). We also reported that overexpression of Rab1b altered the normal development of the CRV by changing its fusogenic capacity. In the present work we have analyzed the development of the CRV in conditions of disrupted secretory pathway. As a first strategy, pharmacological ERGolgi traffic disruption by Brefeldin A (BFA)-treatment was applied. BFA is an uncompetitive inhibitor of Arf1 activation. Cell treatment with BFA results in the specific inhibition of an Arf1 guanine-nucleotide exchange factor, and efficiently and reversibly blocks membrane traffic to the Golgi. As a second strategy, we blocked the secretory pathway at early steps by overexpression of GDP- and GTP-restricted mutants of Sar1 (Sar1 T39N and H79G) which strongly inhibits vesicle budding from the ER. The CRV diameter was dramatically altered under both conditions, indicating that a functional secretory pathway is essential for the normal *C. burnetii* intracellular development.

CB-C04.
MODELING RAB-DEPENDENT INTRACELLULAR TRANSPORT
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A fundamental feature of eukaryotic cells is the presence of distinct membrane-bound compartments having unique protein and lipid composition. These compartments are interconnected by active trafficking mechanisms that must direct macromolecules to defined locations, and at the same time maintain the protein and lipid composition of each organelle. It is well accepted that Rabs play a central role in intracellular transport regulating the recognition, fusion and fission of organelles. However, how the transport is achieved is not completely understood. Our aim was to propose a model where a soluble component in the luminal compartment is transported along different Rab-containing organelles that interact according to the following simple hypothetical principles: i) only organelles with the same or compatible Rab domains can fuse, ii) after fusion, an asymmetric fission occurs rendering a tubule and a round-shaped vesicle, and iii) Rab domains distribute asymmetrically in the two resulting organelles. When this model was tested in a simulation, an efficient and unidirectional transport was observed; and at the same time, the compartment identity was preserved. All three principles were absolutely necessary for transport. The model is compatible with Rab dynamics and it predicts that a misbalance in Rab composition, either for an excess or a defect, affects cargo transport.

CB-C05.
GLUCOSIDASE II BETA SUBUNIT MODULATES N-GLYCAN TRIMMING IN FISSION YEASTS AND MAMMALS

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Glucosidase II (GII) is a key player in glycoprotein biogenesis in the endoplasmic reticulum (ER). It catalizes the sequential removal of the two innermost glc residues from the $Glc_3Man_9GlcNAc_2$ glycan transferred to Asn residues in proteins. GII is involved in the calnexin/calreticulin cycle as it removes the single glc unit added to folding intermediates and misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. GII is an ER heterodimer whose α subunit (GIIα) holds the glycosyl hydrolase active site whereas its β subunit (GIIβ) role is controversial and has been suggested to be responsible for GIIα ER retention and folding. Here we report that in the absence of GIIβ, the catalytic subunit GIIα of the fission yeast *Schizosaccharomyces pombe* (an organism displaying a glycoprotein folding quality control mechanism similar to that occurring in mammalian cells) has an active conformation able to hydrolyze p-nitrophenyl α-D-glucopiranoside. However, the heterodimer is required to efficiently deglucosylate the physiological substrates $Glc_2Man_9GlcNAc_2$ (G2M9) and $Glc_1Man_9GlcNAc_2$ (G1M9). The interaction of the mannose 6-phosphate receptor homologous domain (MRH domain) present in GIIβ and mannoses in the B and/or C arms of the oligosaccharide mediates glycan hydrolysis enhancement. We also present evidence that in mammalian cells

CB-C06.
A ROLE FOR EIF4E ON mRNA TURNOVER IN PROCESSING BODIES

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New functions for the eukaryotic translation initiation factor eIF4E have been recently described in mRNA metabolism, namely nuclear export and ribonucleoprotein complex (RNP) remodelling. RNPs remodelling occurs when mRNAs are translocated from active polysomes to cytoplasmic processing bodies (PBs). To elucidate the pathway of RNP remodelling involving eIF4E we have studied the functional consequences of the interaction between eIF4E, mRNAs and PBs components in vivo. *Drosophila melanogaster* eIF4E isoform 1 is present in PBs. We have generated eIF4E mutants on key tryptophan (W) residues by replacing them with alanine (A). W100 and W147 are required for mRNA 5´cap binding, and W117 is required for protein-protein interactions. We transfected *Drosophila* S2 cells with wild type and mutant eIF4E-1 of variants to evaluate the requirement for PBs localization. Only the mutation of W117 produced relocalization of eIF4E. Similar results were obtained by mutation of the equivalent W in the mouse eIF4E. Therefore, protein-protein interactions rather than cap binding are required for PBs localization. To determine whether eIF4E-interaction is required for PBs assembly we mutated the eIF4E-binding sites of 4ET-Like and me31b, both present in PBs. We will present a model for the remodelling pathway.

CB-C07.
SEARCH FOR ENDOGENOUS SMALL RNAs AFFECTING ALTERNATIVE SPLICING BY TGS

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Small 21- to 25-nucleotide RNAs have diverse biological roles in eukaryotes, including the silencing of repetitive sequences, antiviral defense by small interfering RNAs (siRNAs) and developmental gene regulation by microRNAs (miRNAs). When these small RNAs are fully complementary to their targets can cause specific mRNA cleavage, whereas those with mismatches can mediate translational inhibition. More recently another pathway has emerged: siRNA-induced transcriptional gene silencing (TGS), where small double stranded RNA molecules have been implicated in gene silencing at the level of transcription through DNA methylation, modifications in histone tails and chromatin structure. We have found that exogenous siRNAs are able to affect alternative splicing (AS) events located closely to their target sites by means of the TGS process. In the search for endogenous examples of this process we performed ChIP-seq (deep sequencing) against AGO proteins (key players of TGS) and we've analyzed these data in connection with different small RNA population databases available on “Gene Expression Omnibus” from NCBI. Moreover, we've used deep sequencing databases from ChIP-seq experiments against histone tail modifications and Pol II and filter our previous data with this information
This analysis will allow us to find endogenous small RNAs that could be affecting AS in different genes.

CB-C08.
ROLE OF KRÜPPEL-LIKE FACTOR 6 IN PSG GENE EXPRESSION DURING TROPHOBLAST DIFFERENTIATION

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Krüppel-like factor 6 (KLF6) is a zinc finger transcription factor involved in differentiation, cell cycle control and proliferation in several cell systems. It is highly expressed in human term placenta, and its mRNA and protein levels increase during human trophoblast cell differentiation. Along this process, KLF6 has a nuclear and cytoplasmatic localization. Pregnancy Specific Glycoproteins (PSGs) are early biochemical markers of trophoblast differentiation and are required for pregnancy protection. They have been considered as target genes of KLF6 regulation, although there is little experimental evidence supporting such a role. Here, we confirmed by immunohistochemistry that KLF6 is expressed on human term placental villi. Immunofluorescence assays revealed that KLF6 is expressed before PSG during in vitro trophoblast cell differentiation. KLF6 over expression led to an increase in PSG mRNA and protein levels. In addition, KLF6 transactivated PSG3 promoter activity in non differentiated cells, and this activation was enhanced when cells were differentiated. Moreover, gel shift assays revealed that KLF6 is linked to the PSG promoter region. Altogether these data provide further evidences that KLF6 is a direct regulator of PSG gene expression during placental differentiation.
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CB-C09.
CCN6-DEPENDENT REGULATION OF ZEB1 IN HUMAN MAMMARY EPITHELIAL CELLS

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ZEB1 is a crucial transcription factor that mediates the EMT (Epithelial to Mesenchymal Transition) and tumor invasion by inhibition of the epithelial phenotype. It is known that IGF-1 can up-regulate ZEB1 expression in an epithelial prostate cancer cell line. Our lab has reported that CCN6 loss triggers EMT and invasion through up-regulation of ZEB1 and downregulation of E-cadherin in breast cells. We hypothesize that CCN6 may regulate EMT in breast cancer by modulating IGF-1 signaling and thereby regulating ZEB1 expression. WB and RT-qPCR analysis in CCN6 knock-down HME (Human Mammary Epithelial) (CCN6 KD) cells revealed an up-regulation of ZEB1 and IGF-1 in both transcripts and protein levels. CCN6 KD cells and the aggressive malignant breast cancer cells MDA-MB-231(which express low CCN6) secrete high levels of IGF-1 in the media as well as activation of the IGF-1 signaling pathway. The treatment of CCN6 KD cells with 500 ng/mL rhCCN6 induced down-regulation of ZEB1 and IGF-1 transcripts and proteins levels, with reduced IGF-1R and IRS1expression and activation. Concomitantly, the CCN6 treated cells shown a lower expression of the mesenchymal marker vimentin (WB and IF) and decreased invasive abilities. These results suggest that CCN6 regulates ZEB1- dependent EMT and cancer invasion through IGF-1 signaling in breast epithelial cells.

CB-C10.
THE SERINE-ARGININE RICH PROTEIN SF2/ASF REGULATES PROTEIN SUMOYLATION

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Post-translational protein modification by SUMO is involved in diverse biological functions such as transcription regulation, subcellular partitioning, stress response, DNA damage repair and chromatin remodeling. SUMO conjugation to target proteins proceeds by an enzymatic pathway involving an E1 activating enzyme, an E2 conjugating enzyme and E3 ligases. Ser/Arg-rich (SR) proteins, initially described as splicing regulators, also play important roles in other steps along mRNA metabolism. Considering that the SUMO E3 ligase PIAS1 localizes to nuclear speckles, resembles scaffold-attachment factors shown to interact with RNA polimerase II and SR proteins, and is part of the human spliceosome, we decided to investigate a possible involvement of SR proteins in the SUMOylation process. We found that the SR protein SF2/ASF is a regulator of the SUMO pathway, in vitro and in vivo. Its over-expression stimulates while its knockdown inhibits SUMO conjugation. SF2/ASF interacts with the E2 enzyme Ubc9 and enhances the SUMOylation of specific target proteins, behaving as an E3 ligase. It also interacts with the SUMO E3 ligase PIAS1, regulating its activity. The RNA recognition motif 2 of SF2/ASF is necessary and sufficient for SUMOylation enhancement. These results add a further level of regulation to the SUMOylation pathway and also a new role for the multifunctional SR protein SF2/ASF.

CB-C11.
DNA DAMAGE AND CELL DEATH PATHWAYS: ROLE OF POLY-ADP-RIBOSYLATION IN *Trypanosoma cruzi*

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The generation of genomic lesions triggers different pathways aimed at the restoration of the DNA integrity, except for those cases in which the extension of the damage impedes its reparation, ultimately unleashing cell death mechanisms. The poly(ADP-ribosyl)ation of nuclear proteins by the poly(ADP-ribose) polymerase (PARP) is a post-translational modification that occurs in response to DNA single strand breaks, allowing the recruitment and accession of repairing factors to the lesion site. In *Trypanosoma cruzi*, the only PARP present catalyzes the formation of poly(ADP-ribose) polymers (PAR) in a dose-dependent manner when epimastigotes are treated with H_2O_2 or UVC light. We have also found that PARP moves into the nucleus only after incubation with these agents; in contrast, poly(ADP-ribose) glycohydrolase (PARG) is present in the nucleus even in the absence of DNA damage, as opposed to what has been reported for other organisms. Above a certain threshold, these damaging agents can induce the death of the parasites, possibly through different mechanisms, as suggested by the different morphology observed and the determination of apoptotic and necrotic characteristics assessed by flow cytometry analysis. The importance of PAR in these pathways is reflected in the differential cell survival after treatment with these agents, either in presence or absence of PARP and PARG inhibitors.

CB-C12.
PHOSPHOLIPID BIOSYNTHESIS IS DISPENSABLE FOR BACTERIAL CELL DIFFERENTIATION

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In the absence of nutrients *Bacillus subtilis* initiates a differentiation process called sporulation. After an asymmetrical division two compartments are created, the larger one called mother cell and the smaller one, prespore. σ^E factor, which is essential for sporulation, is proteolitically activated only in the mother cell by the membrane protease SpoIIGA which requires SpoIIR, a protein synthesized in the prespore.
We have previously shown that inhibition of fatty acid biosynthesis precludes σ^E activation, what interrupts the differentiation process. Analyzing a conditional mutant, we observed that membrane lipid biosynthesis is dispensable for artificial activation of σ^E during exponential phase of growth.
To study requirement of phospholipid biosynthesis during sporulation (a membrane remodeling process), we constructed strains that conditionally express *plsY* or *plsC*, coding for phosphatidic acid synthesizing enzymes. Although growth was completely arrested in both uninduced mutants, we found efficient σ^E activation and normal sporulation frequencies. Analyzing lipid extracts of depleted cultures by thin-layer chromatography we confirmed a block in phospholipids synthesis and concomitant fatty acid accumulation. Our results demonstrate, for the first time, the dispensability of phospholipid biosynthesis during this complex differentiation process.

CB-C13.
SPHINGOSINE KINASE 1 IS REQUIRED FOR MIGRATION AND GROWTH OF MELANOMA

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Sphingosine 1-phosphate (S1P) is a lipid mediator that elicits multiple cellular processes, including cell migration, survival, and angiogenesis. S1P levels inside the cells are regulated by the balance between its synthesis by two sphingosine kinases isozymes (SphK1 and SphK2), and degradation by S1P lyase and S1P phosphatases. Activation of SphK by a variety of agonists increases S1P levels, which in turn can function intracellularly as a second messenger or in an autocrine/paracrine fashion to activate cell surface S1P receptors.
Melanoma is the most aggressive type of skin cancer which causes the majority of the deaths. Although the mechanisms involved in the progression of the disease are not fully understood, increased SphK1 activation has been recognized as a hallmark of many cancers. Here we have identified Filamin A (FlnA) as a SphK1 interacting protein, which controls cell migration through the activation of $S1P_1$ receptor. SphK1 activation is required for heregulin-induced migration, lamellipodia formation, activation of PAK1, and subsequent FlnA phosphorylation in melanoma cells. Moreover, we also demonstrated that SphK1 has a critical role in the activation of NF-κB, which in turn is necessary for cell proliferation. Taken together these results suggest a unique role for SphK1 in two main characteristics of melanoma: cell migration and proliferation.

CB-C14.
INVOLVEMENT OF P19INK4D IN REPLICATIVE SENESCENCE

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Senescence is a cellular program that leads to an irreversible cell cycle arrest and is accompanied by phenotypic changes. This mechanism is activated in response to physiological aging and different types of stress. The factors responsible for establishing cellular senescence are pRb and p53. However, the factors involved in its maintenance, as well as those that determine cell fate, apoptosis or senescence, are still unknown. We have demonstrated that p19INK4d, a member of the INK4 family, is upregulated in response to stress-induced senescence, suggesting that p19 might play a role in this process. The aim of this work is to study the involvement of p19 in replicative senescence. Primary fibroblasts from human and mouse enter replicative senescence after 5 to 10 passages and were used as a cellular system for this work. Both p19 mRNA and protein levels became upregulated from passage 5 onward. Increased transcription of the p19 promoter was confirmed by gene reporter and run-on experiments. The E2F1 transcription factor, that is involved in the induction of p19 following stress-induced senescence, does not play a role in replicative senescence, indicating the existence of different pathways. In both cases, however, the response appears to be ATM/ATR-dependent. Our results support a role for p19 in replicative senescence, in addition to that induced by genotoxic drugs.

LI-C01.
DIFFERENTIAL REGULATION OF PHOSPHATIDYLCHOLINE-DERIVED SIGNALING PATHWAYS IN SYNAPTIC MEMBRANE RAFTS

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Membrane rafts are specialized microdomains that compartmentalize several signal transduction processes. In this work we investigate the presence of phosphatidylcholine (PC)-derived signaling in synaptosomal membrane rafts (SMR) isolated from adult and aged rats. The SMR isolation was based on their relative insolubility in cold Triton X-100 (1%). Western blot assays showed that SMR were enriched in Caveolin and c-Src. In addition, SMR presented significant higher sphingomyelin and cholesterol content than purified synaptic endings (Syn). Moreover, SMR phospholipids presented a more saturated fatty acid composition. The presence of PC-specific phospholipase C (PC-PLC) and phospholipase D (PLD) in Syn was previously reported (Mateos et al., 2006 and 2008). Both signaling pathways generate the lipid messenger diacylglycerol (DAG) by catalyzing PC hydrolysis. DAG generated by PC-PLC was measured in the presence of 2% ethanol yielding a specific activity 60% higher in SMR than that found in Syn. PLD1 localization was also increased in SMR; however, GTPS only stimulated PLD activity in SMR from aged rats. On the other hand, PLD2 was excluded from the SMR fraction. Our results show a selective localization and an age-dependent regulation of PC-derived signaling in SMR and Syn.

LI-C02.
PGD2 INCREASES PTDCHO SYNTHESIS BY INTRANUCLEAR CCTα MOBILIZATION - PARTICIPATION OF ERK 1/2

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Prostaglandin D2 is essential for the maintenance of PtdCho synthesis and, therefore, for renal physiology. The PGD2 maintenance of PtdCho biosynthesis includes PLD and PLC-PKC-MEK 1/2-ERK ½ activation, where a short time stimulation depends on the activation of PLD and ERK 1/2, while at longer times the stimulation is dependent on PLD activation. The rate-limiting step of PtdCho synthesis is catalyzed by the amphitropic enzyme, CCT. Only CCTá isoform is expressed in renal papillary collecting duct cells and the enzyme level and its intracellular location depend on endogenous prostaglandins. PGD2 induces increase of PtdCho synthesis by an ERK 1/2 dependent CCTá mobilization, from soluble fraction and PLD activation is required for the further membrane location of the enzyme (ER or nuclear envelope). Respect to the nucleoplasmic CCTá, here is demonstrated for the first time that in absence of endogenous PGs, CCTá was within intranuclear sub-organelles so call "speckle" from where PGD2 induces its mobilization to be further activated in an ERK dependent mechanism. We suggest that intranuclear CCTá within speckles plays a central role in enzyme activation.

LI-C03.
LIVER FATTY ACID BINDING PROTEIN (L-FABP) PARTICIPATES IN DIFFERENT CELL PROCESSES IN THE ENTEROCYTE

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The intestine assimilates and processes a large quantity of lipids incorporated with the diet. Lipids are absorbed mainly as free fatty acids (FA), cholesterol and monoacylglicerol to be then reesterified inside the enterocytes and secreted as Quilomicrons into the lymph. The classical functions attributed to FABP are as cytosolic buffer and transporter of hydrophobic ligands. Nevertheless, new functions have been suggested related to control of gene expression. It is suggested that intestinal FABP would facilitate the intracellular transport of FA and that they could also have a role in the regulation of lipid metabolism and other cellular processes. In this work we obtained an L-FABP knock-down model in Caco-2 cells by anti-mRNA overexpression. We analyzed the effect on the assimilation, metabolism and secretion of FA as well as on the cellular proliferation and differentiation processes. No compensation by I-FABP was observed in differentiated cells. Knock-down clones showed a marked decrease in oleate assimilation, while palmitate assimilation was increased. Differences in oleate distribution were observed at short times, but they disappeared after hours and no change in oleate distribution was observed in secreted lipids. On the other hand, cell proliferation and differentiation were slowed. These results indicate that L-FABP is an important factor of enterocyte functionality.

LI-C04.
EFFECT OF THE HYDROPHOBIC POCKET STRUCTURE ON CATALYTIC PROPERTIES OF THE β-KETOACYL-ACP SYNTHASES

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ß-ketoacyl-acyl carrier protein (ACP) synthases (KAS) catalyze fatty acid chain elongation by the addition of two-carbon units to an acyl-ACP group. These enzymes are potently inhibited by the fungal mycotoxin cerulenin, a 12C fatty acid analog, which binds covalently to the KAS active site thiol. *Bacillus subtilis* contains only one elongation KAS encoded by the *fabF* gene. We have previously described a *B. subtilis* strain containing the *fabF1* allele, which encodes the cerulenin-insensitive protein FabF[I108F]. This strain had a cerulenin MIC 8-fold higher than the wild type strain. Although *B. subtilis* FabF[I108F] is functional both in vivo and in vitro, the *E. coli* FabF[I108F] enzyme is reported to be unable to elongate medium- and long-chain acyl-ACPs. Here we report the characterization of another two spontaneous mutants of *B. subtilis* that exhibit 4-fold increase in the cerulenin MIC. These mutants carry the *fabF2* (L111F) and *fabF3* (I108M) alleles. Biochemical and genetic studies were performed to understand the different properties of wild type and mutated FabFs from *B. subtilis* and *E. coli*. Also, the high resolution structure of FabF1 alone and complexed with cerulenin was obtained.
These studies provide major insights into the key elements that contribute to the activity of this important class of enzymes and the mechanisms associated with the resistance to cerulenin.

LI-C05.
E2F TOGETHER WITH SP1 REGULATES THE MURINE CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE ALPHA (CCTα) EXPRESSION

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The major route for phosphatidylcholine (PC) production is the CDP-choline pathway and the supply of CDP-choline is governed by the activity of CCT. Three isoforms are differentially expressed in tissues, but CCTα is the dominant isoform that is ubiquitously expressed. Stimulation of PC synthesis is associated with cell cycle progression and is driven by transcriptional activation of CCTα. In this sense, we have identified an E2F binding site (-218/-212) in the proximal promoter region of this gene that was shown to activate CCTα transcription during the S phase of the cell cycle. Gel shift analysis using CCTα promoter derived probes led to the identification of several multiprotein complexes that revealed an association between E2F and Sp1 transcriptional factors. These findings were confirmed using TranSignal TF-TF Interaction Arrays. Further analysis using CCTα promoter luciferase reporter plasmids, and plasmids designed to overexpress or inhibit (siRNA) E2F and Sp1 expression were used to study CCT promoter activity. These results suggested that E2F interact with Sp1 and cooperatively activate CCTα transcription in the S phase.
Interestingly this regulatory mechanism evidenced in phospholipids synthesis, is shared with other genes involved in cell cycle progression and DNA replication, and is required to achieve cell cycle dependent regulation.

LI-C06.
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND NEURONAL DIFFERENTIATION

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Acceleration of membrane phospholipid synthesis is associated with neurite elongation in mammalian cells. Our results indicate that membrane biogenesis is driven by the elevated expression of PC biosynthetic genes such as CCTα and CKα following retinoic acid (RA)-induced differentiation of Neuro2A cells. Cell lines that overexpress these enzymes showed high levels of PC biosynthesis and morphological appearance similar to Neuro2a control cells treated with RA. These cells have higher percentage of differentiated cell (+/-RA), longer neurites and they express high basal levels of βIII-tubulin and α-tubulin. These results indicate that PC or any metabolites of the pathway could act as a signal to promote differentiation. To identify how differentiation is turned on in these cell lines, we analyzed the levels of ERK as the kinase required for RA-induced neuronal differentiation. Furthermore, we studied how the levels of cell differentiation and gene expression are affected by the ERK inhibitor. In an attempt to evaluate the role that PC exerts during this process we investigated if exogenous PC could mimic the observed effect. We supplemented cells with PC, PE and LPC and measured the level of cells differentiation, βIII-tubulin expression and ERK phosphorilation. Together these results allow us determining the role that PC metabolism play during neuronal differentiation.

LI-C07.
DIFFERENTIATION-RELATED CHANGES IN LIPIDS WITH VERY LONG CHAIN POLYENOIC FATTY ACIDS IN GERM CELLS

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Pachytene spermatocytes, round spermatids, and epididymal spermatozoa were isolated to determine differentiation-related changes in their sphingomyelins (SM) and ceramides (Cer) very long chain polyenes (VLCPUFA). The gametes were also examined to study the topological distribution of these lipids. Non-hydroxy VLCPUFA predominated in the SM and Cer of spermatocytes, whereas 2-OH VLCPUFA prevailed in the SM and Cer of spermatids and spermatozoa. During the progression spermatocytes, spermatids spermatoza, 28:4n-6 decreased, though less than did 30:5n-6, thereby becoming the major VLCPUFA. Concomitantly, 2-OH 30:5n-6 increased, though more than did 2-OH 28:4n-6, thus becoming the main 2-OH VLCPUFA in both lipids. Spermatozoa had SM with 28:4n-6 and 2-OH VLCPUFA, mostly located to the head, and Cer with 2-OH VLCPUFA, mostly situated in the tail. These lipids and fatty acids, in similar proportions, were the same that appeared in round spermatids. Thus, SM and Cer with non-hydroxy and 2-OH VLCPUFA are generated in spermatogenic cells within seminiferous tubules. Both lipids accompany the gametes almost without changes from their last modification, in round spermatids, to their almost complete maturity after their passage through the epididymal epithelium. The possible functions and properties of the unusual SM and Cer of rat spermatozoa will be the aim of future studies.

LI-C08.
NUCLEAR RECEPTORS, HEPATIC LIPID DROPLETS IN SUCROSE INDUCED LIPIDOGENESIS: REVERTION BY N-3 PUFA

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The sucrose rich diet (SRD) that mimicks non insulin dependent diabetes revealed dependence of liver triacylglycerols (TG) and cholesterol esters (CE) levels with lipidogenic LXR antilipidogenic PPAR-a and dietary polyunsaturated fatty acids (PUFA) of n-3 series. Plasmatic and hepatic increases of TG and NEFA were produced after SRD feeding with normal insulinemia correlated to decreases of PPAR-α and its RE dependent enzymes and increases of lipogenic LXR-a and FA synthase (FS) whereas the stearoyl-CoA desaturase-1 was depressed. Hepatic TG and CE were mainly compartmentalized in lipid droplets. Administration of cod liver oil (7%) reverted the abnormal increases normalizing PPAR-α and its RE dependent enzymes as well as depressing LXR-a and FS and glucose-6-phosphate dehydrogenase. In liver lipids 20:4n-6 was depressed whereas n-3 PUFA were enhanced without variation of 18:1 n-9/18:0 ratio. In hepatic lipid droplets of SRD fed rats the high levels (33%) of 16:0 and 18:1 n-9 of TG were unmodified while 18:2 n-6 was depressed to half (9%) and low 20:4 n-6 was replaced by similar proportion of n-3 PUFAs. In CE the low 20:4 n-6 was little decreased and n-3 PUFA appear. Therefore hepatic lipidogenesis is mainly regulated by PPAR-α and LXR-α, and neutral lipids of lipid droplets behave rather as selective dynamic reservoir of monoenoic and saturated FAs but not of PUFA.

LI-C09.
GLUCOSYLCERAMIDE SYNTHASE IS ESSENTIAL IN MDCK CELL DIFFERENTIATION

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In a previous study we observed that the synthesis of glycosphingolipids (GSLs) was increased under hypertonic conditions. This increase was inhibited by PDMP, an inhibitor of glucosylceramide synthase (GCS); but neither cycloserine (CS), a serine palmitoyltransferase inhibitor, nor fumonisin B1 (FB1), a ceramide synthase inhibitor, were able to reduce GSL levels. We now demonstrate by molecular analysis (RT-PCR and Immunoblot) that GCS is up-regulated by hypertonicity at the transcriptional level. Moreover, the distribution analysis of differentiation markers by immunofluorescence showed that MDCK cells could develop a hypertonicity- induced differentiated phenotype even in the presence of CS or FB1. Since FB1 is able to inhibit both the *de novo* and salvage pathways, we used other metabolic strategy to determine the source and mechanism involved in the hypertonicity-induced glucosylceramide (GC) increase. MDCK cells were labeled with ^{14}C palmitic acid for 1 or 24h. ^{14}C GC was significantly increased under hypertonic conditions in long-term labeling but not in pulse conditions. All together, these results indicate that hypertonicity induces an increase in the synthesis of GSLs by the regulation of GCS expression and the activation of the salvage pathway of GC formation, and that such mechanism is specific and essential to MDCK cell differentiation

LI-C10.
NUCLEAR NEUTRAL LIPIDS ARE ORGANIZED IN DISCRETE FUNCTIONAL DOMAINS

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Cellular nuclei (N) are an evolutionary acquisition of eukaryotic cells with different functional domains immersed in a nuclear matrix (Mx), surrounded by a double nuclear membrane (NM).We have already determined that lipids represent 16% of nuclear components, (PL: 84% and neutral lipids (NL): 16%). PLs are mainly found in NM whereas endonuclear lipids are enriched in NL. The aim of this work was to analyze nuclear NL organization taking into account that they may represent alternative sources of nuclear fatty acids and signaling lipids. With this purpose lipid droplet (LD) isolation method (ultracentrifugation in a sucrose density gradient) was applied to N isolated from liver cells. A unique white upper band was isolated: 80% lipids and 20% proteins. NL: 37%TAG, 34% CE and 28% Col and a minor proportion of PL. TAG fatty acids are: saturated>polyunsaturated (n-6, n-3)>monoenoic and CE: saturated> monoenoic> polyunsaturated (n-6, n-3). LD were stained with Sudan Red, and OsO4 as well as observed by negative staining - TEM microscopy. Inhomogeneity of LD sizes was observed in: isolated band, N, Mx and nucleoplasts (outer membrane depleted nuclei).
In conclusion, neutral lipids are organized in discrete non polar domains inside the nuclei, which consist of a hydrophobic core of NL covered by a monolayer of PL and associated proteins (LD).

LI-C11.
MELATONIN EFFECT ON $FE2^+$ INITIATED PEROXIDATION OF SONICATED LIPOSOMES MADE WITH RETINAL LIPIDS

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Retina is a highly susceptible tissue to oxidative damage due to its high content of polyunsaturated fatty acids (PUFA), mainly 22:6 n3. Melatonin, produced in a limited number of organs in mammals including the pineal and retina, is a free radical scavenger that protects against lipid peroxidation in many experimental models (A. Catalá, Chem Phys Lipids 2009, 157: 1-11 Review). To our knowledge, no studies have been done on the antioxidant ability of this indoleamine against peroxidation of liposomes made with retinal lipids. With a view to gaining information on the antioxidant role of melatonin, its effect on lipid peroxidation was evaluated using sonicated liposomes made with lipids isolated from bovine retina. Liposomes characterized by dynamic light-scattering (Hydrodynamic radii (nm) 81, 00 ± 3, 98; Polydispersity index 0, 280 ± 0,023) were submitted to peroxidation, under air at 22 °C, with $Fe2^+$ as initiator. Conjugated dienes (CD), determined by absorption at 234 nm, and thiobarbituric acid reactive substances (TBARS) were measured as a function of time. The changes in fatty acid content were analyzed by gas chromatography–mass spectrometry. Our results showed that butylated hydroxytoluene, a classical antioxidant, inhibited CD and TBARS formation in a concentration dependent manner, changing both lag phase and initial reaction rates more efficiently than melatonin.

MI-C01.
A B-CELL MITOGEN INVOLVED IN IMMUNOMODULATION AND CHRONICITY IN BRUCELLOSIS

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One of the outstanding characteristics of *Brucella* as well as other pathogens, is the ability to establish a chronic infection that persists over the host lifespan. The fact that a pathogen can survive in a host with an ongoing immune response, depicts the existence of mechanisms capable of subverting or avoiding it. One common way of manipulating the immune response is by interfering with the fine tuned balance of cytokines and thus affecting the number and function of immune effector cells. Understanding this cross-talk could result promising for the development of new strategies for vaccination, immunotherapy and drug design. We have characterized a *B. abortus* protein (PrpA) with strong immunomodulating abilities. We have demonstrated that recombinant PrpA stimulates B-cell polyclonal activation and IL 10 and IFNg secretion *in vitro*.
By conducting experimental infections, we characterized the immune response of mice infected with *B. abortus prpA* mutant and its parental strain (*2308*). We observed that B-cell number, IgG titers, IFNg, IL–10 and IL-4 levels were manipulated by *Brucella* in a *prpA* dependent manner. We also show that *prpA* is responsible for a transient and reversible anergic state during the course of the infection. Finally, mutation of *prpA* resulted in a 100-fold reduction in bacterial number recovered from mice spleens during the chronic phase of infection.

MI-C02.
THE LIGHT ACTIVATED PATHWAY TO VIRULENCE IN *Brucella abortus*

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Brucella is responsible of one of the world's most widespread zoonotic diseases, causing miscarriage and infertility in animals and a febrile disease, known as Malta Fever in humans. Animal brucellosis is a enormous problem worldwide and is endemic in most areas of the world. Light, oxygen and voltage (LOV) proteins are a subset of the large and diverse PAS family that bind FMN and blue light receptors in plants, fungi and bacteria. *Brucella* genomes encode a 463 amino acid histidine kinase protein (LOV-HK) that contains three domains, a LOV domain at the N-terminus, a histidine kinase (HK) at the C-terminus and a PAS domain in between. Blue light absorption by the LOV-HK activates the autophosphorylation of the HK domain. LOV-HK works as photoreceptor that increase the virulence of *B. abortus* upon illumination. Using bacterial two-hybrid we have identified a single domain response regulator (RR) protein as the partner of LOV-HK. It was named as CheY-L because the similar organization to chemotaxis CheY proteins. The interaction between LOV-HK and CheY-L was confirmed by phosphotransfer assays. Bacterial two-hybrid assays also shown that CheY-L interacts with cytoplasmatic components of the flagella, FliM and FliG. We have identified CheY-L and flagellar proteins FliM and FliG as components of the signal transduction pathway initiated by light that regulates the virulence of *Brucella.*

MI-C03.
BLUE-LIGHT DRIVEN ENZYMATIC ACTIVITIES IN EXTREMOPHILIC BACTERIA FROM HIGH-ALTITUDE ANDEAN LAKES

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A collection of 200 extremophilic strains from High-Altitude Andean Lakes (HAAL; between 3000 and 6000 masl) at the northwest of Argentina has been built (www.limla.com.ar). These indigenous extremophiles should produce biomolecules adapted to their unusual living conditions that may represent valuable sources of novel bioproducts. In this work, we search for genes coding photolyases and photoreceptores containing LOV (Light-Oxygen-Voltage) domains in the UV-resistant extremophilic strains of our collection. Genes involved in blue-light driven enzimatic activities were screened in: *Rhodococococcus* sp. A5, *Brevibacterium* sp. Ap13, *Stenotrophomonas* sp. N38, N50, *Acinetobacter* spp. N40, Ver3, Ver5, Ver7 and *Pseudomonas* sp. V1; all of which showed resistance to UV-light and efficient photoreparation mechanisms. Using specific subsets of PCR primers we detected that Acinetobacter sp. Ver3 and Ver7 harboured photolyase genes quite divergent from those deposited in database. Similarly, application of a LOV-domain specific DNA microarray indicated the presence of LOV domains in *Rhodococococcus* sp. A5, *Stenotrophomonas* sp. N50, *Acinetobacter* sp. Ver5 and *Pseudomonas* sp. V1. These studies demonstrated that HAAL are environments where light-inducible flavoproteins are widespread suggesting that light-driven enzimatic activities can be of ulmost importance for survival of the indigenous communities.

MI-C04.
THE LOV HISTIDINE KINASE OF *R. leguminosarum* REGULATES THE SECRETION OF EPS AND FLAGELLAR PROTEINS

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Rhizobium leguminosarum is able to fix nitrogen and form symbiotic interactions with pea plants. Fixed nitrogen is made available to the plant which in turn provides carbon nutrients to the bacteria. Light, oxygen and voltage (LOV) domain are blue light photoreceptor modules that bind FMN as cofactor and undergo a photocycle upon illumination. In a previous work we have identified and characterized a LOV histidine kinase (LOV-HK) from the related bacteria *Brucella*. Searches in genomic databases of *R. leguminosarum bv. viciae 3841* allowed us to identify a gene that encodes for a 345 amino acid protein that has a N-terminal LOV domain and C-terminal histidine kinase domain and lacks the central PAS domain present in *Brucella* LOV-HK. The production of exopolysaccharide (EPS) in *R. leguminosarum* is down regulated by light and this effect is not observed a LOV-HK mutant strain. The flagellin proteins are down regulated in *R. leguminosarum* grown in light as compared with the bacteria grown in the dark. The pattern of flagellar proteins from the *R. leguminosarum* LOV-HK mutant was not different between light and dark and it is similar to the one of *R. leguminosarum* wt cells grown in the dark. Using bacterial two-hybrid assay we indentified two single domain response regulators as partners of LOV-HK protein. These results confirm that LOV-HK is working as blue light photoreceptor in *Rhizobium.*

MI-C05.
INTRACELLULAR PROTEOME OF *Brucella abortus*
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Brucella ssp. is facultative intracellular pathogen that causes brucellosis, a worldwide zoonosis that affects a wide range of mammals and humans. The understanding of pathogenesis of infectious diseases requires comprehensive knowledge of the proteins expressed by the pathogen during intracellular life. In this study, we isolated *Brucella abortus* from infected cells by differential centrifugation and density Percoll gradient centrifugation. Chemical isobaric tagging iTRAQ, was used to study changes in *B. abortus* protein expression during the intracellular life. As *Brucella* protein profile control we compared the intracellular proteome with protein expression of bacteria grown in culture media. Our results revealed several *B. abortus* proteins that are differentially expressed during intracellular life. Interestingly, some of them were described related to virulence in other bacterial pathogens, suggesting a conserved role in pathogenesis. We also identified *Brucella* proteins that have been already involved in *Brucella* virulence and taken together these results validate our proteomic approach to identify *Brucella* virulence factors. The intracellular proteomic analysis of *B. abortus* will provide new strategies to prevent brucellosis infection, new targets for antimicrobial therapy and new insights into mechanisms utilized by *Brucella* to establish intracellular infections.

MI-C06.
***Serratia marcescens* INVADES EPITHELIAL CELLS AND FINDS A SUITABLE NICHE TO SURVIVE AND REPLICATE**
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S. marcescens is an opportunistic human pathogen associated mainly with urinary and respiratory tract infections. This microorganism produces several extracellular proteins but there are evidences that only haemolysin ShlA and protease PrtA are able to act as virulence factors. However, the mechanisms that *Serratia* employs to disseminate in eukaryotic cells are presently unknown. In this work, we demonstrated that a clinical *S. marcescens* isolate was able to invade and multiply in CHO epithelial cells. Confocal microscopy analysis revealed that, since early stages after entry, intracellular bacteria colocalized with markers of early and late endosomes and this colocalization was maintained until six hours post-infection. In addition, *S. marcescens* colocalized with the autophagosomal marker LC3, being induction of autophagy blocked by inhibition of bacterial de *novo* protein synthesis. Moreover, *Serratia* was unable to replicate in *atg5* knock-out mouse embryonic fibroblasts. ShlA expression from a non-pathogenic *E. coli* strain was able to induce autophagy from the extracellular space, suggesting that this factor is involved in the *Serratia* invasion process. Based on these findings, we propose that *S. marcescens* delays phagosome maturation and actively induces an authophagy-like process that might favour the intracellular survival and proliferation of the bacteria inside the host.

MI-C07.
VIRULENCE GENE REGULATION IN *Shigella sonnei* SPONTANEUS MUTANTS
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The pathogenesis of *Shigella* is focused in the capacity to invade intestinal epithelial cells and for this purpose *Shigella* has a virulence plasmid of~220 kb (pINV) that encodes essential products, as the Ipa B, C, D, A proteins. In previous works we characterized clinical isolates of *S. sonnei* ipaBCDA negative by PCR and western blot; these mutants showed ability to invade in vitro Hep-2 and Caco-2 cells. In the present work we studied the gene regulation of virB gene and icsB-ipaBCDA operon. VirB is an essential transcriptiional activtion factor of the icsB-ipaBCDA operon and it was reported to be regulated by temperature and quorum sensing. Two reporter fusions were constructed using virB and icsB-ipaBCDA promotor regions and the luxCDABE operon of *Photorabdus luminescens*. Each construction was cloned in pUC19 and electroporated in *S. sonnei* ipaBCDA negative. Temperature regulation studies at 30 y 37°C were followed by OD and relative luminescence units (RLU). Quorum sensing regulation was assayed using conditioned media (CM) (OD: 1.5-2.5) measuring both OD and RLU. The autoinducer 2 (AI-2) was detected by the *Vibirio harveyi* BB 170 strain. Both virB gene and icsB-ipaBCDA operon respond to temperature increasing their expression at 37°C. Even though both strains showed an increased expression at higher OD, CM assay showed only quorum sensing regulation of virB expression.

MI-C08.
REGULATION OF THE SECRETED PHOSPHOLIPASE PhlA AND FLAGELLAR EXPRESSION IN *S. marcescens*
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Serratia marcescens is a gram-negative enteric bacteria, that can become an opportunistic pathogen in immunocompromised hosts. Despite numerous reported *S. marcescens* infections and the emergence of antibiotic-resistant strains, the virulence mechanisms of this organism are poorly understood. *S. marcescens* secretes many extracellular proteins. Among these, the secreted phospholipase PhlA is the only described exoprotein that belongs to the flagellar regulon and is also secreted by the flagellar apparatus. We conducted a generalized insertional mutagenesis and screened for PhlA-deficient strains. We found that three independent mutations in the *wec* cluster, which impair the assembly of enterobacterial common antigen (ECA), provoked the inhibition of PhlA expression. We determined that in the ECA-defective strains, the transcriptional cascade that controls flagellar assembly was turned off due to the down-regulation of *flhDC* expression. We performed a second mutagenesis to identify the regulatory mechanism involved in this flagellar repression. Our results show that the inactivation of the ECA biosynthesis leads to the activation of the RcsCDB phosphorelay system which in turn represses *flhDC*. Although highly conserved in all enteric bacteria, the role of ECA remained largely uncharacterized. These findings open a new perspective on its physiological role.

MI-C09.
MabR, A NOVEL TRANSCRIPTIONAL REGULATOR OF MYCOLIC ACID BIOSYNTHESIS IN MYCOBACTERIA
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Mycolic acids, the dominant feature of the *Mycobacterium tuberculosis* cell wall, are essential for the survival, virulence, and antibiotic exclusion of this human pathogen. Mycobacteria, unlike most bacteria, have two fatty acid synthases (FAS-I and FAS-II). Both of them are involved in the biosynthetic pathway of mycolic acids which regulation mechanism is still unknown. We discovered a new transcriptional regulator that controls the expression of *fasII* genes, named MabR. We found that MabR binds specifically to the *fasII* operon promoter region, *pfasII*. Moreover, DNAsaI footprinting analysis verified that the protein under study protects a highly conserved 21 bp motif in *pfasII*. Two 6 bp inverted repeats form part of this motif and are essential for the interaction of the regulator with the consensus sequence. Construction and further characterization of a *mabR* conditional mutant in *Mycobacterium smegmatis* demonstrated that this regulator modulates the expression of *fasII* genes *in vivo* and confirmed that it is an essential component of the complex regulatory network involved in maintaining lipid homeostasis. Taking into account that MabR homologues are highly conserved in actinomycetes and are not present in eukaryotes or in the gut flora, it is tempting to speculate that MabR could be an attractive target for the development of new and specific antimycobacterial agents.

MI-C10.
BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF PHOSPHOTRANSACETYLASE FROM *E. coli*
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The successful adaptation of *Escherichia coli*, and other organisms, to constant nutritional changes, which range from feast to famine, relies primarily on the organism's ability to 'switch off' carbon flux to acetate excretion and to 'turn on' acetate utilization. Acetate enters central metabolism as Ac-CoA, either directly through the activity of Ac-CoA synthetase (ACS) or in a two-step pathway involving phosphotrans-acetylase (PTA) and acetate kinase (AK). Sequence analysis of microbial genomes reveals two classes of Pta enzymes. Class I enzymes (PtaI) are 350 residues in length, whereas class II enzymes (PtaII) are twice as long as PtaI (700 residues). PtaI enzymes share end-to-end homology with the C-terminal domain of PtaII enzymes; hence, it is inferred that the active site of PtaII enzymes is located within their C-terminal domain. However, this assignment generate the question of what the function of the N-terminal domain of PtaII might be. Previous results from in vivo genetic and in vitro studies suggest that the N-terminal domain of Pta from Salmonella is a sensor for NADH and pyruvate. We evaluate this hypothesis through the study of three deletion mutants of Pta gene from *E. coli*. Kinetic analyses and the behavior against different metabolites reveal the essentiality of this additional domain on Pta.

MI-C11.
CLONAL ANALYSIS OF *S. aureus* ISOLATES CARRYING THE *cap5* COMPARED WITH THOSE CARRYING THE *cap8* ALLELE
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Most *S. aureus* isolates from humans produce capsular polysaccharide (CP) of serotypes 5 (CP5) or 8 (CP8).The present study was conducted using those 99 isolates to ascertain the presence of selected genes (*pvl, agr, mecA, agr, hla, hlb*), slime production and.Twenty-seven SmaI PFGE types were defined arbitrarily (85% similitude). All isolates bearing the cap5 and cap8 alleles were distributed into two SmaI PFGE clusters. Isolates with pulsotypes A through M carried the cap5 allele whereas isolates of pulsotypes M through AA carried the cap8 allele.A significantly ($p=0.0005$) higher proportion of MRSA isolates bearing the cap5 allele (37/47) was found, compared with those bearing the cap8 allele (n=10/47). The hla and the lukS-PV/lukF-PV genes was also significantly ($p<0.0001$ and $p= 0.0001$, respectively) associated to the presence of the cap5 allele. No significant association was found between disease course and pulsotype, meticilin-resistance, agr type or presence of the lukS-PV/lukF-PV, *hla* or *hlb* genes. Whereas agr I isolates were evenly distributed among strains bearing cap5 and cap8, most agr group II isolates carried the cap5 allele and most agr group III isolates carried the cap8 allele. Our results are consistent with an increased fitness of S. aureus bearing the cap5 allele in patients with osteomyelitis. Two *S. aureus* genomovars, namely Cap5 and Cap8, is suggested.

MI-C12.
PHOSPHATE-ENHANCED STATIONARY PHASE FITNESS OF *E. coli* IS RELATED TO POLYPHOSPHATE LEVEL
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Cellular polyphosphate (polyP) level plays an important role in diverse basic metabolism and in stress responses. The main enzymes associated to polyP metabolism are polyphosphate kinase (encoded by *ppk*) and exopolyphosphatase (*ppx*). We have previously shown that *E. coli* cells grown in media containing >37 mM phosphate displayed an unusual stationary phase. They maintained the expression of several respiratory and defense genes, had high viability, low oxidative damage and elevated tolerance to exogenous H_2O_2. Here, we examined the relationship between the medium´s phosphate concentration, the intracellular polyP level and the stationary phase events. The polyP level was determined in cells of wild type and *ppk ppx* mutant strains grown in media with different phosphate concentrations. This level was maintained up to 72 h only in M9-grown wild-type strain. The *ppk ppx* mutant exhibited low polyP level regardless of the medium used or growth stage. In the wild type, the expression of *sdhC*, *ubiC*, and *rpoS* genes, the enzymatic activities of NDH-2, SDH and catalase and the tolerance to H_2O_2 remained high in stationary phase, but in the *ppk ppx* mutant strain they progressively decreased. Our results demonstrated that a critical phosphate concentration maintains in stationary phase a high polyP level, which could account for the enhanced stationary phase fitness in *E. Coli*.

MI-C13.

***Pseudomonas aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE CONTAINS TWO SITES FOR QUATERNAY AMMONIUM**

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Pseudomonas aeruginosa phosphorylcholine phosphatase (PchP) catalyzes the hydrolysis of phosphocholine to choline and inorganic phosphate (Pi). Phosphocholine is produced after hemolytic phospholipase C (PlcH) acts upon phosphatidylcholine or sphingomyelin. Hence, PlcH and PchP are involved in the pathogenesis of *P. aeruginosa*. PchP belongs to the HAD superfamily as it contains, three conserved sequences motifs named I, II, and III that in mature PchP are ^{31}DMDNT^{35}, ^{166}S, and ^{242}K/^{261}GDTPDSD^{267}, respectively. The enzyme activity is dependent on the presence of various divalent cations but, up to now, we focused our attention on Mg^{2+}, Zn^{2+} and Cu^{2+}. This ions act as Lewis acids stabilizing an octahedral complex with different oxygen atoms contributed by ^{31}D, ^{33}D, ^{262}D, two water molecules and an O of the phosphate moiety. With large differences in enzyme activity, Zn^{2+} and Cu^{2+} are better activators than Mg^{2+}. Kinetic experiments performed in wild type and site directed mutants (^{42}E, ^{43}E, ^{82}YYY^{84}) with phosphocholine as substrate plus inhibition by ammonium quaternary compounds with *p*-NPP as substrate led us to propose that PchP contains two different sites for the interaction with the ammonium quaternary moiety of different compounds. These results can probably be the starting point for designing new drugs to avoid the action of *P. aeruginosa* through the action of PlcH and PchP.

MI-C14.

EXPRESSION OF AN ALCOHOL DEHYDROGENASE FROM *L. mesenteroides* IN *E. coli* REDOX MUTANTS

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Metabolic alterations related to the expression of *Leuconostoc mesenteroides adhE* (alcohol dehydrogenase) were assessed in an *E. coli arcA* and *creC* mutant growing on glycerol under microaerobic growth conditions. Expression of *adhE* resulted in a 1.4-fold enhancement in ethanol synthesis. The *ldhA* gene (lactate dehydrogenase) was deleted, and this strain synthesized up to 6.5 ± 0.3 g/L ethanol in 48 h. Metabolic evolution allowed isolation of derivatives which tolerate ethanol up to 25 g/L, and fermentation patterns were investigated in minimal medium containing additives which modify carbon skeletons and NAD(P)H availability. Pyruvate and acetaldehyde increased both biomass and ethanol concentrations. Yeast extract also promoted growth and ethanol synthesis, and this effect was mainly dependent on its vitamin supply. Calcium D-pantothenate was added at 100 µg/mL replacing complex additives. Two-stage bioreactor cultures were conducted to evaluate acetyl-CoA synthesis under aerobiosis followed by a switch to fermentative conditions. Ethanol reached 15.4 ± 0.9 g/L with a volumetric productivity of 0.34 ± 0.02 g/(L•h). *E. coli* thus responded to overexpression of Adh by funneling carbon and NAD(P)H into a highly reduced metabolite. Broadening the knowledge of the microaerobic metabolism of *E. coli* would help to implement efficient industrial processes, especially for biofuels synthesis.

MI-C15.

ARCHAEA IN EXTREME HIGH ALTITUDE ANDEAN LAKES (HAAL)

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The aim of this study was to examine the halophilic archaea communities in high altitude lakes from the Andes Mountains and Puna in South America (HAAL). Due to their extreme conditions, these environments are unique for studying archaeal biodiversity. Water and sediment samples were collected from different lakes: Catal, Chiro, Brava, Vilama and Socompa to perform physicochemical analysis and culture enrichments; these lakes are located between 3000 and 4500 masl at the Northwest of Argentina. The sediment samples were cultivated in MGM 18 % medium added with chloramphenicol (50 µg/mL). The archaeal community of enrichment cultures and original sediment and water samples were studied by 16S rDNA PCR-DGGE. Most of the DGGE bands obtained showed closest sequence similarity with non-identified uncultured archaeon previously published in the Genbank database and only a few bands gave similarities (83 to 98 %) to identified taxa such as *Halorubrum spp., Haloarcula marismortui, Halovivax sp., Natronorubrum spp* and *Haloarchaeon spp*. It became evident from these results that HAAL harbour a great diversity of potentially novel archaea.

MI-C16.

RAPID AND SENSITIVE *Xanthomomas citri* DETECTION BY LAMP COMBINED WITH NAKED EYE EVALUATION METHODS

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Xanthomomas citri pv. Citri is the bacterial pathogen that causes the canker disease in citrus plants worldwide. The early and accurate identification of this bacterium in citrus is critical for the protection of this crop industry. Detection methods based on antibodies or PCR has been developed previously, but this approaches are time consuming, laborious and requires expensive laboratory equipment. Loop mediated isothermal amplification (LAMP), a novel isothermal DNA amplification technique, is very sensitive, specific, fast and does not require specialized laboratory equipment.

A loop mediated isothermal amplification assay for the detection of *Xanthomonas citri* pv. citri (Xac-LAMP) was developed and evaluated. The assay was optimized for the amplification of a portion of the pthA gene, a patogenicity determinant of Xac. A tipical ladder like pattern on gel electrophoresis was observed in all positive samples. Amplification products were detected by visual inspection using SybrGreen and using a lateral flow dipstick, eliminating the need of time consuming gel electrophoresis. The sensibility and specificity of the assay were evaluated in different conditions. Not cross reaction was observed with pure DNA of other phytopathogenic bacteria.

The Xac-LAMP assay is a simple, fast, sensitive and specific alternative for the accurate detection of *Xanthomonas citri* pv. citri.

MI-C17.

NEW GENERIC PRIMERS LEAD TO THE CHARACTERIZATION OF HPV 115, A NOVEL Beta-Papillomavirus species 3

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Papillomaviruses (PV) are DNA tumor viruses with tropism for mucosal and cutaneous epithelia. Human PVs (HPV) have been taxonomically classified based on the phylogenetic relationships of their L1 ORF. Recently, a link between UV radiation and HPV infection has been established in the early pathogenesis of skin cancer.

In this study we explored the HPV type spectrum in skin diseases from 71 subjects. CUT primers targeting 88 mucosal/cutaneous HPV were designed and compared to FAP primers, using the "hanging droplet" PCR technique. Overall, 69 different HPV types/putative types were identified, being 17 of them novel putative types. Phylogenetic analysis of the novel putative types grouped them into Beta-PV (GC02, GC17), Gamma-PV (GC01, GC04 to GC16) and Mu-PV (GC03) genera. CUT primers showed broader capacity in detecting different genera/species and novel putative types than FAP primers. Using overlapping PCR, the full-length genome of GC02 isolate was characterized. The new virus, designated HPV 115, has a 7476 bp genome potentially encoding 5 early genes (E6, E7, E1, E2, E4) and 2 late genes (L2, L1). L1 ORF phylogenetic analysis indicated HPV 115 as the most divergent type within the genus Beta-PV species 3. This report is the first providing data on cutaneous HPVs circulating in South America. The characterization of HPV 115 expands our knowledge of the Papillomaviridae family.

MI-C18.

CHARACTERIZATION OF Fijivirus P9-1 VIROPLASM PROTEIN: SIMILARITIES TO ANIMAL REOVIRUS COUNTERPARTS

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Mal de Río Cuarto virus (MRCV) is a plant virus of the Fijivirus genus within the Reoviridae family, that infects several monocots and is transmitted by planthoppers in a circulative and propagative manner. Reoviruses replicate in viral inclusion bodies (VIBs), or viroplasms, formed in the cytoplasm of infected plant and insect tissues. In this study, we showed that the protein coded by the first ORF of MRCV segment S9 (P9-1) was able to establish cytoplasmic inclusion bodies resembling viroplasms when expressed in insect cells. Furthermore, MRCV P9-1 self-aggregated giving rise to high molecular weight complexes when expressed in bacteria and strongly interacted with itself in yeast two-hybrid assays. Finally, biochemical characterization showed that MRCV P9-1 binds single-stranded nucleic acids, and has ATPase activity. The results suggest that the formation of viroplasms during MRCV infection may be nucleated by a P9-1 proteinaceous matrix. Furthermore, P9-1 capacity to bind single-stranded nucleic acids could mediate viral RNA retention in VIBs. When also considering its ATPase activity, MRCV P9-1 may be required for genomic RNA replication and packaging into virions. Our work deepened into the properties of Fijivirus P9-1 viroplasm protein and revealed, for the first time, extensive functional and biochemical similarities between plant and animal reovirus viroplasm proteins.

PL-C01.

ANALYSIS OF *RPL10* GENES IN *Arabidopsis thaliana*: ROLES IN DEVELOPMENT, TRANSLATION, AND UV-B RESPONSE

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UV-B exposure was shown to increase expression of a number of maize ribosomal protein genes, including the single copy gene *RPL10*. The Arabidopsis genome contains three *RPL10* genes encoding RPL10A-C. *A. thaliana* RPL10s are expressed with tissue and developmental stage specificity, showing high levels of expression in tissues with active cell division. RPL10C shows increased levels after UV-B, RPL10A expression is not UV-B regulated; and RPL10B is down-regulated by UV-B in all organs studied. Co-immunoprecipitation studies indicated that RPL10s in Arabidopsis associates with translation proteins, demonstrating it is a component of 80S ribosome; the presence of nuclear proteins suggest that at least one of the isoforms may have an extra-ribosomal role. Characterization of T-DNA insertional mutants indicates that *RPL10* genes are not functionally equivalent. *rpl10a* and *rpl10b* mutant plants have developmental phenotypes: knockout *rpl10A* mutants are lethal, *rpl10a* heterozygous plants are particularly deficient in translation under UV-B conditions, and knockdown homozygous *rpl10b* mutants show abnormal growth. The results demonstrate *RPL10* genes have different functions in *A. thaliana*, being involved in plant development, translation and UV-B response.

PL-C02.

REDOX REGULATION OF GLYCOLYTIC TRIOSE-P BRANCH POINT IN THE CYTOSOL OF WHEAT CELLS

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In plant cells, one of the key points in cytosolic glycolysis involves the conversion of glyceraldehyde-3-phosphate (Ga3P) into 3-PGA. In order to understand the fate of triose-P, we studied post-translational modifications of two different plant cytosolic Ga3P dehydrogenases: the phosphorylating enzyme (p-Ga3PDHase, EC 1.2.1.12) [that followed by the 3P-glycerate kinase derives triose-P to the synthesis of ATP and NADH], and the non-phosphorylating enzyme (np-Ga3PDHase; EC 1.2.1.9) [that leads triose-P to the synthesis of NADPH]. We found that both enzymes can be regulated via redox modification (by ROS and RSN), being the p-Ga3PDHase markedly more sensitive to thiol oxidants than np-Ga3PDHase. Concurrently, p-Ga3PDHase is oxidized by H_2O_2 with a k" of 119 $M^{-1}s^{-1}$, which is 63-fold higher than that obtained for np-Ga3PDHase oxidation. The loss of activity was effectively reversed after incubation with physiological reductans as GSH, reduced TRX-h and reduced GRX. After results, it is tempting to speculate that under oxidative conditions the cytosolic fate of triose-P is routed to NADPH generation rather than to ATP and NADH. The redox modification mechanism, together with the regulation of both Ga3PDHases by reversible phosphorylation (and interaction with 14-3-3 regulatory proteins) strongly suggests that the cytosolic Ga3P oxidation step in plant cells is under strict control.

PL-C03.

MUTATIONS IN TWO PUTATIVE PHOSPHORYLATION MOTIFS IN LePRK2 SHOW ANTAGONISTIC EFFECTS ON TUBE LENGTH

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The tip-growing pollen tube is a useful model for studying polarized cell growth in plants. We previously characterized LePRK2, a pollen-specific receptor-like kinase from tomato (*Solanum lycopersicum*). When transiently overexpressed in tobacco pollen tubes, LePRK2-eGFP significantly decreased pollen tube length and increased pollen tube tip width, relative to eGFP tubes. Tubes that expressed a mutation in a lysine essential for kinase activity showed the same length and width as the eGFP control. LePRK2 is present as multiple phosphorylated isoforms in mature pollen membranes. Using comparative sequence analysis and phosphorylation site prediction programs we identified two putative phosphorylation motifs in the cytoplasmic juxtamembrane domain. Site-directed mutagenesis in these motifs, and overexpression in tobacco pollen, showed that both motifs have opposite effects in regulating pollen tube length. Relative to LePRK2-eGFP pollen tubes, alanine substitutions in residues of motif I, S277/S279/S282, resulted in longer pollen tubes, but alanine substitutions in motif II, S304/S307/T308, resulted in shorter tubes. We conclude that pollen tube length can be negatively and positively regulated by phosphorylation of residues in motif I and II respectively. These results suggest that LePRK2 may have a role in pollen tube growth through regulation of its own phosphorylation status

PL-C04.

NON-CANONICAL PROCESSING OF miRNAs IN PLANTS

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MicroRNAs (miRNAs) are small RNAs of ~21 nt that recognize partially complementary sites in target mRNAs and guide them to cleavage or translational arrest. They are transcribed as larger precursors that contain fold-back structures that are processed by RNAse III complexes. The precursor contains spatial clues which determine the position of the miRNA along its sequence. The first step in microRNA biogenesis usually involves a cleavage at the base of its fold-back precursor. Here, we describe a non-canonical processing mechanism for microRNAs miR319 and miR159 in *Arabidopsis thaliana*. We found that their biogenesis begins with the cleavage of the loop, instead of the usual cut at the base of the stem-loop structure. DICER LIKE 1 proceeds then with three additional cuts until the mature miRNA is released. We further show that the conserved upper stem of the miR319 precursor is essential to organize its biogenesis, while sequences below the miRNA/miRNA* region are dispensable. The biogenesis of miR319 is conserved in the moss *Physcomitrella patens* indicating that its processing mechanism has an ancient origin. These results provide new insights into the plasticity of small RNA pathways.

PL-C05.
HAHB10 SUNFLOWER TRANSCRIPTION FACTOR IS INVOLVED IN PHYTOHORMONE MEDIATED RESPONSE TO BIOTIC STRESS

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The transcription factor HAHB10 belongs to the sunflower HD-Zip II subfamily and it has been previously associated with the induction of flowering.
A gene expression analysis performed with HAHB10-constitutively expressing Arabidopsis plants indicated that specific flowering transition genes, such as *FT, AGL8* and *AGL9* were several fold induced whereas genes related to biotic stress responses, such as *PR1, PR2, ICS1, AOC1, EDS5, PDF1-2a*, were repressed. Specific gene induction and repression was validated by qRT-PCR, both in Arabidopsis transgenic plants and in transiently transformed sunflower leaf discs. The expression of *HAHB10* and the flowering genes *HaAGL9* and *HaFT* was up regulated by both SA treatments and infection with a virulent strain of *Pseudomonas syringae*. Together, the results indicated that HAHB10 participates in the induction of flowering and defense responses by activating and repressing respectively specific genes involved in these two processes.
On the other hand, basal SA and JA levels in Arabidopsis plants ectopically expressing HAHB10 were similar to control plants, however SA levels differentially increased in the transgenics after wounding and infection with *P. syringae* while JA levels differentially decreased. Moreover, Arabidopsis plants co-expressing 35S:HAHB10 and 35S:NahG also showed early flowering, suggesting that HAHB10 acts downstream of SA signaling.

PL-C06.
PHYLOGENETIC AND EXPRESSION ANALYSES OF WRKY TRANSCRIPTION FACTORS PROVIDE INSIGHTS INTO SUNFLOWER

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Sunflower is a highly economically important crop for which genomic and molecular biology tools are very limited and whose genome sequence is unknown. Transcription factors (TFs) are key proteins regulating many signal transduction pathways. WRKY TFs are involved in the plant abiotic and biotic stress responses. The goal of this survey was to identify and characterize sunflower WRKY TFs through in silico and expression gene analyses.
Performing a bioinformatic approach, the existence of 97 putative sunflower WRKY members derived from NCBI EST database was estimated. Phylogenetic trees constructed with WRKY domains indicated that these genes could be classified in the 7 groups assigned to *Arabidopsis* and resolved a novel clade with traits apparently specific to *Asteraceae*. Expression patterns of one selected member of each group indicated that these genes are regulated by biotic and abiotic stresses as well as by the hormones associated to these stresses. One of them, *HAWRKY5*, is differentially expressed in sunflower lines tolerant to *Sclerotinia infection*.
This study indicates that Asteraceae WRKY family has suffered a particular diversification, which could be the source of specific new functions. On the other hand, the sunflower WRKYs would play a role in the biotic stress response and could be markers of tolerance to necrotrophic pathogens, useful for the improvement of this crop

PL-C07.
CHARACTERIZATION AND REGULATION OF A MAIZE FLAVONOL SYNTHASE

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Flavonoids, a broad collection of specialized compounds containing the C6-C3-C6 carbon framework, are widely distributed throughout the plant kingdom. These compounds have diverse functions in plants, and they are benefitial for human's health. Flavonols are synthesized from the dihydroflavonols dihydrokaempferol (DHK), dihydroquercetin (DHQ) or dihydromyricetin by the action of flavonol synthase (FLS) enzymes. We identified and characterize a maize FLS (ZmFLS1). ZmFLS1 can use DHK and DHQ as substrates but show preference for DHK: it has a higher catalytic efficiency and a lower (Km value than for DHQ. *fls-1* Arabidopsis mutant plants complemented with ZmFLS1 partially restored the anthocyanin levels and production of kaempferol and quercetin. By combining transient expression assays in maize Black Mexican Sweet (BMS) cells and chromatin immunoprecipitation (ChIP) experiments, we demonstrate that *ZmFLS1* is a direct target of the P1 and C1/R regulators of flavonoid biosynthesis. Highlighting a role of flavonols in UV-B protection, we show that the expression of ZmFLS1 is significantly enhanced by UV-B in maize leaves. The results presented here demonstrate the activity, regulation and role of the first FLS characterized from maize in the flavonoid pathway.

PL-C08.
EFFECTS OF gammaCA2 OVEREXPRESSION ON MITOCHONDRIAL FUNCTION IN ARABIDOPSIS CELL SUSPENSIONS AND LEAVES

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We had previously shown that ectopic expression of CA2, one of the gamma-Carbonic Anhydrase integrated Complex I subunits, causes male sterility by anther non-dehiscence. Also, green leaves from overexpressing plants show an alteration in respiration rates. Particularly, O_2 uptake via mitochondrial complex I is nearly null. These data suggested a mitochondrial dysfunction by CA2 overexpression. In the present work, we studied the mitochondrial Complex I functionality in dark-grown, heterotrophic cell suspensions, obtained from both Col-0 and overexpressing CA2-FLAG tagged plants. It was observed that Complex I from overexpressing cell suspension contains all the required subunits for normal activity. Indeed, NADH dehydrogenase activity remains unaltered. In contrast to the results obtained in green leaves, 35S::*CA2-FLAG* cell suspension shows a higher respiration rate through Complex I compared to Col-0 cell suspension probably leading to more fresh weight after one week growth. Thus, CA2 overexpression has different effects on mitochondrial functionality (in particular, on Complex I function) depending on growth and light conditions. These results suggest that mitochondrial gamma-CAs function is directly related to a light-related mechanism, i.e. Photorespiration.

PL-C09.
ENDOCYTOSIS DURING SEED GERMINATION

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Endocytosis is the internalization of plasma membrane proteins and lipids, extracellular molecules, fluids, particles and exosomes. This process has only recently been analyzed in plants and proved to be crucial in cells that experience polarized growth as pollen tubes and root hairs. We have previously demonstrated that an extracellular lipid transfer protein from sunflower seeds, Ha-AP10, is also detected intracellularly during early germination. The aim of this work was to evaluate if endocytosis takes place upon imbibition and could be responsible for the appearance of Ha-AP10 inside the cells. We observed that the intracellular localization of Ha-AP10 was not affected by the presence of cycloheximide during seed imbibition, suggesting that de novo synthesis was not at the origin of intracellular Ha-AP10. Incubation of seeds with the endocytic marker FM4-64 allowed the visualization of the dye intracellularly, reveling that endocytosis occurs upon early imbibition. This process probed to be conserved between species, since Arabidopsis seeds also internalized the dye during imbibition. To our knowledge this is the first demonstration of an endocytic process in seeds, suggesting that the recycling of membrane components and/or protein internalization could be important cues during early germination.

PL-C10.
SPERMINE ACCUMULATION AND OXIDATION INCREASES *A. thaliana* TOLERANCE TO *P. viridiflava*

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Apoplastic polyamine oxidation was previously found by our group to play a role in the defense of tobacco plants against necrotrophic and biotrophic pathogens. In addition, *A. thaliana* plants that accumulate the tetraamine spermine by overexpression of a *SPERMINE SYNTHASE* gene (35S::*SPM*) are less susceptible to *P. viridiflava* than *spermine synthase* null mutants (*spm1-1*) and wild type plants (WT). The aim of this work was to determine whether spermine-mediated tolerance of *A. thaliana* to *P. viridiflava* depends on the oxidation of this tetraamine and which polyamineoxidase (PAO) isoform is involved in this response.
Feeding *spm1-1* and WT *A. thaliana* plants with spermine led to increased tolerance to *P. viridiflava*, reaching a similar level to that of 35S::*SPM* plants. On the other hand, supplementation with the PAO inhibitor SL11061 led to increased susceptibility to *P. viridiflava* in the three Arabidopsis lines. Susceptibility of *A. thaliana pao4-1* and *pao5-1* null mutants was similar to that of WT plants, while *pao1-1* null mutant exhibited increased susceptibility. The results of this work suggest that spermine-mediated tolerance of *A. thaliana* to *P. viridiflava* is due to oxidation of this tetramine by PAOs and the PAO1 isoform seems to play a specific role in this defense response. The relevance of two other PAO isoforms not evaluated in the present work remains to be established.

ST-C01.
THE HYDRATION LEVEL OF A TRANSMEMBRANE HYDROPHILIC MOTIF MODULATES DesK ACTIVITY

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The histidine kinase DesK from *Bacillus subtilis* is a paradigmatic example of a sensor specifically tailored to remodel membrane fluidity when the temperature drops below ~30°C. In this work, we address the process by which DesK transmembrane segments (TMS) transmit temperature signals across the membrane by engineering the 5 TMS domain of the DesK into a single-TMS chimeric sensor. This so-called Minimal Sensor (MS) fully retains in vivo and in vitro the sensing input and transmission output of the parental system. Progressive deletions of TM segments revealed that only the first TM segment (TM1) is essential to regulate the kinase activity. Therefore, our engineered MS combines the N-terminal 17-residue portion of TM1 with the C-terminal 14-residue portion of TM5 which is naturally fused to the cytosolic catalytic domain. The MS N-terminus contains three hydrophilic aminoacids near the lipid-water interface creating an instability hot spot. This boundary-sensitive motif controls the sensing and transmission activity. Accordingly, we hypothesize that membrane thickness is the temperature agent that determines the signaling state of the cold sensor by dictating the hydration level of the meta-stable hydrophilic spot. This conjecture is supported through the study of the signaling behavior of MS variants purposely constructed.

ST-C02.
ROLE OF LIPID BIOSYNTHESIS IN THE ACTIVATION OF THE YycFG SIGNAL TRANSDUCTION PROTEINS

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The YycFG two-component system is the only signal transduction pathway in *B. subtilis* known to be essential for cell viability. This system is highly conserved in low G+C gram-positive bacteria, including several human pathogens, and regulates important processes such as cell envelope homeostasis, but the signal modulating its activity is not clearly defined. The YycF response regulator activates the expression of *yocH* which encodes a putative autolysin. We have previously shown by microarrays analysis that inhibition of fatty acid biosynthesis by cerulenin induces *yocH* expression. These data pinpointed YycFG as the responsible for the increased *yocH* expression by responding to the inhibition of lipid biosynthesis. We confirmed this hypothesis analyzing the *yocH* expression in an *yycFG* conditional mutant. We found that YycFG depletion in cerulenin-treated cultures avoids *yocH* overexpression. Inhibition of fatty acid and phospholipid biosynthesis by cerulenin is accompanied by higher intracellular levels of malonyl-CoA and a decrease in fatty acid chain-length in membrane phospholipids. Using mutants in several genes of lipid biosynthesis we found that the activity of YycFG responds to alterations in membrane phospholipid contents. These results provide the first evidence that YycFG signal derivates from membrane lipid homeostasis.

ST-C03.
CELL SIGNALING IN *Trypansoma cruzi*: CLONING AND EXPRESSING A CLASS I PI-3 KINASE (TCPI3K)

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The life cycle of *Trypanosoma cruzi*, protozoan parasite agent of Chagas' disease, comprises three different morphological and functional forms: amastigotes, epimastigotes and trypomastigotes. Under stress conditions, intermediate forms between epi- and trypomastigotes are also observed. Previously, we have found that epimastigotes possess PI3K activity which is a component in the signaling pathway activated by hyperosmotic stress. In adition, this activity increases in the intermediate forms respect to epimastigotes. The aim of this work was to define if class I PI3K is functional in this parasite, through cloning and expressing a putative *T. cruzi* class I PI3K (TcPI3K) in BL 21 *E. coli* cells. Primer sequences were designed from Tc00.1047053510167.10 gene and PCR product was sequenced and compared with the original sequence found at GeneDB. We used pET22 b(+) expression vector and protein induction with IPTG was performed at 25ºC to obtain enough soluble protein for its purification. Southern and Northern blot analysis showed that TcPI3K is a single copy gene and its mRNA is expressed in epimastigote forms. Moreover, recombinant TcPI3K recovered from soluble fraction was assayed in order to determinate kinase activity, confirming a functional PI3K. Our results suggest for the first time, the presence of class I PI3K signaling pathway system in trypanosomas.

ST-C04.
ALPHA-2M/LRP-1 SYSTEM INDUCES Shc PHOSPHORYLATION AND NFkB ACTIVATION IN MACROPHAGE CELLS LINES

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Alpha2-Macroglobulin (α2M) is a broad specific proteinase inhibitor, which is recognized by LDL receptor-related protein (LRP1), an endocytic receptor belonging to the LDL receptor gene family. Previously, we demonstrated that α2M/LRP1 induces intracellular signaling activation characterized by the activation of PKC and MAPK-ERK1/2 and the expression of MMP-9 in macrophage-derived cell lines. In this work, we investigated the molecular intermediates in the regulation of the intracellular signaling activation induced by α2M/LRP1 system in J774 and RAW 264.7 macrophage derived cell line. By Western blot we observed that α2M induces p52-Shc phosphorylation concomitantly with PKC α/ß activation. The α2M-induced Shc activation was fully blocked by GÖ-6976, an inhibitor of PKC α/ß, and RAP, an antagonist of the α2M binding to LRP1. In addition, we observed that α2M induced IkBa degradation and NFkB p65 nuclear translocation. Both intracellular pathways induced MMP-9 synthesis, which were fully abolished by the presence of PD98059 and BAY, inhibitors of MEK and NFkB, respectively. In conclusion, our data demonstrate that α2M/LRP1 system promotes the intracellular p52-Shc and NFkB activation, which are responsible for the MMP-9 expression in macrophages.

ST-C05.
CHARACTERIZATION OF ANG II AT2 SIGNALING PATHWAY IN PND15 RAT HINDBRAIN

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The classical effects of Ang II are well known but new evidences involve this peptide in development. The aim of the present study was to elucidate the non-classical signaling pathway of Ang II AT2 receptors in PND15 rat hindbrain, a critical developmental stage. Membrane preparations were stimulated with Ang II (10-7M) in the presence or not of competitors and the c-Src inhibitor PP2 or its inactive analog PP3, solubilized and immunoprecipitated with anti-AT2 or anti-SHP-1. Following Ang II stimulation, SHP-1 associates to AT2 receptors but not to AT1 subtype. Immunocomplexes obtained with anti-AT2 or anti-SHP-1 exhibited PTPase activity, blocked by PD123319 (AT2 antagonist) or PP2. SHP-1 activation was correlated with tyr-phosphorylation level of SHP-1 in immunocomplexes. Both immunocomplexes contained c-Src and PP2 blocked SHP-1 tyr-phosphorylation as well as activation and association SHP-1 to c-Src. Immunocomplexes also contained a high molecular weight protein (125-130 kDa). To further investigate this protein we assayed the participation of FAK and paxillin, which were not present in immunocomplexes. Thus Ang II stimulation via AT2 subtype, induced tyr-phosphorylation of SHP-1 and formation of immunocomplexes AT2-SHP-1-Src. These results demonstrate SHP-1 activation by Ang II AT2 receptors and thus a potential physiological role of these receptors in developing hindbrain.

ST-C06.
DOPAMINE ACTIVATES ERK1/2 IN PROXIMAL TUBULE CELLS: ROLE OF PKC, PKA AND REACTIVE OXYGEN SPECIES

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In the proximal tubule, dopamine (DA) regulation of Na^+,K^+-ATPase involves the activation of D1- and D2-like receptors, stimulation of PKC and PKA, PLA2-mediated arachidonic acid (AA) release and generation of hydroxylated metabolites of AA. Since the activity of cytosolic PLA2 is regulated by protein phosphorylation, we aimed to study the effect of DA on ERK1/2 activity and its possible role in PLA2 activation. Opossum kidney cells were stimulated with DA or selective D1 (SKF-38393) or D2 (PPHT) agonists. DA (1 µM, 5 min) induced ERK1/2 activation through D1 and D2 receptors, an effect abolished by an antioxidant or by monoaminooxidase (MAO), PKC and PKA inhibition but not by PLA2 inhibition. Immunocytochemistry and Western blot analysis showed that MEK1/2 inhibition blunted the effect of DA on ERK1/2 and PLA2. DA-induced ERK1/2 activation promoted no cell proliferation, measured 24, 48 and 72 hours after incubation. We conclude from our results that DA, acting through D1 and D2 receptors, promotes ERK1/2 activation via a mechanism that involves PKC and PKA activities and the production of ROS by MAO-dependent DA metabolism. We also suggest that PLA2 is a downstream effector of ERK1/2.

ST-C07.
HORMONE-DEPENDENT PHOSPHORYLATION OF ACYL-COA SYNTHETASE 4 (Acsl4) IN STEROIDOGENIC CELLS

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Steroid synthesis is modulated by different hormones or factors through PKA or PKC-mediated protein phosphorylation. Acsl4, whose substrate is Arachidonic Acid (AA), is a key enzyme in steroid synthesis. We previously described that Acsl4 and Acyl-CoA thioesterase I (Acot2) regulate the intracellular levels of AA and steroidogenesis in a concerted manner. Since Acsl4 aminoacidic sequence shows phosphorylation consensus sites for PKA and PKC, the aim of this study was to determine whether Acsl4 is substrate of these kinases. Recombinant Acsl4 results to be suitable substrate for PKA and PKC kinase. Then, we studied Acsl4 phosphorylation in Y1 adrenal cells. By $^{32}P[H_3PO_4]$ incorporation, immunoprecipitation, two-dimensional gel electrophoresis, autorradigraphy and western-blot we demonstrated that ACTH regulates the phosphorylation of Acsl4. We demonstrate for the first time that Acsl4 is a phosphoprotein and that this phosphorylation is hormone regulated.

ST-C08.
THE ACTION OF TYROSINE PHOSPHATASES ON StAR PROMOTER ACTIVITY IS MEDIATED BY ARACHIDONIC ACID

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LH/CG stimulation of steroidogenesis by Leydig testicular cells is dependent on protein tyrosine dephosphorylation mediated by PKA-activated tyrosine phosphatases (TPs), the release of arachidonic acid (AA) and the induction of StAR protein. Since StAR protein expression is regulated by AA through the activity of its promoter, the aim of this work was to analize the relationship between TPs, AA and StAR protein promoter. MA-10 Leydig cells were transfected with a plasmid containing luciferase gene under the control of StAR promoter 48 hs before treatment with or without AA, benzylphosphonic acid (BPA, TP inhibitor) and 8 Br-cAMP. As already known, 8Br-cAMP (0.1 or 0.25 mM) increased the activity of the promoter (approximately 2-and 3-fold% respectively). Addition of AA further increased the action of cAMP (approximately 30%). BPA (0.20 mM) significantly reduced the promoter activity increased by cAMP (25-50%). Treatment of the cells with 8Br-cAMP, TPs inhibitor and AA all together showed that AA is able to overcome (85-95%) the inhibition produced by BPA on the cAMP-activated promoter activity. Steroid production varied accordingly in all cases. This study shows that TP action affects steroidogenesis acting on StAR promoter activity. The mechanism involves AA release, since exogenous AA is able to overcome the inhibition of TPs on downstream events like StAR promoter activity.

SB-C01.
CYCLODEXTRIN GLUCANOTRANSFERASE FROM *Bacillus circulans* DF 9R: PRODUCT SPECIFICITY AT THE -6 SUBSITE

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Cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19) is a member of the GH13 family that catalyses the conversion of starch into cyclodextrins which are important for industrial applications. Nine substrate binding subsites have been described in CGTases. Interactions at the -6 subsite (Tyr167, Gly179, Gly180, Asn193, Asp196) activate the enzyme via an induced-fit mechanism. All the CGTases whose primary structure is already known have Gly at position 179 and it has been suggested that the absence of side chains in this residue is a requirement for substrate binding favoring such mechanism. Nevertheless, we have demonstrated that CGTase from *Bacillus circulans* DF 9R is the only one possessing Gln instead of Gly at position 179. The gen of this CGTase was cloned into pET22b(+) plasmid and two mutants were obtained: Gln179Gly and Gln179Leu. The corresponding recombinant proteins were expressed in *E. coli* BL21 and purified by affinity-cromatography. The Gln179Gly mutant showed higher hidrolytic activity than both the wild type recombinant and the Gln179Leu mutant enzymes. This work is a contribution to the knowledge of CGTase structure-function relationship, particularly about product specificity.

SB-C02.
FITNESS LANDSCAPE OF METALLO-β-LACTAMASE-MEDIATED ANTIBIOTIC RESISTANCE

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Protein evolution is crucial for organismal adaptation and fitness. This complex process relies on a subtle interplay between sequence, structure, functionality and stability. The identification of structural traits acquired through evolution could be valuable for anticipating the molecular features that enhance bacterial resistance to β-lactam antibiotics mediated by the expression of β-lactamases, enzymes whose evolution is continuously challenged by the indiscriminate use of antibiotics. Metallo-β-lactamases (MBLs) are the latest generation among these enzymes, whose structural diversity and broad substrate profile, allows them to inactivate most β-lactam antibiotics. Directed evolution on the MBL BcII from *Bacillus cereus* yielded an optimized variant, that harbors four mutations remote from the active site, which is able to confer greater antibiotic resistance. Two of these mutations display sign epistasis. One of them improves the hydrolysis rate by stabilizing a catalytic intermediate. The second mutation bestows a more flexible scaffold, resulting in a enzyme with broader substrate spectrum. The fitness landscape of metallo-beta-lactamases is such that the stability threshold has not been exhausted. We have also identified structural determinants of the evolution, therefore this approach can be exploited to design mechanism-based inhibitors with an evolutionary perspective.

BT-P01.
USE OF ZEBRAFISH EMBRYOS FOR THE IMPLEMENTATION OF TOXICITY TESTS
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Environmental contamination with chemicals represents a serious risk to ecological and human health. Information about the toxicity of chemicals, pesticides, biocides and pharmaceutical drugs is required. Common eco-toxicity tests that make use of adult fish specimens have increasing ethical objection and demand to be replaced. One choice is the "fish embryo toxicity assay" that correlates other common eco-toxicity assay. This test is performed with non-hatched embryos of the zebrafish *Danio rerio* and allows to determine toxicity by using 24-well multiplates. We have optimized our zebrafish facility for the test conditions and designed a variant test using a single 96-well multiplate to perform the complete assay, that allows to rapidly obtain the LC_{50} for a drug or liquid sample using low volumes. We assayed commercial formulations of the broad-spectrum herbicide glyphosate and the insecticide cypermethrin obtaining LC_{50} values that are, respectively, 1000-fold and 2-fold lower than the concentrations used in farming lands. Additionally, taking advantage of the zebrafish as an excellent organism for the study of physiological function, here we present the design and construction of molecular tools for the generation of transgenic fish lines that will result useful to detect liver cellular damage *in vivo* by using bimolecular fluorescence complementation strategy.

BT-P02.
CHROMIUM (VI) BIOREMEDIATION AT VARIOUS ENVIRONMENTAL CONDITIONS USING CHROMATE-RESISTANT YEASTS
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Contamination by chromium compounds is caused by a variety of industrial applications eventually leading to heavy pollution of soil, surface water and atmosphere. Two chromium resistant yeast strains, *Pichia jadinii* and *Pichia anomala*, converting toxic Cr(VI) to the less toxic and immobile Cr(III), were evaluated at different culture conditions in order to optimize heavy metal removal. Cultures were carried out in YNB medium supplemented with sucrose and ammonium sulfate during 120 h. The influence of Cr(VI) initial concentration, temperature and agitation conditions on Cr(VI) detoxification were evaluated. For both yeast strains biomass production and Cr(VI) removal was positively affected by increasing incubation temperature (up to 30ºC) and under shaken culture conditions (up to 250 rpm). Complete Cr(VI) disappearance was retarded as initial Cr(VI) concentration increased. Whilst at 0.5 mM strains were able to completely remove Cr(VI) within 12 and 8 h of incubation, at 2 mM, full removal required 96 and 72 h, for *P. jadinii* and *P. anomala* respectively. These optimized parameters would be applicable at fermenter scale in order to perform a succesful Cr-removal potentially valuable for bioremediation purposes.

BT-P03.
HARNESSING GLYCEROL FROM BIODIESEL FUEL PRODUCTION TO OBTAIN VALUABLE INDUSTRIAL COMPOUNDS
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Glycerol (Gro) has many appliances in the cosmetic, food, and pharmaceutical industries. However, Gro has become an inexpensive carbon source due to its generation as inevitable by-product of biodiesel production, a process with outstanding growing within the last decade. In addition, the excess of Gro has environmental problems since it is not innocuous and its disposal turns complex. Thus, conversion of low-priced Gro streams to higher value products, such as 1,3-propanediol or dihydroxyacetone (DHA), has been proposed as relevant biorefinery strategies. Many organisms are able to metabolize Gro, and their enzymes are key targets in the bioconversion of this compound. In this work we have cloned and expressed the gene encoding for Gro dehydrogenase (GroDHase) in *Escherichia coli*. The enzyme converts Gro into DHA. The recombinant enzyme was purified to near homogeneity either by IMAC or heat shock treatment. We have analyzed the activity assay conditions and determined the $S_{0.5}$ values for both substrates, NAD^+ (0.5 mM) and Gro (100 mM). The maximum activity was reached at pH 11.0 and 40 ºC. NH_4Cl was found to be an activator of GroDHase, reducing (4-fold) the $S_{0.5}$ for Gro and increasing (1.5-fold) the V_{max}. Gro waste derived from biodiesel production was an effective substrate for the enzyme, which supports the biotechnological application of the recombinant GroDHase.

BT-P04.
NATURAL HYDROCARBON-BIODEGRADATION ACTIVITY IN AGED CONTAMINATED SOILS FROM NEUQUÉN, ARGENTINA
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Petrochemistry activities usually produce contamination events. Soils in areas where petroleum is extracted are impacted by the presence of hydrocarbons and frequently also heavy metals. *In situ* action can not be taken without previous evaluation of the biological potential of the contaminated soil at laboratory scale. In this work, intrinsic hydrocarbon biodegradation activity in soils from Centenario oilfield (Neuquén) was evaluated *in vitro* using Erlenmeyers flasks containing 10 g of soil and 50 ml of saline medium. Soil was characterized in texture, hydrocarbon concentration and some heavy metal content. Flasks were incubated at 25°C and 250 rpm in a rotatory shaker for 8 d. Total heterotrophic aerobic bacteria (THAB) were evaluated by plate count and total petroleum hydrocarbons (TPH) by IR after alkaline extraction (pH 13) of samples at 0 and 8 d. Soil was sandy and although the analyzed metals (Pb, Hg, Cd, Zn, As and Cr) showed no levels of environmental concern TPH value was high (18840 ppm). Whereas abiotic control at d 8 was not statistically different compared with system at 0 d, system having biological activity reduced TPH to 7925 ppm (57.9% of removal) in eight days. THAB values increased from $7.2x10^5$ UFC/g to $8.7x10^9$ UFC/g ($p<0.001$). Results indicated that bioremediation of this soil would be possible using the degradative potential of the indigenous microflora.

BT-P05.
A NOVEL FUSION TAG FOR RECOMBINANT PROTEINS PURIFICATION

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Affinity chromatography (AC) is the most effective method for direct isolation and purification of biomolecules from complex mixtures. Short peptides are excellent ligands for AC as they are not likely to cause an immune response in case of leakage into the product, they are more stable than antibodies to elution and cleaning conditions and they usually have very acceptable selectivity. Hydropathically complementary peptides show enough selectivity to be successfully used as ligands for protein purification in AC.
In this work we design a system for recombinant protein purification using as model the Green Fluorescent Protein (GFP) expressed in *E. coli* and two complementary peptides: The peptide LSAAIIIPFLLH was synthesised by solid phase using the Fmoc chemistry and immobilised on NHS-agarose and GFP was expressed with the peptide KNYPKKKMEKRF as a fusion tag in the carboxi termini. After cell disruption, the extract was directly applied to the complementary peptide-agarose chromatographic matrix equilibrated with 20 mM sodium phosphate buffer, pH 7.0. The GFP fused with the peptide tag was adsorbed and eluted specifically with 50 mM sodium acetate buffer, pH 3.0, 1 M Tris. The method designed can be applied for purification of other recombinant proteins.

BT-P06.
ENHANCEMENT OF RECOMBINANT PEROXIDASE EXPRESSION IN BACULOVIRUS SYSTEM

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Baculovirus-insect cell system is a popular choice for heterologous gene expression when an eukaryotic environment is required. *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV) is by far the most widely used baculovirus expression vector. Many attempts were done in order to increase recombinant protein expression-levels. In this work a new recombinant virus was constructed with the aim of enhancing horseradish peroxidase (HRP) expression level by introducing a 15 pb element from the 5´end of polyhedrin gene (PPHS) in the 5´ of the peptide-signal GP67-HRP fusion protein gene. HRP follows the secretion route. This virus (AcMNPVHRP PPHS) was compared with a lacking-PPHS one (AcMNPVHRP) for HRP expression. In vitro experiments were performed in Sf9 cell line infected with MOI 1. In vivo assays were done on *Spodoptera frugiperda* and *Rachiplusia nu* larvae infected by intrahaemocoelical injection with 50 ml of 8.106 pfu. ml^{-1} of both viral suspensions. Although HRP level in vitro was the same with both viruses, enzyme levels achieved with AcMNPVHRP PPHS were 2 and 3 times higher than those reached with AcMNPVHRP. Thus, the PPHS introduction enhances secreted HRP expression level up to 132 and 298 mg. Kg^{-1} in *S. frugiperda* and *R. nu* respectively.

BT-P07.
OPTIMIZATION OF BOVINE LACTOFERRIN ENZYMATIC HYDROLYSIS FOR LACTOFERRICIN B ISOLATION

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Antimicrobial peptides, particularly cationic peptides have received increasing attention as new pharmaceutical products. Bovine lactoferricin is a small positively-charged fragment of a larger protein called lactoferrin (Lf), which is found in cow's milk and whey, a cheese manufacture subproduct.
The aim of this work is to optimize the conditions for pepsin digestion of Lf in order to obtain a high yield of lactoferricin for its further purification by affinity membrane chromatography. Preliminary assays were performed in solution with standard bovine Lf. A 10 mg/ml Lf aqueous solution was adjusted to pH 1.3 or 3.0 and digested with 0.04 mg/ml porcine pepsin at 37 ºC for different periods, between 0-20 h. The reactions were stopped by heating at 80ºC for 15 min and the pH adjusted to 7.0 by addition of 1 M NaOH. Hydrolysates were tested for its antibacterial activity by using the susceptible strain *Bacillus subtilis* ATCC 6633. For the pH 3.0 digest, the results evidenced time dependence with the highest antimicrobial activity after 20 h incubation. For the pH 1.3 digest, the maximum antimicrobial activity appeared at 1 h and remained constant in the period studied. For Lf adsorbed onto chromatographic matrix hydrolysis, the pH 3.0-based process is the best choice attended to the chemical stability of the support.

BT-P08.
PURIFICATION AND CHARACTERIZATION OF HORSERADISH PEROXIDASE FROM *Spodoptera Frugiperda* LARVAE

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Horseradish peroxidase is useful in many biotechnological fields, including diagnostics, biocatalysis and biosensors. The aim of this work was to study different purification strategies for the recovery of recombinant horseradish peroxidase (HRPr) directly from *Spodoptera frugiperda* larvae extract. The cDNA, supporting, two tags (6XHis and 6XArg), was cloned under the polyhedrin promoter and the signal peptide sequence GP67. *Spodoptera frugiperda* larvae were injected with 50 µl of recombinant baculovirus stock (1.4x107 $pfu.ml^{-1}$). HRPr expression level was 137 ± 17 mg HRPC Kg^{-1} larvae at day 6 post infection. HRPr produced by a single larva was around 41 µg. The whole larvae extract was directly subjected to ion exchange chromatography on SP-Sepharose and HRPr was purified in only one step with a yield of 75% and a purification factor of 117. On the other hand, HRPr was also purified by inmobilised metal ion affinity chromatography on Ni-Sepharose with a yield of 86% and a purification factor of 29. Both purification methods yielded virus-free enzyme. HRPr glycosylation pattern agreed with that expected for invertebrates. The kinetic parameters using guaiacol as substrate at 20ºC were determined. The apparent Km was 12.1 ± 1.7 mM and Vmax was 2673 ± 107 U mg^{-1}, similar to standard HRP. The process herein described is an excellent way to obtain high levels of active HRPr.

BT-P09.
A CULTURE SYSTEM DESIGN TO STUDY SHEAR STRESS EFFECTS ON *Rubia Tinctorum* CELL SUSPENSION CULTURES

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Shear stress is used as a mechanical elicitor to induce secondary metabolism in plants. We previously found that shear stress elicits anthraquinones (AQs) production in *Rubia Tinctorum s*uspension cultures growing in a stirred tank bioreactor. To study mechanical elicitation mechanisms a large number of experimental units working in parallel are necessary. The aim of this work was to design a down-scaled and simple culture system which allows replacing bioreactor experiments with shaked flasks. To induce a similar intensity of shear stress to the one reached into the bioreactor, 100 mL baffled shake flasks on an orbital shaker at 360 rpm were used. Controls were carried out in 100 mL baffled flasks agitated at 100 rpm. Cultures were grown at 25 °C in presence or absence of light. Biomass concentration, cell death and AQs production were evaluated. A decrease in biomass concentration (28%) compared to control cultures was observed. Cell death increased in 60% and in 40% after 7 days of shear stress in presence and absence of light respectively. AQs accumulation increased up to 155% and 216% compared with control after 14 days of shear stress in presence and absence of light respectively. These results show that baffled flasks system elicit AQs production in a similar way as bioreactors in R. tinctorum suspension cultures and could be useful in the study of mechanical elicitation mechanisms.

BT-P10.
BEHAVIOR OF AN ATRAZINE DEGRADING BACTERIAL CONSORTIUM IN SHAKE FLASK AND BIOREACTOR CULTURE SYSTEMS

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Shake-flasks have been used for decades as simple bioreactors to allow the development of effective growth media for cellular growth processes. On the other hand, stirred tank bioreactors are widely implemented in the industrial production to obtain high cell biomass or desired products. The transfer of shake-flask derived processes to standard stirred tank bioreactors is typically problematic since growth and yields fall significantly at this stage. Bioremediation is a well known tool used to degrade or transform contaminants. To conduce an efficient bioremediation process it is necessary a bioaugmentation phase in order to produce an inoculum with high biomass concentration. In this work we studied the performance of an atrazine degrading bacterial consortium isolated from soils of the Argentinean Humid Pampa in both, erlenmeyers and stirred tank bioreactors. Shake flask experiments were carried out in 250 ml erlenmeyers utilizing previously optimized conditions. Alternatively, 1.5 l stirred tank bioreactor, operated at the same conditions as in shake flask, was employed. The evolution of biomass production and media pH showed similar behavior in both culture systems. Complete degradation of atrazine was observed in all experiments. Results show that the transfer of erlenmeyer optimized conditions to bioreactor was effective and can be used in future bioaugmentation processes.

BT-P11.
EXPLORING DIFFERENT STRATEGIES TO EXPRESS DENGUE VIRUS ENVELOPE PROTEIN IN A PLANT SYSTEM

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The utilization of plants as bioreactors presents several advantages related with its simplicity, biosafety and low cost. It retains the advantages of eukaryotic expression systems, as post-translational modifications, and prokaryotic expression systems, as scalability and economical production. However, long time-scale and low yield of recombinant proteins have been the main bottlenecks in plant-made biopharmaceuticals. A recently developed technique known as Magnifection can offset these disadvantages. This transient expression technology is based on replication of viral vectors delivered to the plant by *Agrobacterium* and allows very fast production, high recombinant protein expression levels. Dengue virus (DV) serotype 2 envelope glycoprotein (E) is the main protein associated with viral entry and the induction of immunity. In order to produce a candidate for subunit vaccines and to provide an antigen for diagnostic kits, E protein was expressed using deconstructed viral modules. An E truncated version was designed to be expressed alone and co-expressed with DV structural proteins. As well, the critical Domain III of E protein was fused to Hepatitis B core antigen. The recombinant proteins were produced successfully in *Nicotiana benthamiana* plants and were reactive with anti-E polyclonal antibody and the fusion was reactive with anti-E polyclonal and anti-HBcore antibodies.

BT-P12.
DEVELOPMENT OF A RAPID STRATEGY FOR TESTING OF CANDIDATE GENES AFFECTING PLANT METABOLISM

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Since the beginning of the post-genomic era efforts have been focussed on the development of rapid strategies for testing plant genes with unknown function. Viral Induced Gene Silencing (VIGS) becomes one of the most promising strategies due to its simplicity and rapidness. However, side-effects intrinsic to VIGS vectors and/or to reporter genes on the metabolism of silenced cell have not been investigated. By using a VIGS system based on the Tobacco Rattle Virus vector we study the effect of silencing the phytoene desaturase gene on the metabolism of *Arabidopsis* leaves and tomato fruits. We found dramatic effects on soluble sugars and pigment metabolisms in *Arabidopsi*s leaves associated with the photo-bleached phenotype produced by the silencing of the pds endogenous gene. However, no apparent effects on primary carbon metabolism were detected in the tomato fruits. In contrast, VIGS targeted tissues of *Arabidopsis* and tomato constitutively expressing a GFP protein as marker, beside constitutes a traceable and rapid strategy for transient silencing, it avoids the limitation of noisy effects on silenced tissues. These results indicate that the gfp-based VIGS system presented here can be used for reverse genetic studies aimed to investigate the function of genes operating in metabolic networks both in vegetative (*Arabidopsis* leaves) and reproductive (tomato fruits) tissues.

BT-P13.
IMPORTANCE OF LOOP IN VASOPRESSIN ANALOGS FOR THE INTERACTION WITH V2 VASOPRESIN RECEPTOR

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Desmopressin (DDAVP) is a vasopressin (VP) analogue with antitumoral activity. This cyclic nonapeptide was punctually substituted in positions 4 and 5 to improve its biological activity and half life. These positions are included in the loop formed between the disulphide bridge of the molecule. We suspected that the loop is strongly related to the interaction of desmopressin and its analogs with the V2 receptor of endothelial and some tumor cells. In order to demonstrate this hypothesis we analyzed the biological activity of the loop tetrapeptides (fragment 2-5) of DDAVP and two analogs (VQ and AQ) and compared them with the lead molecules. Because the expression of V2 vasopressin receptor has been associated with antiproliferative behavior, we analyzed the peptides ability to modify in vitro cell proliferation of mammary carcinoma cells MCF7 expressing V2 receptor. The incubation with VQ analog (100-1500 nM) during 72 h significantly reduced cell proliferation ($p<0.001$), showing a better performance than DDAVP and AQ analogue in doses of 500-1500 nM ($p<0.05$). Fragments 2-5 showed lower ability to reduce cell proliferation in low concentrations (100-500 nM) and the effect was completely abolished at higher concentrations. These results suggest that loop conformation by disulphide bridge formation is essential for the biological activity of the molecule.

BT-P14.
SYNERGISM BETWEEN AZOLES AND STATINS ON THE GROWTH OF OPPORTUNISTIC PATHOGEN YEASTS

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Candida bloodstream infections are an important cause of mortality. A few opportunistic fungemia cases due to *Candida utilis* have been reported up today. Likewise, numerous cases of infections, particularly in immunocompromised patients, caused by *Saccharomyces cerevisiae*, a safe regarded microorganism, have been also described. Increased incidence of these opportunist fungal infections, added to the emergence of resistance to several antifungal drugs have focused interest in developing new alternatives for treatment. In this work the synergism between statins, cholesterol-lowering agents, and azoles against *S. cerevisiae* and *C. utilis* strains was evaluated by using a diffusion-agar bioassay. Growth inhibition was significantly enhanced, from 11.2 to 77.5%, for azole combinations with lovastatin, simvastatin, rosuvastatin and atorvastatin. Miconazole in combination with simvastatin exhibited the best *in vitro* activity. Sinergism was confirmed by determining a reduction in cellular sterol levels. Our findings suggested that the combined use of azoles and statins may represent a viable alternative for fastidious mycosis treatment, not only by increasing the fungicidae effects but also by reducing the azole doses to be administrated.

BT-P15.
CLONING, EXPRESSION AND PURIFICATION OF DIFFERENT VARIANTS OF AN UV-SPECIFIC DNA REPAIR ENZYME

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UV radiation produces mutations in skin cells that correlate with the onset of actinic keratoses and basal and squamous cell carcinomas. Xeroderma pigmentosum (XP) is a heritable disease characterized by an extreme sensitivity to UV radiation. Previously, we have described the expression of an UV-specific DNA recombinant endonuclease (aUveA) from *Micrococcus luteus.* XP fibroblasts transfected with aUveA gene increased UV radiation resistance. However refolding yields from the aUveA inclusion bodies (IBs) remain low, limiting further studies. The aim of this work was to analyze the influence of the entire domain a and the cysteines within this domain in refolding yields. Four proteins were designed, one lacking domain a and three other with cysteine mutations in this domain. aUveA variants were built by PCR and cloned into Gateway and pET plasmids for expression in *E. coli* systems. Cysteine mutants and UveA were expressed as IBs. Cysteine mutants didn't improve either refolding yields or specific activity. In contrast specific activity from UveA was higher than aUveA. These results indicate that domain a prevents the correct aUveA folding. Alternative expression systems, avoiding the formation of IBs, could be tried so as to set up a technological platform heading to the development of a pilot scale.

BT-P16.
CUPRIC REDUCTASE ACTIVITY AS PART OF THE COPPER RESISTANCE MECHANISM IN *Amycolatopsis tucumanensis*

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Amycolatopsis tucumanensis DSM 45259T, has been studied for its remarkable copper resistance as well as for its high bioaccumulation abilities. Nevertheless, there is no specific information about proteins involved in the copper resistance mechanisms in actinobacteria. The aim of this work was to studied the cupric reductase activity of this novel strain as part of its copper resistance mechanisms.
The strains under studied were *A. tucumanensis* DSM 45259T and *Streptomyces* sp. AB5A and as sensitive controls, *Streptomyces coelicolor* DSM 40783T and *Amycolatopsis eurytherma* DSM 44348T. Strains were grown in Minimal Medium (agar or broth) with 0.25, 0.5 and 1 mM $CuSO_4$ (MMCu) during four days. Cupric-reductase activity (RACu) under different incubation temperatures (10, 25, 30, 45 ºC) were analyzed using a modified colorimetric technique with bicinchoninic acid reagent while growth was assessed by dry weight. The highest RACu was detected in *A. tucumanensis* (220 mU/mg of cells), which also was the most copper resistant strain of all selected, because it was able to grow in the maximal copper concentration tested (1 mM). Cupric-reductase activity was detected in both, copper adapted cells and non adapted cells of all strains but this activity was up to 4-fold higher in pre-adapted cells of DSM 45259T. This is the first time that RACu was demonstrated in *Amycolatopsis* strains.

BT-P17.

CHROMATE REDUCTION BY *Burkholderia cepacia* IMMOBILIZED IN SOL-GEL SILICA MATRICES

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Cr(VI) is highly soluble and bioavailablei, its carcinogenic and teratogenic potential is well known. Cr(III) on the contrary is less soluble and less toxic. Cr(VI) pollution mainly results from industrial activities. Chemical methods for the reduction of chromate are expensive, thus recently biological treatments arouse great interest. In this work we proposed the use of sol-gel silica matrices obtained from sodium silicate to immobilize a chromate reducing bacteria. The advantages of bacteria immobilization are the possibility to recycle the beads, protect bacteria and reduce their presence in the resulting product. Bacteria were cultured aerobically at 35 ºC in different media amended with chromate concentrations from 25 to 500 ppm. Cr(VI) was estimated by the diphenyl carbazide method. Experiments were performed in the presence of various electron donors such as glucose and in LB media. Best results were obtained with LB media. No difference was observed for pH 7 and pH 9. *B. cepacia* was resistant to 1000 ppm of Cr (VI) and reduced more than 98% of Cr (VI) within 48 hs when 50 or 100 ppm were added. After 8 days, bacteria reduced more than 89% and 60% of 400 and 500 ppm respectively. No differences were observed between bacteria in suspension and the immobilized ones. Further studies are being performed with real samples obtained from metal plating waste and contaminated soils.

BT-P18.

CONSTITUTIVE EXPRESSION OF A POLYGALACTURONASE FROM *Aspergillus kawachii* IN *Saccharomyces cerevisiae*

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Pectinases catalyze the hydrolysis of pectin and/or pectic acid. Among pectinases, endopolygalacturonases (PGase; E.C. 3.2.1.15.) are the most important biocatalysts. *A. kawachii* expresses a PGase, namely PG1, active at acidic pH values. Low expression levels of wild type PG1 require cloning and over expression for its industrial applications. PG1 ORF was cloned into p416 and p426 *S. cerevisiae* expression vectors (after intron removal by PCR and partial digestion with restriction enzymes), generating the constitutive expression constructions p416GDP:PG1ΔI and p426GDP:PG1ΔI. *E. coli* Top10 cells were then transformed and clones were tested by restriction analysis and colony PCR using specific primers for Pg1. Positive clones showed no mutations and correct ORF. The p416GDP:PG1ΔI and p426GDP:PG1ΔI were used to transform *S. cerevisiae* INVSc1 and W303. Transformed yeast clones were analyzed by colony PCR. Positive clones were obtained and tested in terms of PG1 expression. Both strains expressed and exported an active PGase in YPD and SC URA- media. Expressed recombinant protein was recovered as a unique extracellular protein, has a MW of ~60 kDa and was identified as PG1 by hydrolysis of polygalacturonic acid at pH 2.5 but not at pH 5.

BT-P19.

PRODUCTION AND CHARACTERIZATION OF AN ACID PGASE FROM *Aspergillus kawachii* CLONED INTO *S. cerevisiae*

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Polygalacturonase (PG; EC 3.2.1.15) catalyses the hydrolysis of pectin and/or pectic acid and it is useful for juice clarification and pectin extraction. *Aspergillus kawachii* produces an acidic PG called PG1. This enzyme was cloned into *S. cerevisiae*. Heterologous expression and growth of recombinant yeast in a synthetic medium was studied in batch and fed batch cultures. It was determined a maximum of PG1 production between 20 and 24 hours of batch growth (2 U/ml and 15 ppm of secreted proteins). Specific growth rate was determined as 0,28 h^{-1} and carbon balance was 1.05, which indicates that there is no other product but ethanol generated. When grown on fed batch system, PG1 activity obtained was 16 U/ml. A purification process was developed. At pH 3.0, the acid PGase was adsorbed to a cationic matrix; meanwhile major contaminating proteins were eliminated. After that, a size exclusion chromatography was performed, recovering the purified protein. From SDS PAGE and IEF analysis it was determined a molecular weight of 60 kDa and an Ip of 3.6. Optimum pH was 4.0. After trypsin hydrolysis and MALDI ToF-ToF analysis, interrogation against the NCBI nr database identified peptide similarities with a PPase-AS from *A. Awamori*, a PG from *A. kawachii* and a PG from *A. niger*. These results indicated successful expression of the gene encoding PG1 activity from *A. kawachii* by *S. Cerevisiae*.

BT-P20.

GENERATION OF RECOMBINANT FORMS OF A UV-SPECIFIC ENDONUCLEASE WITH POTENTIAL TRANSDUCING ABILITY

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Exposure to UV radiation produces mutations that correlate with the onset of dermatological disorders such as actinic keratoses and basal and squamous cell carcinomas. Xeroderma pigmentosum (XP) is a heritable disease characterized by an extreme sensitivity to UV radiation. A recombinant fused-form of UveA, a stable UV-specific DNA endonuclease from *Micrococcus luteus* (aUveA) previously described in our lab, showed to increase resistance to UV radiation of XP fibroblasts transfected with aUveA gene. UveA has the potential to be part of a topical enzymatic formulation capable of penetrate skin cells and repair DNA lesions. In order to overcome the difficulties to intracellular delivery, UveA was fused to Tat protein transduction domain. It is known that fusion proteins carrying Tat peptides can translocate across the plasma membrane and facilitate cell internalization. This fused-enzymes has enhanced potential to enter the cell and repair UV-damaged DNA. In this way, two molecules were engineered: TatUveA and ßTatUveA. Fusion proteins were cloned into pET vector and expressed. Both were obtained in the form of inclusion bodies and solubilised assessing alternative refolding and purification protocols. With the aim to determinate its DNA repairing capacity in UV irradiated cells, UV-DNA repair activity was characterized in vitro and transduction ability evaluated.

BT-P21.
DETECTION OF COLD-ACTIVE MOLECULES PRODUCTION BY ANTARCTIC MARINE BACTERIA

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Among the adaptation to cold, psychrophilic bacteria produce cold-active molecules with high activity at low temperatures, which are of industrial interest. We isolated Antarctic marine bacteria, identified them to the genus level and tested for antibacterial (ABA) and enzymatic activities (EA) and exopolysaccharide (EPS) production. Isolation was made either in marine (without selection pressure) or skim-milk agar (direct isolation of proteolytic bacteria). Enzyme production was evidenced as a clear zone of hydrolysis around colonies in solid media containing the corresponding substrates as sole carbon source: crystalline cellulose, carboxymethilcellulose, xylan, pectin and starch. Proteolytic activity in marine-agar isolates was detected in skim-milk agar plates and EPS by production of mucoid colonies in marine agar with glucose. ABA was tested against reference strains grown in overlay-agar. Among the predominant genera, *Pseudoalteromonas* represented the higher proportion of isolates producing EA and EPS, while *Psychrobacter* had few positive ones. ABA could not be detected in any of the isolates. It was observed that production of EPS and multiple EA by bacteria isolated in skim-milk agar was twice as much as that found among the non-selectively isolated ones. A deep knowledge of the features of these cold-molecules would help the genetic engineering of commercial enzymes.

BT-P22.
ISOLATION OF PATHOGEN MICROORGANISMS USING IMMOBILIZED ANTIBODIES ONTO MAGNETIC SILICATE BEADS

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In medical and food microbiology, specific microorganisms must be detectable in the presence of dominant background microflora. Our aim was to develop a versatile system for pathogen bacterial isolation from contaminated media. For this purpose *Salmonella* anti-OSA polyclonal antibodies (Ab) were immobilized onto home-made magnetic silicate beads by the sol-gel aqueous route. A $FeCl_3$ and $FeCl_2$ aqueous mixture was coprecipitated with NaOH. The ferrofluid thus generated was mixed with a sodium silicate solution and used as the aqueous phase for an inverse microemulsion system. Thus magnetic beads were generated by an aqueous route sol-gel process. The obtained beads were functionalized with APTES to provide NH_2 groups and treated with glutaraldehyde, a bifunctional reagent that attaches to both APTES and Ab free NH_2 groups. These beads can be recovered by means of a magnet after an assay.
Different mixtures of generic *Salmonella spp* and generic *Escherichia coli* were used to evaluate the specificity and sensibility of the system. Specificity was demonstrated with a minimum background. Sensibility was demonstrated with a high recovery of *Salmonella* OSA even from a 90:10 mixture of *E. coli*:*Salmonella* OSA.
The system appears as a promising technology for early detection of pathogenic bacteria in a much shorter time compared to the classical methodology (approximately 48 hours).

BT-P23.
PRODUCTION AND PURIFICATION OF A FIBRINOLYTIC ENZYME FROM *Bionectria* sp.

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Several thrombolytic agents such as urokinase, alteplase (t-PA), and streptokinase (SK) are currently available for clinical usage, although they still suffer significant shortcomings, including requeriment of large therapeutic dose, short plasma half-life, limited fibrin specificity, reocclusion and bleeding complications. Among microbial sources of *Bacillus*, *Aspergillus*, *Fusarium* and *Streptomyces* have been reported as fibrinolytic enzyme producers. In the present study we isolated a novel *Bionectria* sp. LY 4.1 fibrinolytic enzyme from Las Yungas forest. After cultivation at shake-flask scale, the supernatant was precipitated with cold acetone, and purificated by gel filtration chromatography with Sephacryl S-300 HR. Fibrinolytic activity was monitored by the fibrin plate method, and quantificated by the amount of tyrosine released from fibrin clots per min. The effect of pH and incubation temperature was studied. Finally, *in vitro* activity over human blood clots was also analyzed to verify thrombolytic effects. Results confirmed that the purified fibrinolytic enzyme obtained from cultures of *Bionectria* sp LY 4.1 showed thrombolytic effect over human blood clots and constitude a promising candidate for treating thrombolytic diseases with the additional benefits of its more specific mode of action (plasminogen independent) and probably associated to less side effects.

BT-P24.
MICROCAPSULES CONTAINING EXTRACT OF AN EXTREMOPHILE PLANT WITH BIOLOGICAL ACTIVITIES

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Baccharis incarum is an extremophile plant that grows in Argentina Puna. Its geographical distribution is exclusively American, grows at 3800 meters above sea level in areas of arid climate, low atmospheric pressure, wide range of temperatures and high solar radiation. In previous works we reported the antibiotic effect on multiresistant bacteria, antifungal, antioxidant and antiinflammatory activity of ethanolic and aqueous extract of *Baccharis*. The objective of this study was to prepare *B. incarum* microcapsules, and to characterize the obtained microparticles.
B. incarum extractive solution was encapsulated by spray-drying technique using gum arabic (GA) as wall-forming polymer. The average microencapsulation efficiency was ca. 80%, as determined by phenolic compounds extraction with ethanol and by exhaustive extraction with an aqueous solution and ethanol. The morphology of the microcapsules was evaluated by scanning electronic microscopy (SEM). The absence of apparent cracks or porosity indicated a good covering protection of the core material. The biological activities (antioxidant and antibacterial activities) of *B. incarum* extract, showed stability when the extract was encapsulated with GA. The microcapsules containing antioxidant and antimicrobial natural agents would be useful as another dosage form in the pharmaceutical industry.

CB-P01.
THE SMALL HEAT SHOCK PROTEIN 20 IS PALMITOYLATED "IN VIVO" IN *Toxoplasma gondii*
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The small heat shock protein 20 in *Toxoplasma gondii* (TgHSP20) does not contain any transmembrane domain or hydrophobic regions. However, it is localized to the plasma membrane and to the inner membrane complex. TgHSP20 displays a behavior similar to an integral membrane protein, since this localization is not altered by incubation with high salt concentrations. In fact, membrane localization is only altered by treatment with detergents. TgHSP20 was predicted to be palmitoylated by "in silico" analysis. Palmitoylation is the post-translational attachment of palmitate to cysteine residues of a protein. This modification plays a critical role in the localization and activity of the protein that is modified. Metabolically labeled parasites showed that TgHSP20 is palmitoylated "in vivo". Mutation of candidate cysteine residues revealed that palmitoylation plays a critical role in TgHSP20 localization. Studies on the importance of this modification in the biology of *T. gondii* are being carried out.

CB-P02.
APT-1-INDEPENDENT DEACYLATION OF GAP-43
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An acylation/deacylation cycle is required to maintain the steady state subcellular distribution and biological activity of S-acylated proteins. The cycle steps are catalyzed by protein acyl tranferases (PATs) and acyl-protein thioesterases (APTs), respectively. PATs has been recently identified and substrates are being characterized. In contrast, proteins and mechanisms involved in protein deacylation are poorly understood. We studied in CHO-K1 and HeLa cells the deacylation step of the full-length ($GAP\text{-}43^{full}$) or the acylation motif (^{N13}GAP-43) of diacylated GAP-43 fused to YFP as well as their single acylated mutants $GAP\text{-}43^{full}$(C3S) or ^{N13}GAP-43(C3S). Using biochemical assays and live cell fluorescence microscopy, it was demonstrated that single acylated mutants, but not dually acylated GAP-43, were deacylated by treatment with an inhibitor of protein palmitoylation. The ectopic expression of APT-1, the unique so far described cytosolic APT, did not affect the acylation state of dually acylated ^{N13}GAP-43 but increased the deacylation rate of the single acylated protein. Surprisingly, APT1 transcripts were detected by RT-PCR in HeLa cells, but not in CHO-K1 cells where deacylation activity was also demonstrated. These results suggest the presence of additional enzymes with acyl-protein thioesterase activity involved in the deacylation of GAP-43 in CHO-K1 cells.

CB-P03.
INTERACTIONS BETWEEN CLUSTERED PROTOCADHERINS AND THEIR ROLE IN CELL ADHESION
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Clustered protocadherins (Pcdh) are surface glycoproteins expressed in specific regions of the central nervous system of vertebrates.Because of their similarity with other adhesion molecules and enriched presence at synapses, they could be involved in determining the specificity of some synaptic connections. They are encoded by three closely related gene clusters: alpha, beta, and gamma. Alternative splicing generates multiple alpha and gamma isoforms, potentially producing enormous variability in cell surface molecules.We are interested in understanding the biological role of this diversity and whether these proteins engage in homo or heterophylic interactions. We propose certain Pcdh isoforms have different roles from other members of the cluster.We have found evidence for this. When overexpressed, the alpha c2 isoform localizes to cell-to-cell contacts in a calcium-dependent manner, retrieving from the plasma membrane to vesicles when extracellular Ca^{2+} levels decrease, as opposed to other alpha isoforms tested. We are confirming these observations in stable cell lines. To test for protein interactions in a different way, we have expressed Pcdh extracellular domains using the baculovirus expression system, challenging cells expressing a certain isoform with the same or different soluble Pcdh.We hope our research helps to understand the nature of the interactions between Pcdh.

CB-P04.
PROTEIN TYROSINE PHOSPHATASE PTP1B REGULATES CELL MIGRATION THROUGH A FAK/SRC DEPENDENT PATHWAY
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To identify molecular pathways regulated by PTP1B in cell-matrix adhesion complexes we searched for conditions that prevented the accumulation of the substrate trapping mutant PTP1B-D181A in these complexes. We started using cell lines in which different mediators of integrin signaling were knocked out or mistargeted. Cells knock out for c-Src or for the adaptors proteins paxillin and p130Cas allows accumulation of the PTP1B-DA. However, accumulation was lost in cells triple knockout for c-Src, c-Fyn and c-Yes. Reconstitution with wild type c-Src or c-Fyn rescued accumulation. Reconstitution with different mutants of c-Src (regulatory tyrosines and/or kinase dead) indicates that PTP1B targets Src-pY527 in cell-matrix adhesions, in addition to not yet identified Src downstream substrates. FAK displacement from cell adhesions and FAK-null cells lost the accumulation of PTP1B-DA. Reconstitution with wild type FAK but not FAK-Y397F (Src SH2-recruiting motif eliminated) rescued accumulation. Imaging of living PTP1B null cells show higher assembly/disassembly kinetics of adhesions, enhanced lamellar instability, and frequent changes in the direction of migration compared to WT cells. We postulate that PTP1B regulates the FAK/Src signaling pathway, regulating the turnover of adhesions, lamellar stabilization, and directional cell migration.
Supported by ANPCyT.

CB-P05.
FUNCTIONAL RELATIONSHIP BETWEEN PTP1B AND SRC ACTIVATION AND LOCALIZATION TO CELL-MATRIX ADHESIONS

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PTP1B is an ER-bound tyrosine phosphatase implicated in the maturation and dynamics of cell-matrix adhesions through Src-dependent pathways. We report a PTP1B-dependent regulation of Src activation and localization to nascent adhesions. PTP1B KO cells (KO) in suspension exhibit basal levels of active Src, which is markedly enhanced by integrin stimulation. In contrast, PTP1B WT (WT) cells show similarly high levels of active Src in either condition. Immunolabeling and FRET analysis reveal that active Src in WT cells shortly plated on fibronectin accumulates in a peripheral ring of nascent adhesions. ER/microtubule dissociation as well as plating on poly-lysine abolishes this distribution, which now remains as a uniform punctate. KO cells plated on fibronectin also show active Src in a uniform punctate. Inactive Src accumulates at the endosome recycling compartment (ERC) in KO cells but distributes uniformly in WT cells. In these cells, expression of a dominant-negative Rab11 (S25N) suppresses active Src localization at nascent adhesions and enhances accumulation of inactive Src at ERC. Complexes of substrate trap PTP1B D181A and Src are visualized in vesicle-like structures using bimolecular fluorescence complementation (BiFC). We conclude that activation and localization of Src at nascent adhesions require the coordinated action of stimulated integrins and PTP1B.
Supported by ANPCyT.

CB-P06.
EXPRESSION AND LOCALIZATION OF AURORA KINASES IN *Trypanosoma cruzi*

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Aurora kinases (AUKs) belong to a family of S/T kinases that play an essential role in many events during cell division. However, recent works suggest that some members of this family are involved in processes that occur in interphase. In mammals the Aurora kinase family includes 3 members, two of them have been found to be over-expressed in some cancer lines. In many protozoan, AUKs have been described and in *Trypanosoma cruzi* we found 3 possible AUK genes (TcAUK). *T. cruzi* is a kinetoplastid parasite with a complex life cycle in which it adopts 3 different forms: epimastigote, amastigote and trypomastigote. We determined by RT-PCR that TcAUKs are expressed in the 3 stages of *T. cruzi* life cycle, showing differences in the mRNA levels of the same AUK among different parasite forms. To study the possible functions of the TcAUKs we generated transgenic parasites that over-express these genes. TcAUK1 also was expressed as a fusion protein with GFP, to determine its localization in epimastigote cells. Because substrates of these enzymes in *T. cruzi* are unknown, we express recombinant TcAUKs to evaluate its substrate specificity. Viewing the increasing role of AUKs in wide cellular events, all related to cytoskeleton dynamics, it would be of great importance to elucidate their function in a protozoan that not only divides but also changes its form during the life cycle.

CB-P07.
PHOSPHORYLATION/DEPHOSPHORYLATION OF C-FOS: WHO DOES THE JOB?

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In T98G cells c-Fos/endoplasmic reticulum association and consequently phospholipid synthesis activation is regulated by the phosphorylated state of c-Fos tyrosine residues. The small amount of c-Fos present in quiescent T98G cells is tyrosine-phosphorylated, is not associated to the ER and does not activate phospholipid synthesis. (Oncogene, 2007 26:3551-8). Herein, we identified which tyrosine-kinase(s) and tyrosine-phosphatase(s) are responsible for maintaining the phosphorylated or dephosphorylated state of c-Fos. An in silico search performed using the Protein Human Data Base to identify putative kinases or phosphatases acting on c-Fos evidenced c-Src and EGFR as putative kinases and TC-PTP as phosphatase, respectively. In vitro, c-Src tyrosin kinase actively phosphoryated recombinant c-Fos which was then dephosphorylated by recombinant TC-PTP. Endogenous immunoprecipitated c-Src isolated from T98G cells phosphorylated recombinant c-Fos. To evidence a possible interaction between c-Fos and different TC-PTP isoforms in vivo, point "substrate-trapping"mutated TC-PTPs were used. These substrate trappers formed stable complexes with c-Fos that co-immunoprecipitated. Taken together, these results indicate that c-Src tyrosin kinase and TC-PTP phosphatases are at least one of the kinase(s) and phsophatase(s) that regulate c-Fos tyr-phosphorylation *in vivo*.

CB-P08.
FRA-1 AND C-FOS ACTIVATES PHOSPHOLIPID SYNTHESIS TO SUPPORT GROWTH OF BREAST TUMORS

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Up to date, no other inducible proteins have been found capable of replacing c-Fos in its capacity to activate phospholipid synthesis for membrane biogenesis. However, an in-silico search revealed that Fra-1 has a BD (Basic Domain) practically identical to c-Fos, varying in a single basic aminoacid. Consequently, the capacity of Fra-1 to activate phospholipid synthesis was examined in NIH and T98G cells and found to be similar to that of c-Fos.
As Fra-1 expression pattern does not overlap with that of c-Fos during normal cell growth, we studied its putative role in breast cancer cell growth, a situation in which Fra-1 overexpression has been reported. Confocal microscopy analysis showed Fra-1 co-localizing with the endoplasmic reticulum (ER) marker calnexin, in growing MDA-MB 231 and MCF7 cells. In addition, cellular fractioning assays revealed that stripping membranes of associated proteins (0.1 M KCl) results in quiescent cell phospholipid synthesis rates which was restored to initial activated rates upon addition of recombinant Fra-1 to these stripped membranes demonstrating that Fra-1 is able per se of activating lipid synthesis. Finally, over expression of Fra-1 was shown in human breast tumor samples as compared to normal tissue which co-localized with ER markers. Our results indicated that c-Fos and/or Fra-1 support breast tumor growth by activating phospholipid synthesis.

CB-P09.
THE INSULIN GROWTH FACTOR TYPE-II RECEPTOR IN RAT MAMMARY GLAND TUMORIGENESIS

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The insulin-like growth factor II receptor (IGF-IIR) is involved in binding, internalization and degradation of IGF-II. It is now considered to act as a tumor suppressor gene. We have previously demostrated that three ip injections of N-Nitroso N-Methylurea (NMU) at 50, 80 and 110 days of animals life induce malignant mammary tumors in rats. The aim of the present study was to investigate the expression of IGF-IIR in mammary tissue of normal (C) and NMU-injected rats (mNMU) during the carcinogenic process. Also, we studied the IGF-IIR expression in tumors of NMU rats and in those of ovariectomized NMU rats (OVX). Breast tissue specimens were processed at 60, 90, 120, 150 and 180 days. Immunohistochemical and Western blot analysis were performed. Results: there was no significant difference in the intensity of IGF-IIR staining and percentage of positivity were high in normal mammary glands at all times, whilst in mNMU gland the expression lowered progressively between 120 and 180 days. Tumors showed a low cytoplasmatic staining vs mammary tissue. Interestingly, tumors regressing post-OVX showed a low malignant grade and moderate IGF-IIR staining, while tumors that not regressed showed scarce or null receptor expression. Conclusion: our results suggest that decreased levels of IGF-IIR are related to the process of carcinogenesis in this experimental model.

CB-P10.
THE INSULIN RECEPTOR SUBSTRATE-1 EXPRESSION IN RAT MAMMARY CARCINOGENIC PROCESS

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It is known that the insulin-like growth factors (IGF) family plays an important role in the growth of both normal and neoplasic cells. Many authors observed that insulin receptor substrate 1 (IRS-1), one of the signalling molecules of IGF-1, is expressed in human breast cancer as an indicator of early disease recurrence. We demonstrated that three i.p. injections of N-Nitroso-N-Methylurea (NMU) at 50, 80 and 110 days of life of rats induce the development of mammary tumors. The objective of this study was to investigate the expression of the IRS-1 in mammary gland during carcinogenesis and in mammary tumors, in correlation with the hystological differentiation grade. Group of rats were randomly separated in control and NMU. Samples of mammary gland at 55, 85, 115, 145 and 185 days, and of all developed tumors were processed for western blot and immunohistochemical technics. All mammary tissue showed low cytoplasmic staining in correlation with a low proliferation rate. In some well differentiated tumors the IRS-1 expression was markedly whereas staining in surrounding normal mammary tissue was nule or very low. Expression of IRS-1 was shown to be down-regulated in poorly differentiated carcinomas. Our study reinforced the importance of IRS-1 activation in carcinogenesis and the idea that the components of the IGF signalling are involved in the regulation of breast cancer.

CB-P11.
HISTAMINE MODIFIES MALIGNANT BIOLOGICAL BEHAVIOUR IN IRRADIATED BREAST CANCER CELLS

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MDA MB 231, a metastatic breast cancer cell line, expresses the four known types of histamine receptors (HAR), which differentially regulate cell proliferation. HA also exerts a radiosensitizing effect when is added to MDA MB 231 cells before irradiation in a way related to the elevation of H_2O_2 levels. However, ionizing radiation (IR) has also been demonstrated to affect malignant biological behaviour depending upon cell type and irradiation characteristics.
The present study was conducted to investigate the action of HA and IR on two events involved in metastatic capacity such as the expression and activity of matrix metalloproteinases (MMPs) and cell motility. HA decreased MMP2 and MMP9 expression assessed by RT-PCR and cytochemistry as well enzymatic activity determined by zimography. This effect was mimicked by H2 agonists, while an opposite action was mainly observed when H4 agonists were employed. Cell motility, evaluated by wound healing assay, was also distinctly modulated through HAR. It was significantly augmented via H4R and to a lesser extent via H1R and H3R, though diminished through H2R. 2 Gy irradiated cells showed an enhanced MMP2 and MMP9 activity and cell motility compared to control cells. However, this effect was counteracted by HA.
Results suggest that HA treatment could improve radiotherapy efficacy regarding the potential development of metastases.

CB-P12.
ANTITUMORAL EFFECTS OF ROSIGLITAZONE ARE INDEPENDENT OF PPAR GAMMA IN NMU-INDUCED TUMORS

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The thiazolidinediones such as Rosiglitazone (R) are antidiabetic agents acting through the activation of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ ligands have recently been identified as potential targets for the treatment of human cancers including breast cancer. We have previously reported that R inhibits the tumor growth rate of mammary tumors induced in rats through the ip administration of N-Nitroso-N-methylurea (NMU). The aim of the present work was to investigate the expression of PPARγ and proliferation markers in mammary normal tissue and NMU-induced tumors of untreated and R-treated (0.06 mg/Kg/day) rats. The percentage expression of PPARγ and proliferating cell nuclear antigen (PCNA) was determined by immunohistochemistry. The PPARγ expression was scarce in mammary tumors while it was significantly higher in normal mammary tissue ($p<0.001$). PPARγ immunoreactivity was observed preferentially in the nucleus of ductal epithelial cells. On the other hand, PCNA expression was low in normal mammary tissue while it was higher in mammary tumors ($p<0.001$). In addition, R treatment significantly reduced PCNA ($p<0.0001$) while did not modify PPARγ tumor expression. On the basis of the present results, we conclude that PPARγ expression decreases during carcinogenesis and that the R antitumoral effects are independent of PPARγ in this experimental model.

CB-P13.
EFFECT OF DEHYDROLEUCODINE IN THE PROLIFERATION OF MELANOMA B16 F0-F10 CELLS

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Dehydroleucodine (DhL) is a sesquiterpene lactone extracted from the medicinal plant Artemisia douglasiana. Results of our laboratory showed that DhL delays the proliferation of HeLa cells, arresting them in the G2 phase of the cell cycle. In this work we proposed to analyze whether DhL inhibits the proliferation of B16-F0 and B16-F10 cells and alters cell cycle proteins. B16 F0 y B16 F10 cells were obtained from a spontaneous mouse C57BL/6 melanoma. Quiescent cells were stimulated with 10% of fetal bovine serum (SFB) in the presence of 0-6 µM DhL by 72 h. The growth index was determined (IC ± ESM) each 24 h and the results were analyzed by the variance test. Proteins of the cell cycle were analyzed by western blot. Results: After 72 h of culture control B16 F0 and B 16 F10 cells showed an IC of 9.7 ± 0.3 and 10.5 ± 1.1 respectively, with 2 µM DhL 5.7 ± 0.5 and 6.4 ± 0.7 respectively, with 4 µM 1.7 ± 0.3 and 1.9 ± 0.1 respectively, with 6 µM 1.0 ± 0.1 and 1.4 ± 0.3 respectively. Statistical analysis showed significant difference among 0, 2, 4 and 6 µM DhL. Also, incubation with 4 y 6 µM DhL reduced the concentration of ciclin B1 and survivin. DhL inhibits the proliferation of the B16 F0 and B16 F10 cells in a concentration dependent way, decreasing the expression of ciclin B1 and survivin.

CB-P14.
CELL AND VIRAL FACTORS CONTRIBUTING TO CELL POLARITY DISRUPTION DURING HPV-ASSOCIATED CARCINOGENESIS

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E6 proteins derived from oncogenic HPV types have the ability to bind PDZ-domain containing proteins involved in control of cell polarity. Among them, human Disc large (DLG1) has been extensively studied and it was demonstrated to be lost in human tumours. We investigated the mechanisms regulating DLG1 expression and showed that Snail transcription factors repress the transcriptional activity of the DLG1 promoter and down-regulate DLG1 levels. Preliminary results indicate an inverse correlation between DLG1 and Snail expression in tumour biopsies, by optimizing an immunohistochemistry assay for Snail proteins.
To further investigate the ability of HPV to disrupt polarity and tissue maintenance, we started to investigate how E6 proteins, through the binding to PDZ proteins, interfere with lipid distribution. This last point considering that the correct localization of phosphatidylinositol phosphates (PIP), in polarized cells, depends on the interaction with PDZ-containing proteins. We cloned E6 proteins derived from different HPV types, introduced them into epithelial cell lines and corroborated their proper cell distribution by immunofluorescence. The differences of PIP distribution in the presence or absence of E6 proteins is being analyzed through the localization of a PIP biosensor, the PH domain of phosholipase C fused to GFP, in stable cell lines expressing this construct.

CB-P15.
BINDING AND PHOTOCLEAVAGE PROPERTIES OF $[CR(PHEN)_2(DPPZ)]^{3+}$ TO DNA. IMPLICATIONS IN PHOTOTHERAPY

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Our aim was to study the ability of $[Cr(phen)_2(dppz)]^{3+}$ (Cr(dppz)) to associate to DNA and to characterize it as photocleavage reagent for Photodynamic Therapy (PDT).
Cr(dppz) binding to DNA was assessed by absorption and fluorescence spectroscopies, electrophoretic studies and competitive binding experiments. The Cr(dppz)-DNA binding constant was detemined as $1.1x10^5$ M^{-1} and this value suggests an intercalation of Cr(dppz) between base pairs of DNA. Competitive binding experiments showed that Cr(dppz) complex displaced ethidium bromide from its binding site in the DNA. Moreover, the Cr(dppz) complex, bound non-covalently to DNA, promoted the photocleavage of DNA under irradiation at specific wavelength. These results indicated that excited state of Cr(dppz) is the responsible for the DNA structure damage. This fact was also corroborated by measuring the transformation index of *E. Coli* with photosensitized plasmid DNA-Cr(dppz), as well as by the same transformation index obtained by irradiation applied to *E. Coli* after the bacterial transformation with plasmid DNA-Cr(dppz).
DNA, $[Cr(phen)_2(dppz)]^{3+}$ and light are the essential elements to induce the DNA damage. In sum, our results allow us to postulate the $[Cr(phen)_2(dppz)]^{3+}$ complex as a very attractive candidate for DNA photocleavage with potential applications in PDT.

CB-P16.
RETINOIDS INDUCE DIFFERENTIAL RESPONSES IN TUMOR-DERIVED LUMINAL AND MYOEPITHELIAL MAMMARY CELLS

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Due to their role in the regulation of cell growth and differentiation through binding to RAR/RXR receptors, retinoids (Rds) are being evaluated in clinical trials for cancer prevention. We focus our studies on LM38-LP, a murine mammary tumor cell line composed by luminal (LEP) and myoepithelial (MEP) cells who express functional RAR/RXR receptors. We have studied the mechanisms involved in Rds effects in LEP/MEP interaction. Rds treatment induced apoptosis (AnnexinV Assay) only in the LEP component. In 3D cultures, we found a decrease in pAkt expression in a group of cells present between the LEP and MEP components in presence of Rds, together with an increase of CK18 expression (confocal). Rds also reduced the migratory capacity of LM38-LP cells (Wound Healing assay), and increased fibronectin (FN) and BSA adhesive ability. Using anti E-cadherin/immunobeads we could temporarily separate LEP and MEP components of LM38-LP cell line. We determined that Rds induced a significant increase in RARb2 only in LEP cells (RT-PCR). We could also determine that after Rds treatment, the increase in the adhesive capacity to FN was dependent on MEP component. These results suggest that Rds induced apoptosis and modulate motility in a differential manner in LEP and MEP components of the mammary LM38-LP cell line.

CB-P17.
PARTICIPATION OF THE GENES ASI2 AND YPG1 IN L-LEUCINE TRANSPORT IN *Saccharomyces cerevisiae*
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The transport of amino acids in the yeast *Saccharomyces cerevisiae* is mediated by permeases with ample or restricted specificity.
In previous communications, we described the isolation of mutants with a decreased transport activity for the amino acid L-leucine and also with deficiency in the reactions of N-glycosylation. The mutants were isolated for their resistance to Sodium Vanadate salt and their growth is inhibited by the antibiotics hygromycine and gentamicine. Six amongst twenty mutants presented a partial resistance for the toxic analogous of L-leucine: Trifluoroleucine (TFL).
Also we characterized by western blott assays the phenotype of the mutants through an antibody (WBP1) raised against a unit of the yeast oligosaccharyltransferase (OST1). This protein is an appropriate reporter for changes in the glycosylation pattern of proteins in the endoplasmic reticulum. The complementation of these mutants with a DNA genomic library constructed on the vector pCT3 allowed us the isolation of higromicine resistant transformants. Afterward, the transformants evidenced a transport activity increased for L-leucine uptake. The isolation of the plasmids that restore the original phenotype and their ulterior sequencing permitted us to identify two genes involved: ASI2 and YPG1.

CB-P18.
INVOLVEMENT OF A SERIN LIKE PROTEASE IN THE *S. cerevisiae* H^+-ATPASE ACTIVATION MECHANISM
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We have shown that tubulin, in its acetylated form, interacts with some P-ATPases inhibiting their enzymatic activities. In *S. cerevisiae*, the plasma membrane H^+-ATPase is inhibited by tubulin and, upon glucose addition, the ATPase is activated and the tubulin is dissociated. Recently, we have shown that tubulin not only is dissociated from the ATPase, but it is also degraded. Our objective was to search a proteolytic enzyme responsible of tubulin degradation upon glucose stimulation. The physicochemical characterization of tubulin interacting proteases was performed by several chromatographic approaches and by using specific protease inhibitors. Haploid knock out yeast strains loosing the ORFs corresponding to several proteases were used. Biochemical experiments showed that in S. cerevisiae there is a citosolic protease that interacts with tubulin, it has a MW of ~45 kDa, a pI of 8-9 and is inhibited by serine protease inhibitors. The strain YOR084, loosing a serin protease of 44 kDa and pI 8.4 is deficient in H^+-ATPase activation, at least, by tubulin degradation when stimulated with glucose, indicating that this protease could be involved in the H^+-ATPase activation mechanism. Even though some authors have already proposed a protease in this activation mechanism, it is the first report where the protease involvement is confirmed and the enzyme is partially identified.

CB-P19.
ROLE OF LIPIDIC ENVIRONMENT IN THE TUBULIN REGULATION OF PMCA
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Our working group demonstrated that tubulin interacts with some members of the family of P-ATPase, (Na^+,K^+-ATPase, H^+-ATPase and Ca^{2+} -ATPase) in different systems. The interactions with tubulin modify the enzymatic activity of these P-ATPases. In this paper we focus on the behavior of PMCA (Ca^{2+}-ATPase) in the presence of acetylated tubulin in different preparations. Here we show that in COS and CAD cells and human erythrocyte intracellular calcium concentration (estimated by Fura 2-AM) decreased with the addition of ethanol, in addition a decrease in the formation of the complex acetylated tubulin/PMCA was observed. Recent experiments suggest that the formation of the complex would be due to a direct interaction of PMCA/tubulina and that the activity-dependent PMCA lipids surrounding the enzyme, this hypothesis is supported by the following results: 1 -preparations of purified PMCA reconstituted into liposomes and subsequent addition of tubulin leads to activation of PMCA. 2 -the increase in PMCA activity depends on the lipids used in reconstitution, and 3 - the alteration of lipid membranes of rat brain produced by treatment with PLC modifies the effect of exogenous tubulin on the activity of PMCA, with an activation of the enzyme. These results show that the effect of tubulin on PMCA activity depends on the lipidic composition where the enzyme is immersed.

CB-P20.
METACASPASE OVEREXPRESSION IMPAIRS CELL CYCLE PROGRESSION IN *Trypanosoma cruzi*
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Metacaspases (MCAs) are distant caspase relatives present in plants, fungi and protozoa, but absent in animals. We have previously determined the catalytic properties of two MCA gene products (TcMCA3 and TcMCA5) in *T. cruzi*. TcMCAs are cysteine peptidases that need an intact His-Cys catalytic dyad but at variance with caspases are enzymatically active without proteolytic processing and display substrate specificity for Arg at P1.
We used the tetracycline inducible vector pTcINDEX to generate cell lines overexpressing TcMCA3, TcMCA5 and their corresponding active site mutants in order to determine if they produce any effects in the different stages of the parasite: epimastigote, trypomastigote and amastigote. Overexpression of TcMCA3, but not its active site mutant, impairs epimastigote growth. By Flow cytometry analysis of a synchronized epimastigote culture overexpressing TcMCA3, we showed an arrest in cell cycle progression at the G1/S transition. Overexpression of TcMCA3 in the parasite mammalian stages also suggests alteration of the parasite life cycle in the Vero cell model. Overexpression of TcMCA5, on the other hand, did not cause similar effects. Thus, our results suggest that metacaspases, although differing from classical caspases in their biochemical properties, might share some of their possible functions in apoptosis, progression of the cell cycle and differentiation.

CB-P21.
ALPHA-2 MACROGLOBULIN/LRP1 SYSTEM INDUCES GLIAL FIBRILLARY ACIDIC PROTEIN IN RETINAL MÜLLER CELLS

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The up-regulation of glial fibrillary acidic protein (GFAP), an intermediate filament protein (IF), is an indicator of "stress" in Müller cells (MC) during proliferative retinal diseases. Previously, we have demonstrated an increased expression of alpha-2 macroglobulin (α2M) in the vitreous of human subjects with proliferative diabetic retinopathy (PDR) as well as in retinas of rats with ischemia-induced neovascularization. In this animal model, the α2M receptor, LRP1, showed a high level of protein expression like to GFAP, in MC. However, the functional significance between α2M/LRP1 system and GFAP is, at the present, unknown. Herein, we investigate the effect of α2M on the GFAP expression in a human Müller cell line, MIO-M1, which expresses LRP1. The GFAP expression was determined by Westen blot and immunofluorescence microscopy. We demonstrated that the α2M/LRP1 interaction induced GFAP expression in MIO-M1 cell line. This expression was significantly blocked by RAP, an antagonist of LRP1-binding ligands. However, vimentin, another IF, showed an uniform pattern of expression which was not modified by the α2M stimulation. In conclusion, our results demonstrate that α2M/LRP1 system promotes a differential IF regulation in MC with a preferential GFAP up-regulation, which suggests that α2M/LRP1 system play a key role in neuroprotective events occurred in retinal disorders as PDR.

CB-P22.
ROLE OF LRP1 ENDOCYTOSIS RESPECT TO THE INTRACELLULAR SIGNALING INDUCED BY ITS LIGAND α2M

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The LDL receptor-related protein 1 (LRP1) is an endocytic receptor involved in the activated α2Macroglubulin (α2M*) internalization by clathrin-coated pits. We demonstrated that LRP1 mediates the α2M*-induced MAPK activation, promoting MMPs expression, cellular proliferation and migration. However, the molecular mechanisms that regulate the intracellular signaling activity of LRP1 are unknown. Considering that in other ligand-receptor systems the endocytosis play a key role in the regulation of the intracellular signaling, the aim of this work was to evaluate whether the LRP1 endocytosis is implicated in the intracellular signaling activity of this receptor interacting with α2M*. For this propose we used different cell lines incubated in the presence of α2M*. The α2M*-induced MAPK-ERK1/2 phosphorylation was evaluated by Western blot, whereas endocytosis was analyzed by immunoflurescence microscopy and flow cytometry. To evaluate the endocytosis effect on the α2M*-induced MAPK activation the cells were cultured in the presence of hiperosmolar sucrose medium, K^+-depleted medium and cytoplasm acidification to block at different levels the clathrin-mediated endocytosis. Our data demonstrate that endocytic function of LRP1 is needed to promote intracellular signaling of α2M*-induced MAPK activation, suggesting similar adaptor proteins are involved in both cellular processes.

CB-P23.
NUCLEAR PROTEIN IMPORT IN *Trypanosoma brucei*

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In eukaryotic cells, the nuclear envelope provides a physical separation between the nucleus and the cytoplasm allowing the precise control of cellular processes as gene expression, signal transduction and cell cycle progression by selectively regulating transport between both compartments. This regulation necessitates the existence of molecular machinery that specifically recognizes a protein in one compartment and translocates it through the nuclear pore to release it in the other compartment. The active transport of macromolecular cargo between the cytoplasmic and nuclear compartments is achieved by specific soluble carrier proteins called karyopherins, with those involved in import termed importins. The alpha subunit of importin binds the nuclear localization signal (NLS) in the cytosol and the beta subunit docks this complex at the cytoplasmatic side of the nuclear pore complex. So far nuclear import machinery in Trypanosomatids has no been described and NLS are barely known in these organisms. We found that *Trypanosoma brucei* genome encodes a putative Alpha Importin of predicted 58 kDa and a putative Beta Importin of predicted 95 kDa. Both proteins were cloned as fusion proteins to YFP, Cherry FP and HA tag to study subcellular localization and dynamics. We are also performing RNAi experiments using p2T7-177 vector to evaluate the viability of the transfected parasites.

CB-P24.
CHO CELLS LACKING EXPRESSION OF THE COG COMPLEX SUBUNIT 2 (COG2) SHOW REDUCED SPHINGOMYELIN CONTENT

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Conserved oligomeric Golgi complex (COG) is a Golgi-associated tethering complex necessary for retrograde trafficking of multiple Golgi glycan processing enzymes. COG deficiencies lead to abnormal organization of the Golgi complex and defective trafficking of these enzymes, which results in pleiotropic glycosylation defects in N-, O- and ceramide linked oligosaccharides. In this work we studied the lipid synthesis status in Cog2 null mutant (ldlC) cells and found that in addition to defective sialylation of lactosylceramide to form the ganglioside GM3, these cells also show defective synthesis of a lipid identified as sphingomyelin (SM). Metabolic labeling experiments showed the synthesis of SM reduced to 25% in ldlC cells compared to wt cells. Sphingomyelin synthase (SMS) activity in vitro was essentially normal in ldlC cells as it were the LacCer- and GM3-synthase activities. SMS1 of ldlC cells was found localized in fragmented Golgi membranes, contrasting with the more compact image of the Golgi that they depict in wt cells. Results indicate that Golgi miss-organization caused by COG deficiency, in addition to affect glycosylation, also affects the synthesis of SM. Considering the importance of SM as a structural component of membranes, this finding is worth of consideration in relation to the clinical phenotype of patients suffering congenital disorders of glycosylation type II.

CB-P25.
MICROTUBULES AND DYNEIN ARE REQUIRED FOR FORMATION OF *Coxiella burnetii*-CONTAINING VACUOLES

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Coxiella burnetii (Cb) is an intracellular pathogen that causes Q fever disease. Cb replicates within a big vacuole called parasitophorous vacuole (PV), which interacts with vesicles from the endocytic and phagocytic pathways. Microtubules (Mt) and Mt motor proteins such as dynein and kinesin are the key components in vesicular trafficking. We analyzed if Mt, dynein and kinesin are involved in PV formation. We tested the effect of nocodazole (Noc), an inhibitor of Mt polymerization, on PV formation. HeLa cells were incubated by 2 h with Cb, then treated for 46 h with DMSO or Noc, fixed and processed for indirect immunofluorescence. Then, the cells were analyzed by confocal microscopy. Interestingly, Noc inhibited PV formation. Furthermore, we also analyzed the interaction between PV and endocytic compartments in Noc-treated cells. We observed a decrease in the colocalization between Cb and dextran-texas red (endocytic marker) and also between Cb and CD63 (lysosomal marker). Then, we analyzed the effect of EHNA, a specific dynein inhibitor, or ATA, a specific kinesin inhibitor. HeLa cells were treated as mention above. EHNA but not ATA inhibited PV formation. Also, like Noc treatment, EHNA diminished the colacalization of Cb with dextran-texas red and with CD63. We could conclude that Mt and dynein are involved in both PV formation and interaction of PV with endocytic compartments.

CB-P26.
JUNÍN VIRUS Z PROTEIN, EXPRESSED IN MAMMALIAN CELLS, IS PACKAGED INTO EXPORTABLE VESICLES

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Junín virus, the etiological agent of Argentine hemorrhagic fever, belongs to the Arenaviridae family. This family has 23 recognized species with an ambisense coding-bipartite ssRNA genome. The enveloped virions include non-equimolar amounts of each genomic RNA, named L (ca. 7200 nt; coding for L and Z proteins), and S (ca. 3500 nt; coding for N and GPC proteins). The Z protein, the matrix protein of the virion, interacts with several proteins in infected cells. The N-terminal region has a conserved G at position 2, which would be myristoilated, and the C-terminal region comprises the late domain, previously described as the main driving force of the budding process.
In this study, we analyzed the Z protein expression and its ability to induce exported vesicle formation. We used a modified Z protein in which the C-terminal aminoacid is fused to GFP protein (Z-GFP), and analyzed its expression and exportation potential in mammalian cells by differential ultracentrifugation followed by western blotting, flow citometry, fluorescent microscopy and immunogold-electron microscopy. Our results showed that Z-GFP protein expression induce virus like particles formation which contains the recombinant protein.

CB-P27.
RAB24 COLOCALIZES WITH CtBP/BARS A PROTEIN INVOLVED IN CELL CYCLE PROGRESSION

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Rab24 is an atypical member of the Rab GTPase family that presents a reticular perinuclear distribution in interphase cells. We have recently shown that during mitosis Rab24 interacts with microtubules and modulates chromosome segregation and cytokinesis. Rab proteins are thought to control different functions through the interaction with specific effector molecules. In a recent publication it has been indicated that Rab24 interacts with CtBP/BARS by a yeast twohybrid assay.The C terminal binding protein (CtBP) family functions in the nucleus as corepressors of transcription and in the cytoplasm as regulators of Golgi fission. Interestingly, depletion of CtBP leads to errors in mitotic chromosome segregation. In the present work we have analyzed the distribution of both proteins and its mutants in transiently transfected cells. In HeLa cells overexpressing CtBPwt, the protein presented a punctate pattern and also we observed aggregates that colocalized with Rab24wt. Similar results were obtained when cells were cotransfected with Rab24 D123I (a dominant negative mutant) suggesting that the nucleotide state of the Rab protein is not critical for association. Preliminary results in our laboratory lead us to hypothesize that Rab24 could be involved in the regulation of mitosis through its association with CtBP.

CB-P28.
AUTOPHAGOSOME FORMATION DEPENDS ON THE SMALL GTPASE RAB1 AND FUNCTIONAL ER EXIT SITES

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Autophagy is an important cellular component degradation pathway present in all eukaryotic cells. Autophagy sequesters portions of cytoplasm and/or organelles in emerging double membrane structures call autophagosomes. In spite of the intense advance achieved in the autophagy´s knowledge little information exists with regard to the formation of the autophagosomes in the mammalian cells. The present work is based on the investigation of the secretory pathway, principally on its early stage, as key route involved in the autophagosome formation. Sar1 and Rab1b are monomeric GTPases that control the anterograde traffic from the Endoplasmic Reticulum to the Golgi apparatus. The analysis of autophagic pathway was realized by the monitoring of LC3, a well known marker of autophagosome, by confocal microscopy and western blot. We demonstrate for the first time the requirement of the Sar1 and Rab1b for the autophagosome formation in mammalian cells and determine that the autophagy and secretory pathway cross at level of the stage controlled for Rab1b. Also by means of the blockage of the action of Arf1, we verify that the transport from the cis/medial Golgi is not necessary for the formation of the autofagosomas.

CB-P29.
THE RAB11 INTERACTING PROTEIN FIP-2 IS RECRUITED TO *Chlamydia trachomatis* INCLUSION

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Chlamydia trachomatis is an obligate intracellular bacterium that replicates in an not acidic vacuole called inclusion. Rab GTPases are the molecules in charge of intracellular traffic control. Rab11, a Rab present on immature phagosomes, is recruited towards the inclusion of *Chlamydia*. A Rab11-Family of interacting Proteins known as FIPs has recently been cloned. Rab11-FIP2 has a RBD (Rab11/25 Binding Domain) domain in its C-terminus which interacts with Rab11 and a C2 domain in its N-terminus that binds phospholipids. We are interested on unravel Rab11-FIP2 involvement in *Chlamydia trachomatis* inclusion biogenesis and development. We examined by confocal microscopy the intracellular localization and function of this interacting protein and its mutants: Rab11-FIP2ΔC2 and Rab11-FIP2ΔC2ΔRBD fused to EGFP in Hela cells infected with *C. trachomatis* biovar LGV. We observed that this protein strongly decorates *Chlamydia* inclusion, whereas Rab11-FIP2ΔC2ΔBD, the mutant that lacks the RBD domain, is not found associated to it. FIP2WT recruitment occurs at early stages of inclusion development, and depends on bacterial protein synthesis. These data suggest that *Chlamydia trachomatis* selectively interferes with critical components of the trafficking machinery of the host cell in order to generate an intracellular niche favorable for its surviving and development.

CB-P30.
***Giardia lamblia* AND RETROMER: TWO EVOLUTIONARY ANCESTORS**

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Retromer is an evolutionary conserved protein complex required for endosome-to-Golgi retrieval of lysosomal hydrolases receptors. These complex is compound by five subunits, SNX1 and SNX2 (Sorting Nexins SNX), Vps35, Vps29, and Vps26 (Vps, Vacuolar protein sorting). By searching the *Giardia*DB, we found genes that codified to the subcomplex gVps35, gVps29, and gVps26 but not SNX1 or SNX2. Analysis of the sequence and structure of these proteins showed high similarities with the ones described in yeast and mammalian cells. However, because *Giardia* does not contain a recognized Golgi apparatus or a typical endosome/lysosome system, it is possible that this complex shows many particularities. Over-expression of fusion proteins in transfected cells showed that gVps35 localized around of the nucleus and in the lysosomal-like peripheral vacuoles (PVs), while gVps26 localized mostly in the PVs. Besides, by yeast-two hybrid assays, we found that gVps35 directly binds to gVps29 and the *Giardia* hydrolase receptor (gHR). Disclosing whether or not this complex plays a critical function in protein recycling and cell viability will contribute to understanding how to control the cell cycle and to the understanding of how the endosomal/lysosomal pathway has evolved during the eukaryotic evolution.

CB-P31.
PROTEIN SYNTHESIS STIMULATED BY THYROTROPIN IN A SECRETORY SYSTEM REQUIRES RAB1B FUNCTION

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The GTPase Rab1b is essential for membrane transport from the Endoplasmic Reticulum (ER) to the Golgi complex. Although Rab1b is ubiquitously expressed, its mRNA levels are increased in some tissues with high secretory activity. Here, a thyroid rat cell line (FRTL5) was used in order to study the role of Rab1b in a secretory system. In FRTL5 cells, the thyroid-stimulating hormone (TSH or thyrotropin), stimulates synthesis and secretion of the plasma membrane protein sodium iodide symporter (NIS) as well as thyroglobulin (TG). We have previously shown (SAIB 2008) that TSH also increased the expression of Rab1b and other Golgi proteins but the role of Rab1b was not analyzed. In this work, we inhibited Rab1b activity in FRTL5 cells by overexpressing a dominant negative Rab1b mutant (Rab1b N121I) and by knocking-down endogenous Rab1b expression levels using small interfering RNA (siRNA). Our results show that there was a significant reduction of NIS and TG synthesis in Rab1b inhibited cells compared with control cells. Moreover, ER to Golgi transport was also inhibited by Brefeldin A (BFA) addition. Interestingly, BFA did not modify NIS levels induced by TSH. Taken together our results indicate that TSH mediated synthesis of NIS in FRTL5 cells requires a Rab1b activity not related to ER to Golgi transport, suggesting a differential Rab1b role in secretory tissues.

CB-P32.
THE CIRCADIAN DEADENYLASE NOCTURNIN INTERACTS WITH A NOVEL FORM OF THE RNA-BINDING PROTEIN KSRP

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Mouse nocturnin (mNOC) is a circadian regulated deadenylase. mRNA deadenylation induces transcript degradation or translational silencing. In order to identify mNOC binding partners, we performed a large-scale immunoprecipitation (IP) screen. The mass spectroscopy analysis of co-IP proteins revealed KSRP (KH homology splicing regulatory protein) as a potential mNOC binding protein. KSRP is an AU-rich element (ARE) containing mRNA binding protein that regulates mRNA turnover. We verified this interaction by co-IP. We utilized three anti-KSRP antibodies, two of them detected two KSRP forms with apparent molecular weigh of 75 and 60 kDa, whereas the other one (monoclonal), only the 75 kDa form. mNOC co-IP and co-fractionate in gel filtration with the 60 kDa form. Since KSRP is also involved in nuclear splicing, we studied the mNOC and KSRP60 subcellular localization to determine where this interaction takes place. ICC and biochemical fractionation showed that both proteins co-localize in cytoplasm, whereas KSRP75 is in the nucleus. We confirmed the identity of KSRP60 by repeating the mass spectroscopy analysis.
Our results suggest that NOC may play a role in regulating ARE-mRNA metabolism. Our current working hypothesis is that mNOC is recruited to ARE-mRNAs by KSRP60, which results in the deadenylation of these messages, and consequently, their degradation or translational silencing.

CB-P33.
CIRCADIAN CLOCK EXPRESSION IS MODIFIED IN THE LIVER OF VITAMIN A-DEFICIENT RATS
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Mammalian liver has its own cellular clock machinery which controls and synchronizes circadian gene expression. Previously, we showed RARα, RXRα and RXRα expression display a daily rhythmicity in the rat liver. Our objective was to investigate whether vitamin A deficiency modifies circadian expression of clock genes in the rat liver, by modifying retinoid receptors daily oscillation. Holtzman rats weaned at 21 d of age were assigned to either the experimental diet, devoid of vitamin A (vitamin A-deficient group) or the same diet containing 4000 IU of vitamin A/Kg diet (control group) during 3 months. Liver samples were taken every 4 h. Total RNA was extracted using the Trizol reagent. Transcript levels of Bmal1, Per1, Cry1 and RevErbβ were determined by RT-PCR. Protein levels were analyzed by immunoblotting. Regulatory regions of clock genes were scanned for retinoid receptors binding sites using the MatInspector software (www.genomatix.de). We observed daily rhythmicity of clock -gene and protein- expression were differentially modified in the liver of vitamin A-deficient rats, probably, as a consequence of temporal changes in RAR and RXR expression. Thus, vitamin A might be essential to establish circadian clock expression in the liver, a peripheral clock with a relevant function in daily metabolism.
This work has been supported by NIH Grant R01-TW006974 funded by the FIC, USA.

CB-P34.
DIFFERENT MOLECULAR MECHANISMS CONTRIBUTE TO THE REGULATION OF HUMAN DISC LARGE PROTEIN EXPRESSION
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Human Disc large (DLG1) has been demonstrated to be involved in the control of cell polarity and maintenance of tissue architecture, and to be lost in human tumors. However, the mechanisms controlling DLG1 expression are not fully understood. We previously performed the cloning and functional analysis of DLG1 promoter region. Using RACE techniques, we identified an alternative splicing event in the 5' region of DLG1 mRNA that generates transcripts with two different 5'-UTRs. We showed, by reporter assays, that the DLG1 5'-UTR containing the alternatively-spliced exon interferes with the translation of a downstream ORF, suggesting that this splicing event can contribute to down-regulation of DLG1. We are analyzing the contribution of an ATG codon present in the extra exon, in reducing translation efficiency, by mutagenesis and luciferase assays. By real time RT-PCR and Actinomicyn D treatment we could determine that the short version of DLG1 5'UTR makes the transcript more stable. Moreover, bioinformatic analysis of DLG1 promoter revealed the presence of two CpGrich regions compatible with CpG islands. We observed an increase of DLG1 expression by immunoblotting and immunofluorescence in low-level DLG1-expressing cells when treated with the methylase inhibitor 5-aza-2'-deoxycytidine. These results might indicate that epigenetic mechanisms may also play a role in DLG1 regulation.

CB-P35.
PRE-IMPLANTATION EMBRYOS AFFECT LEFTY2 GENE EXPRESSION IN THE RAT OVIDUCT
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Accumulating data suggested that embryo-maternal communication exists during preimplantation period within the mammalian oviduct, which provide the optimal microenvironment for embryo development. Signals produced by the pre-implantation embryos affect their transport in the oviduct, and the oviductal physiology and gene expression patterns. Previously, we have found increased levels of *lefty2,* a novel member of TGF-β family, at the 4th day of pregnancy, when the embryos are finishing their passage through the oviduct. In order to distinguish whether the embryo presence or the hormonal levels induce *lefty2* rise in the oviduct, we studied *lefty2* gene expression by using a unilaterally pregnant rat model. This procedure enables us to obtain one oviduct with embryos and other without them. By semiquantitative RT-PCR assays we found that lefty2 mRNA level was significantly higher in the pregnant oviduct containing embryos when compared with the contralateral empty tube. These data suggest that the embryo might participate regulating *lefty2* expression in the rat oviduct during the pre-implantation period of pregnancy. The study strongly suggests that *lefty2* might be an important mediator for preimplantation embryo-maternal dialogue. Thefore, the potential application of LEFTY2 in the culture of embryos deserves further exploration.

CB-P36.
GENE EXPRESSION PATTERN OF BONE MORPHOGENETIC PROTEINS IN THE BOVINE OVIDUCT
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Bone morphogenetic proteins (BMPs) regulate the morphogenesis of tissues and organs during the embryonic development. However, the presence of these factors in the oviduct, where preimplantation events take place, is unknown. In this study we demonstrate the expression of BMPs and some of their signal transduction components in the bovine oviduct. RT-PCR was used for verifying the expression of BMP2,3,4,5,6,7,10,12,13,15; the receptors ALK2, ACTRIIA and SMAD1, one of their signaling pathway molecule, in oviductal samples of adult cows during the preovulatory stage. Total RNA was extracted from: complete oviducts, portions of ampulla and isthmus, oviductal epithelial cells (OEC) freshly obtained from both regions and OEC cultured *in vitro*. Our results showed the expression of BMP2,3,4,5,7,15, ALK2, ACTRIIA and SMAD1 in the complete oviduct. This expression pattern was conserved in ampulla and isthmus, with the exception of BMP5 that was absent in the ampulla region. Moreover, all these factors were highly expressed in freshly obtained OEC from both anatomical regions. In adittion, under *in vitro* culture conditions, OEC conserved a similar expression profile. Considering that BMP system is present in the oviduct epithelium we suggest that BMPs could be active as autocrine/paracrine regulators of the early development events in the oviductal environment.

CB-P37.
HISTONE DEACETYLATION AND DNA METHYLATION INHIBITORS ACTIVATE PSG GENE EXPRESSION
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The human pregnancy-specific glycoprotein (PSG) gene family is composed by 11 highly similar members whose expression constitutes an early biochemical marker of trophoblast differentiation. They are transcriptionally up-regulated during this process and individual PSG members reach different expression levels. Herein, we study the contribution of epigenetic regulation to PSG expression through histone acetylation and DNA methylation. For this purpose, primary cytotrophoblasts, choriocarcinoma JEG3 and cervix carcinoma HeLa cell lines were treated with histone deacetylase inhibitors, Trichostatin A (TSA) and sodium butyrate (NaBu), and an hypomethylating drug, 5-Aza-Deoxycytidine (aza). Both, PSG mRNA and protein levels were dose-dependently induced, as revealed by immunofluorescence and qRT-PCR assays. TSA and NaBu treatment of JEG3 cells transiently transfected with PSG3 promoter constructs enhanced reporter activity. PSG3 transcriptional activation was observed even with the shortest promoter construct bearing a 130 bp promoter sequence overlapping the gene transcription start site. Our results indicate that epigenetic mechanisms are involved in PSG gene regulation, and suggest that the proximal promoter region is recognized by transcription factors which can mediate histone modifications.
Supported by CONICET, FONCyT, MinCyT of Córdoba and SECyT UNC.

CB-P38.
NUCLEAR MORPHOLOGY AND GENE EXPRESSION ARE ALTERED IN HUMAN TROPHOBLAST CELLS BY ORGANOPHOSPHATES
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Gene expression and cellular differentiation are tightly controlled by genetic and epigenetic mechanisms which may be disrupted by toxic compounds. Epidemiological data suggest an association between chronic organophosphate (OP) exposure of pregnant women and an increased risk of spontaneous abortion, low weight offspring and intrauterine growth restriction. To address the molecular mechanism(s) underling these effects, we hypothesized that OPs exposition alter placenta cell morphology and gene expression. Human trophoblast derived JEG3 cell line was used as an in vitro model to test this hypothesis. In an initial approach, cellular viability was assayed to select chlorpyrifos, phosmet and methylazinphos concentrations for further experiments. Cell number with fragmented and/or condensed nuclei increased with OP concentration (50 and 100µM) and incubation time (24 and 48h) instead, no major effect was observed on spontaneous cell fusion. RT-PCR analysis revealed that chlorpyrifos induced a reduction in GCM1 and KLF6 transcription factors and an increase in StarD7 expression. In addition, immunofluorescent StarD7 protein staining was augmented in the presence of OPs, especially in cells with condensed nuclei. These data suggest that the OPs used in these experiments impact on placental cell morphology and function.
Supported by SACyT, CONICET, FONCyT, MinCyT of Córdoba and SECyT-UNC.

CB-P39.
siRNAS AND microRNAs MEDIATED TRANSCRIPTIONAL GENE SILENCING REGULATION OF ALTERNATIVE SPLICING
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When targeting promoter regions, small interfering RNAs (siRNAs) trigger a previously proposed pathway known as transcriptional gene silencing (TGS) by promoting heterochromatin formation. We have seen that siRNAs targeting intronic or exonic sequences close to an alternative exon could regulate its alternative splicing (AS). The effect depends on AGO proteins (TGS key players) and is counterbalanced by factors favoring chromatin opening or transcriptional elongation. Moreover, we have shown the increase of epigenetic heterochromatin marks (H3K9me2 and H3k27me3) and the reduction of RNA polymerase II processivity at the target site. These results suggest a mechanism involving the kinetic coupling of transcription and AS.
On the other hand, it has been shown that microRNAs could trigger TGS. Taking into account that we do not have any physiological example for this process we wonder whether this mechanism could operate endogenously in the cell by means of microRNAs targeting intronic regions near an AS event. In this context, we are cloning microRNAs target sequences in the intron downstream of the alternative exon EDI of the FN1 minigene. These microRNAs are known to be expressed or not in HeLa cells and we are also cloning the sequences in the 3´UTR of a luciferase reporter gene as a control. After transfection of HeLa cells with these constructs we will determine variation on AS.

CB-P40.
ALTERNATIVE SPLICING AND CHROMATIN STRUCTURE IN NEURONAL DIFFERENTIATION
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Changes in RNA polymerase II elongation rates modulate alternative splicing choices, affecting the timing at which nascent pre-mRNA splice sites and regulatory sequences are presented to the spliceosome. This situation could be relevant in regulation of endogenous alternative splicing choices by dynamic changes in intragenic chromatin structure that could affect RNA processivity or elongation rate. Using the exon 18 (E18) of the NCAM gene as a model, we have determined that intragenic histone acetylation and chromatin relaxation, triggered by membrane depolarization of N2a cells, decreases exon inclusion. An opposite effect occurs during neuronal differentiation, which favors the expression of the NCAM 180 isoform, typical of mature and stable synapses. We study this regulation using DMSO-induced differentiation of N2a cells and retinoic acid-induced differentiation of HN9 cells, two murine neuronal cell lines. Treatment of differentiated N2a cells with a DNA methylation inhibitor reverts the effect of differentiation on E18 inclusion, suggesting that an epigenetic component could be also involved in this process. We are currently analyzing the changes in chromatin structure along the NCAM gene and trying to determine their participation in NCAM E18 alternative splicing, in what could be an example of chromatin-dependent alternative splicing modulation associated with development.

CB-P41.
REGULATION OF HYPOTHALAMIC EXPRESSION OF THE *PROOPIOMELANOCORTIN* GENE BY NUCLEAR RECEPTOR FACTORS

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The *proopiomelanocortin* (*POMC*) gene encodes a prohormone expressed mainly in the pituitary and the arcuate nucleus of the hypothalamus. POMC-derived peptides play important roles in responding to stress and controlling food intake and energy balance. We have identified two enhancers - named nPE1 and nPE2 - that control *POMC* expression in the mammalian hypothalamus. To identify transcription factors that might control *POMC* expression, we performed an one-hybrid screening with nPE2 as bait and identified factors of the nuclear receptor superfamily that can bind to an element in nPE2 that follows the consensus binding site for these factors. In addition, this element is conserved in all mammals and is present within a crucial region for nPE2 enhancer activity. Since the factors we identified by one-hybrid screening are not co-expressed with *POMC* in the brain, we sought to find other factors based on the literature. One candidate is oestrogen receptor α (ERα) which is known to be involved in the control of *POMC* expression and energy balance, and is widely expressed in the brain. By immunohistochemistry using a transgenic mouse model, we found that around 30% of POMC-EGFP neurons co-express ERα, indicating that this transcription factor is a strong candidate for regulating the expression of the *POMC* gene. Further functional studies are needed to corroborate this hypothesis.

CB-P42.
UNCOVERING CIS AND TRANS-ACTING CODE REGULATING *PROOPIOMELANOCORTIN* TRANSCRIPTION IN THE BRAIN

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The *proopiomelanocortin* gene (*POMC*) encodes a prohormone that is expressed mainly in the pituitary and the arcuate nucleus of the hypothalamus, playing a central role in the stress response and energy balance, respectively. We have showed that hypothalamic expression of *POMC* is driven by two upstream enhancers, nPE1 and nPE2. To identify the critical secuences of nPE1 (630 bp) we performed a bioinformatic analysis coupled with an expression study in transgenic mice. Using this strategy we unraveled a functionally critical 144 bp central region within nPE1 that is necessary and sufficient to drive reporter gene expression to *POMC* neurons in transgenic mice. Comparison of this critical nPE1 region with those previously identified in nPE2 evidenced a number of short conserved sequences that are present in both enhancers, suggesting that the transcriptional activity of nPE1 and nPE2 might be conferred by a similar set of homeodomain containing transcription factors (HDTF). Data from the literature and from a UNIPROBE search led us to test the ability of several recombinant HDTF candidates to bind to the enhancers' regions in gel shift assays. Our results represent a step forward to elucidate the transcriptional code of *POMC* in the brain and the understanding of the overlapping functions of nPE1 and nPE2.

CB-P43.
FUNCTIONAL COMPARATIVE GENOMICS AT THE VERTEBRATE EXTREMES

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Until recently, the identification of transcriptional enhancers has relied on tedious deletional studies of non coding flanking regions systematically tested in expression assays. The near completion of several vertebrate genomes has dynamized this task by revealing a myriad of conserved potential cis-acting elements such as the neuron-specific enhancers of the proopiomelanocortin gene (*Pomc*), nPE1 and nPE2, that we discovered to be conserved across mammals. To study the molecular evolution of Pomc expression we analyzed the ability of putative enhancer sequences to drive reporter gene expression to transgenic animals of distant vertebrate Orders: the mammal *Mus musculus* (mouse) and the teleost fish *Danio rerio* (zebrafish). To this end, we set up a transgenic zebrafish facility and determined that mouse nPE1 and nPE2 were able to drive EGFP expression to the hypothalamus of transgenic zebrafish embryos. Using the tol2 transposon system, we injected zebrafish eggs with ptol2-nPE1-nPE2-zpomca-egfp and ptol2ΔnPE1-nPE2-zpomca-egfp. Interestingly, both constructs drove EGFP expression to the pituitary and hypothalamus of zebrafish embryos and EGFP colocalized within Pomc neurons as determined by immunofluorescence against ACTH. These results provide a clear example of functional conservation at the vertebrate extremes despite great sequence divergence.

CB-P44.
FUNCTIONAL ANALYSIS OF MATERNAL AND ZYGOTIC CNBP TRANSCRIPTS DURING ZEBRAFISH DEVELOPMENT

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Cellular nucleic acid binding protein (CNBP) is a strikingly conserved protein required for forebrain development. Its depletion causes forebrain truncation due to alterations in craniofacial structures and neural crest derivatives. cnbp-mRNAs are maternally inherited and ubiquitously distributed in the embryo until mid-blastula translation stage (MBT). Afterwards, cnbp expression becomes localized in anterior-most regions of the embryo. We wondered if maternal and zygotic cnbp-mRNAs display differential transcription control. By 5'RACE, RT-PCR and primer extension experiments we found a novel exon sequence located 5' upstream of exon1. We demonstrated the existence of two transcription start sites that generate two transcripts that differ in their 5'UTRs but code for the same protein. Both transcripts were maternally inherited; however, one of them increased beyond MBT whereas the other decreased along embryonic development. Two modified antisense oligonucleotides were designed to specifically knockdown the expression of each transcript. The phenotype comparison lead to figure out the possible differential roles for both cnbp transcripts during zebrafish embryonic development.

CB-P45.
FINDING MOLECULAR TARGETS FOR THE NUCLEIC ACID CHAPERONE CNBP
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Cellular nucleic acid binding protein (CNBP) is a conserved single-stranded nucleic acid binding protein required for vertebrates craniofacial development. CNBP acts as a nucleic acid chaperone, rearranging the secondary structure of its targets. Recently, our group demonstrated that CNBP promotes the formation of G-quadruplexes, stable nucleic acid structures described as novel elements for gene expression regulation. However, the consensus sequence of CNBP targets has not been elucidated yet. The main goal of this work was to gain knowledge about the common features among CNBP molecular targets in order to identify putative genes regulated by this protein. Therefore, we performed a "Systematic Evolution of Ligands by Exponential Enrichment" (SELEX) using zebrafish CNBP and a single-stranded nucleic acid library degenerated in 16 nucleotides. Sequences obtained with this procedure have been analyzed and compared with previous reported targets in order to establish a consensus sequence. On the other hand, we performed a genome-wide screening for zebrafish CNBP binding sites using "inverse monohybrid" strategy on a genomic zebrafish library cloned in yeast. The screening provided the identity of putative target genes that may be controlled by CNBP. These results will help to better understand how CNBP performs its essential biological function in embryonic development.

CB-P46.
ROLE OF CHROMATIN ON THE EXPRESSION AND ALTERNATIVE SPLICING OF THE HUMAN BCL-X GENE
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Progestins stimulate proliferation and inhibit apoptosis of T47D human breast cancer cell line. We found that the progestin R5020 enhances bcl-X expression and increase the ratio Bcl-XL (antiapoptotic)/Bcl-XS (proapoptotic) isoforms. By deep sequencing analysis (Illumina Genome Solexa) five PR binding sites located in intron regions of this gene were identified. Interestingly two of these sites are placed near the 5´alternative splicing (AS) donor site. This finding prompt us to hypothesize that activated PR could locally affects transcription-AS regulation, being the chromatin state a possible link for coupling these processes. Treatment of T47D cells with the histone deacetylase inhibitor (TSA) or the G9a inhibitor (BIX01294) completely inhibited both, the hormone-dependent induction of the bcl-X gene and the change in the Bcl-XL/Bcl-XS ratio supporting a role for the chromatin state on PR regulation of this gene. We found, by chromatin immunoprecipitation assays (ChIPs), that PR binding is accompanied by an increase in different epigenetics marks associated with active transcription such as H3K4me3, H4K8ac and H3K14ac. Moreover, differences among the PR binding sites were observed in the kinetics of PR recruitment and in the appearance of the epigenetics marks, suggesting a role for Pol II elongation rate during these events.

CB-P47.
GENE EXPRESSION PROFILE IN STARD7 siRNA-TRANSFECTED JEG-3 CELLS
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StarD7 protein is a member of the StarD2 subfamily of START domain proteins, whose function remains poorly defined. We have recently reported that StarD7 is expressed in trophoblast cytoplasm and is partially re-localized in in vitro differentiating cytotrophoblast cells. Furthermore we described that ß-catenin activates human StarD7 through Wnt/ß-catenin signalling. Herein, we used StarD7 siRNA to block down its expression in JEG-3 cells to pursue its function. Labeled complementary ribonucleic acid from StarD7 siRNA- and scrambled siRNA-transfected JEG-3 cells was hybridized to a custom Affymetrix oligonucleotide DNA microarray. Identification of altered gene expression in both conditions was assessed by using the Significance score (S-score) method. The data demonstrated differential expression of 89 genes (46 increased, 43 decreased). Among them, several Wnt signalling pathway-associated genes were identified. The mRNA levels of six differentially expressed genes (ß-catenin, Cnx43, iNOS, MBD2, RII) were validated using qRT-PCR. From this genome-wideSmurf2 and TGF siRNA study, novel aspects of the molecular background of StarD7 were revealed, and thus may lead to the identification of candidate StarD7-dependent genes probably related to its cell function.
Supported by SACyT, MinCyT of Córdoba, CONICET, FONCyT and SECyT-UNC.

CB-P48.
STARD7 IS A FUSOGENIC PROTEIN THAT BINDS CARDIOLIPIN AND PHOSPHATIDYLSERINE
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StarD7 is a novel protein of unknown function that belongs to the START lipid domain family. It was previously found that StarD7 is able to interact in lipid monolayers with phosphatidylserine (PS). Moreover, StarD7 is partially relocated from the cytoplasm to the plasma membrane during in vitro cytotrophoblast differentiation into syncytiotrophoblast (STB), a process characterized by an enrichment of PS into the STB surface. This study further investigates the function of StarD7 in trophoblasts. Here we demonstrate an inhibition of BeWo cell proliferation by incubation with recombinant StarD7 at 5, 10 and 20 µg/ml. Fluorescent microscopy indicated that there were few intercellular desmosomes between adjacent BeWo cells after treatment with StarD7 at 20 µg/ml. Similar results were found in control cultures treated with 5 µM forskolin. In addition, a protein-lipid overlay assay was performed by an ELISA method using cardiolipin (CL), PS, cholesterol (Chol), ceramide (Cer) and phosphatidylinosytol (PI). The results indicate that StarD7 binds CL and PS, but no PI, Chol or Cer. Altogether, these findings and previous data indicate that StarD7 is a fusogenic protein leading us to conclude that StarD7 can initiate/facilitate the syncytialization of BeWo cells through transport of PS to the STB membrane surface.
Supported by SACyT, MinCyT of Córdoba, CONICET, FONCyT and SECyT-UNC.

CB-P49.
HYPOTHYROIDISM MODIFIES THE EXPRESSION OF GENES RELATED TO OXIDATIVE STRESS IN RAT HEART

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Different studies on the effects of thyroid status on oxidative stress in heart have produced conflicting results. In this work the effects of hypothyroidism on gene expressions of antioxidant enzymes and Nrf2, a redoxsensitive transcription factor, were determined in heart ventricles of female virgin Wistar rats. Also, the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and the TBARs and Carbonil groups levels in heart were measured. The hypothyroid state was induced in rats weighing 150180 g by administration of 6npropyl 2thiouracil (100mg/L) in the drinking water for 30 days. Age-matched euthyroid rats were used as controls. The mRNA expressions were determined by RTPCR, using β-actin as internal control, and the enzyme activities by spectrophotometric assays. Heart mRNA levels of NADPH oxidase2 (NOX2) and its modulate factor NRF2 increased ($P< 0.002$ and $P<0.03$, respectively). The mRNA expressions of SOD increased ($P<0.005$), CAT decreased ($P<0.02$) and GPx was unchanged, compared with their respective controls. The activity of GPx increased ($P< 0.04$), while that of CAT and SOD was unchanged in relation to control. No difference in TBARs and Carbonil levels was observed. The results suggest that hypothyroidism induces a prooxidant state at the gene levels in heart ventricles.

CB-P50.
IDENTIFICATION OF P19INK4D PHOSPHORYLATION SITES INVOLVED IN DNA REPAIR FUNCTION

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We have reported that p19INK4d (p19), a member of INK4 cell cycle inhibitors, has a role in DNA repair and becomes phosphorylated upon DNA damage. We identified putative phosphorylation sites replacing serine or threonine by alanine in predicted sites. We found two mutations that abrogates p19 DNA repair capacity. In this work, we aimed to study first the phosphorylation ability of other three p19INK4d mutants and second to test if the replacement of serine or threonine by glutamic acid, in those mutants that lose the DNA repair function, can mimic the phosphorylation event and recall DNA repair ability. p19 mutants were made replacing serine or threonine by alanine in predicted sites. To evaluate in vivo phosphorylation we performed metabolic labeling with 32P. We found that none of the mutants containing alanine diminishes p19 phosphorylation level concluding that this sites aren´t phosphorylated upon DNA damage. As for the mutant bearing glutamic acid in both sites linked to DNA repair, the phosphorylation event was entirely supressed. However, this mutant didn´t lose its DNA repair ability as measured by UDS. We conclude that glutamic acid mimics the phosphorylation state of p19 after DNA damage, that these are the only two phosphorylation sites upon DNA damage and the phosphorylation event is absolutely necessary for exerting an effective DNA repair function.

CB-P51.
EGR-1 INDUCES P19 EXPRESSION IN NEURAL TYPE CELLS

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Neuronal terminal differentiation is directly linked to cell cycle exit. Previous data suggests that two CDK inhibitors, p19INK4d and p27Kip1, are responsible for neural postmitotic arrest. Why both proteins show early expression during the central nervous system development and their levels remain high even in adulthood is still to be determined. The aim of this work is to study the mechanisms that are implicated on maintaining the high levels of p19 in terminal differentiated neurons. For this, SH-SY5Y and HN9 cells were treated or not with retinoic acid to induce differentiation, and p19 mRNA stability was analyzed by northern blot after treatment with actinomycin D. This experiment showed that p19 half-life on differentiated cells doubles that of cells in G1/S. Moreover, we observed that EGR-1, a transcription factor involved in the neuronal differentiation process, causes a 2.5-fold induction on p19 levels. Of note, several potential EGR-1 binding sites were found on the p19 promoter, suggesting a direct effect. Our results indicate that an increased transcription of p19, mediated by EGR-1, added to an enhanced mRNA stability, could contribute to the elevated expression levels of p19 in postmitotic neurons.

CB-P52.
TRANSCRIPTIONAL REGULATION OF E2F FACTORS IN DNA DAMAGE RESPONSE

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Following genotoxic stress, the E2F1 transcription factor has been shown to undergo post-translational modifications that correlate with a stabilization and increase in its protein levels. The aim of this work is to examine whether this increase in protein levels is only due to its gained stability or is also a result of enhanced transcription of the E2F1 gene. This study also includes the other family members, E2F2 to E2F5.
We subjected HEK293 and HN9 cells to genotoxic stress -such as UV, H_2O_2 and neocarzinostatin- and allowed them to trigger a DNA repair response and recover for different time periods. Northern Blot analyses were carried out using E2F1 to E2F5 probes. Quantifications revealed that E2F1 mRNA levels were induced between 4 to 8 hours following genotoxic exposition in both cell lines for all types of stress, reaching a 3 to 4.5-fold increase in HEK293 and a 2 to 3.5-fold increase in HN9 cells. Surprisingly, E2F2 transcription was enhanced 2.5 to 4-fold from 4 to 12 hours of recovery only in neuronal HN9 cells after every stress. E2F3, E2F4 and E2F5 mRNA levels remained unchanged. Preliminary results suggest that MAPK and ATM/ATR pathways are involved in this response.
Our results reveal a new mechanism in which E2F1 and E2F2 respond to genotoxic stress by increasing their transcript levels.

CB-P53.
SUB-NUCLEAR IRRADIATION, *IN-VIVO* MICROSCOPY AND SINGLE-MOLECULE IMAGING TO STUDY A DNA POLYMERASE

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When the DNA is damaged in cells progressing through S phase, replication blockage can be avoided by TLS (Translesion DNA synthesis). This is an auxiliary replication mechanism that relies on the function of specialized polymerases that accomplish DNA damage bypass. An example of a classical TLS polymerase is Pol η(eta). The current model implies that Pol η activity is circumscribed to S-phase. Here we perform a systematic characterization of Pol η behaviour after DNA-damage. We show that Pol η is recruited to UV-induced DNA lesions in cells outside S phase including cells permanently arrested in G1. This observation was confirmed by different sub-nuclear damage strategies including global UV irradiation, local UV irradiation and local multi-photon laser irradiation of single nuclei in living cells. By local UV irradiation and alpha particle irradiation we evaluated the potential connection between Pol η recruitment to DNA lesions outside S phase and Homologous recombination repair (HRR) or Nucleotide excision repair (NER). Finally, we employ a single-molecule imaging approach (known as DNA fiber-assay) to determine how Pol η influences the progression of the replication fork. Our data reveals that the re-localization of Pol η to DNA lesions might be temporally and mechanistically uncoupled from replicative DNA synthesis and from DNA damage processing.

CB-P54.
MODULATION OF THE ANDROGENETIC RESPONSE IN DIVERSE SKIN CELL TYPES: THE PILOSEBACEOUS UNIT

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Androgens play a central role in diverse morphogenetic processes of the skin. Hair growth and follicular cycle are regulated in part by androgens. Androgens also play a key function, together with other receptors such as the PPARs receptors family, on the proliferation and differentiation of the sebaceous gland that forms part of the pilosebaceous unit and influences hair growth and skin wellbeing. UV radiation may affect androgens regulation of skin homeostasis.
Objectives: to study the modulation of androgenetic response related to UV radiation on the pilosebaceous unit, in two skin conditions: androgenetic alopecia and acne, both affecting skin and constituting major concerns for affected individuals.
Methods: primary cultures of cells and established cell lines from the pilosebaceous unit: dermal papillae cells, keratinocytes and sebocytes. Analysis of lipid content, inflammatory response and proliferation of cells under the influence of androgens, PPARs ligands and UVR.
Results: sebocytes primary cultures were obtained from human sebaceous glands. Proliferation and differentiation, as well as the expression of proinflammatory molecules (IL-1, TNF alpha, iNOs) and lipogenic enzymes (FASN) under androgens and UV treatment were assessed. The response to androgens under UV exposure was also analyzed in dermal papillae cells in culture.

CB-P55.
SUBSTRATE RECOGNITION BY ER CHAPERONES *IN VIVO* IS DETERMINED BY THEIR CONFORMATIONAL STATUS

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Protein folding is a complex process highly prone to fall into irreversible errors. Chaperones and folding assisting enzymes are in place to increase the efficiency of this process. Although we know the structural determinants recognized by many chaperones in cell free systems, the conformational pattern recognized *in vivo* remains mostly unknown. The most abundant chaperones in the endoplasmic reticulum of *T. cruzi* are BiP, and calreticulin (CRT, a lectin-chaperone central in the glycoprotein folding quality control system). We show that in vivo these chaperones display a sharp difference regarding the folding status of their substrates. As a model substrate we employed cruzipain (CZ), an abundant lysosome protease. We followed its maturation *in vivo* and its association with different chaperones by using a combination of thiol group modification and co-immunoprecipitation. We found that BiP binds CZ during its first folding stages, when the protein adopts an expanded conformation, while CRT binding occurs during advanced stages, when the protein adopts a compact conformation. This substrate specificity and sequential action ensures the optimal assistance all along the protein folding pathway. In addition, a parasite in which the access to CRT has been abolished displays a much more lasting BiP association, illustrating the competition and cooperation between both folding systems.

CB-P56.
INTRACELLULAR LOCALIZATION OF CALRETICULIN STUDIED USING A BIOLOGICAL APPROACH

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Calreticulin (CRT) is an abundant protein resident of endoplasmic reticulum (ER) that works as a lectin-chaperone and is one of the main intracellular calcium buffers. Although CRT displays an export peptide and an ER retrieval signal, several reports have found it in the cytosol.
By using a biochemical approach we previously reported that the presence of CRT in the cytosolic fraction is regulated by ER calcium levels.
In order to gain further insight into the mechanism of translocation, we used CRT GAL4-P53, a fusion protein that was expressed in mamallian cells (COS-7). GAL4 is a DNA binding domain of the yeast protein while P53 is a classical activator of POL II. We cotransfected these cells with GAL-P53-dependent luciferase reporter plasmid, which has the firefly luciferase gene downstream a promoter and UAS region. When the GAL4 DBD present in the fusion protein binds this region the luciferase gene switches on. Using this system, we were able to indirectly measure the amount of CRT GAL-P53 present in the cytoplasm by measuring the level of luciferase activation. Under conditions in which the ER is depleted of Ca^{2+}, i.e. after a 30 min treatment with thapsigargin which inhibits the ER Ca^{2+} pump, the signal was increased by 10-20%. This alternative approach provided further evidence supporting the modulation of cytoplasmic CRT levels by ER Ca^{2+} concentration.

CB-P57.
ARGINYLATED CALRETICULIN DOES NOT INTERACT WITH INTEGRINS AND AFFECTS CELL ADHESION
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Posttranslational arginylation of proteins consists in the covalent union of an arginine into an acidic amino acid at the NH2-terminus by arginyl-tRNA protein transferase (ATE1). We demonstrated the arginylation of calreticulin (CRT) "*in vitro*" and in living cells. The arginylated form of CRT (R-CRT) was observed in the cytoplasm in contrast to the ER-CRT.
It has been demonstrated that CRT affects cell adhesion by interaction with α-subunits of all integrin receptors. This interaction occurs via the highly conserved KxGFFKR sequence and is therefore hypothesized to play a crucial role in integrin function. In this work, by affinity chromatography "*in vitro*", we show that when CRT is arginylated does not interact with integrins. Moreover, under stress conditions, R-CRT increased and cells had less adhesiveness to substrata compared to control cells. In addition, when ATE1-/- cells were transfected with full length CRT that overexpressed ER-CRT, attachment to substrate was improved. This results lead us to speculate that the increased levels of R-CRT is the responsible for impair adhesiveness. Thus, posttranslational arginylation of CRT seems to regulate its intracellular localization and cytoplasmic function.
Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

CB-P58.
CRT ARGINYLATION AFFECTS THE SENSITIVITY TO TRYPSIN DIGESTION COMPARED TO ER-CRT
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Posttranslational arginylation of proteins consists in the covalent union of arginine in an acidic amino acid at the N-terminus position. We demonstrated that both "in vitro" and "in vivo" assays calreticulin (CRT) is modify by arginylation. Arginylated calreticulin (R-CRT) showed a different subcellular localization that the one described for CRT within the ER. In this work we show that transfected CRT-/- cells with full length CRT containing the ER signal peptide, leads to the appearance of a cytoplasmic pool of R-CRT. Since the site for arginylation of CRT arises from the cleavage of the signal peptide in the ER, the increased substrate in the cytoplasm must be a consequence of CRT retrotranslocation from the ER. We also confirmed that once CRT is in the cytoplasm it is posttranslationally argininated by ATE1.
In previous experiments "in vitro" and in cell culture, was shown that CRT arginylation is inhibited by Ca^{2+}. It has been demonstrated that in the ER CRT adopts a compact conformation and has a protease resistant core identified as the N-terminus region (Corbett, 2006). In this work we show that R-CRT is resistant to trypsin digestion compared to ER-CRT, even in the absence of Ca^{2+}. In conclusion, we propose that CRT arginylation could be affecting the trypsin resistant digestion due to a conformational change.
Supported by SECyT-UNC, CONICET, ANPCyT-PICT.

CB-P59.
ROLE OF TYROSINE KINASE RECEPTOR erbB2 IN HYPERTROPHIC DILATED CARDIOMYOPATHY
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The activity of erbB2 and erbB4 heterodimers is essential for the maintenance of myocardial function. Ventricular specific erbB4 deletion (erbB4 CKO) leads to dilated cardiomyopathy, characterized by the eccentric hypertrophic growth of cardiomyocytes with a 50% reduction in contractility. The erbB2-dependent hypertrophic pathway was revealed to be active in erbB4 CKO hearts as monitored by differential gene expression in DNA microarrays. Therefore, we investigated the relative incidence of erbB2 in the hypertrophic growth of erbB4-deficient myocardium. Towards this aim, the erbB4 CKO mouse was crossed into erbB2 heterozygous mice resulting in a murine model (named, erbB4 CKO; erbB2+/-) with a 50% partial reduction of erbB2 protein. In this setting, we found that the hypertrophic growth reflected as a 1.5 fold increase in of erbB4 CKO was not detected in erbB4 CKO; erbB2+/- mice. In addition, the remarkable expression level of hypertrophy-related genes (ANP, BNP) determined in erbB4 CKO was kept to a 1.5x increase in erbB4 CKO; erbB2+/- myocardium. The overall heart morphology was, to some extent, preserved compared to erbB4 CKO, indicating that the hypertrophic process was attenuated in the partial reduction of erbB2. This study suggests that in the absence of erbB4, the erbB2 may be active in a pathologic growth through EGF receptor and G-protein coupled receptors.

CB-P60.
***Phytomonas*: RELATIONSHIP BETWEEN BIOSYNTHETIC PATHWAY, POLYAMINE CONTENTS AND TRANSPORT OF PRECURSORS**
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Polyamines (putrescine and spermidine) are essential for cell survival and proliferation. Putrescine can be synthesized: a)-from ornithine (orn) by ornithine decarboxylase (ODC); or b)-from arginine (arg), indirectly, by arginine decarboxylase (ADC) or by arginase. Spermidine is formed from putrescine via spermidine synthase. *In vivo* assays with Phytomonas Jma 066, a plant parasite, have shown that both arg and orn are precursors of putrescine synthesis.However, arg was only converted into orn, indicating the presence of arginase activity, the absence of ADC and that polyamines are synthesized via ODC in Phytomonas. The ODC activity was dependant on the growth phase, being 6.4 and $0.7 nmol.h^{-1}.mg^{-1}$prot at early log and late phases, respectively. At stationary phase endogenous Spermidine, detected by HPLC, and growth rates were both reduced. Arg and orn were taken up at a rate of 7.6 and $0.84 pmol.min^{-1}.10^{-7}$cells respectively. Arg competed with orn uptake but the latter did not affect the arg transport.This suggests that they share a transporter with a higher affinity for arg. Lysine was also decarboxylated by ODC (at a rate 100 times lower than orn) and was taken up at 6 $pmol.min^{-1}.10^{-7}$cells showing no competence by the orn uptake. This work confirms that levels of polyamines and transport of precursors, as well as ODC activity are involved in cell proliferation of Phytomonas Jma 066.

CB-P61.
ANDROGENS ALTER DERMAL PAPILLA SECRETED FACTORS INVOLVED IN HAIR FOLLICLE STEM CELL DIFFERENTIATION

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Hair follicle (HF) formation begins when signals from the mesenchyme-derived dermal papilla (DP) reach multipotent epidermal stem cells in the bulge region. The growth of human HF from beard, axillas and scalp is susceptible to androgen action by modulation of DP activity. In androgenetic alopecia (AGA) androgen action causes HF miniaturization and baldness through a mechanism(s) which remain unclear.
Circulating androgens act on DP cells probably by altering the regulatory paracrine factors involved in the differentiation and proliferation of the HF multipotent cells. Bidimensional gels performed with proteins from conditioned culture media of DP cells revealed differences in the profile of DP secreted protein in response to androgen action. In order to evaluate the role of epithelial-mesenchymal interactions in HF cell differentiation, we established a co-culture model with DP cells from human biopsies of patients suffering AGA and HF stem cell line (Tel E6/E7). The expression levels of K6hf, keratin used as hair differentiation marker, were lower in Tel E6/E7 co-cultured with androgen-treated DP cells, both by western blot and real time PCR.
We conclude that androgens, modulating the DP secretory activity, would alter the expression of specific genes involved in normal HF stem cell differentiation, de-regulating hair follicle regeneration and inducing miniaturization.

CB-P62.
DEHYDROLEUCODINE INHIBITS MIGRATION OF HeLa CELLS

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It is important to identify chemical compounds with cell antimigratory activity to control tumor metastasis. The present study was designed to test the effect of a sesquiterpene lactone, dehydroleucodine (DhL), on the migration of HeLa cells in culture. Before use, DhL solution was freshly prepared by diluting with DMEM F12 medium a stock solution of 0.2 M DhL in DMSO. For wound healing assay, cells were grown to confluent monolayer in medium supplemented with 10% fetal bovine serum (FBS) in a 12-well plate. After 24 h of incubation in serum-free medium, the wound was made by scraping the cell monolayer with a pipette tip. The cells were cultured with 0-20 μM DhL in medium supplemented with 1.5% FBS containing 2 mM hydroxyurea. Wounds were imaged at 0 and 24 h of treatment, at the same spots by direct microscopic visualization. Migration of cells in 0 μM DhL was considered 100%. Cells treated with 5, 10 and 20 μM DhL migrated 93% ±0.7, 75% ±2.5 and 60% ±5.5 (x ± SEM of 3 experiments) respectively. Recently, it has been shown in HeLa cells in culture, that DhL inhibits cell-cycle progression for arresting cells in G2. In this study, DhL effectively inhibited migration of HeLa in culture. DhL looks like a new promising candidate as an antimetastatic agent.

CB-P63.
***Lactobacillus reuteri* REDUCES APOPTOSIS AND ERK 1/2 ACTIVITY IN PBMC WITH DISRUPTED RAFTS**

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The immunomodulatory capacity of lactobacilli were previously studied but the molecular mechanisms involved in this effect remain unclear. Our previous studies showed that *L. reuteri* CRL 1098 reduced TNF-α production by human peripheral blood mononuclear cells (PBMC), and this effect depended on lipid rafts integrity. The aim of this work was to investigate the apoptotic effect of *iL. reuteri* on control and disrupted-rafts PBMC (dr-PBMC) and the modulation of ERK1/2 and p38 activities. Also, IL-10 regulation by *L. reuteri* was investigated in order to determine the anti-inflammatory capacity of this strain. Kinases activities and Il-10 were detected by ELISA and apoptosis by flow cytometry. When PBMC were co-incubated with *L. reuteri*, no modification on IL-10 production was observed. These results, with the previous one of diminished TNF-α level, indicate that *L. reuteri* exerts an anti-inflammatory effect on PBMC. Apoptosis was reduced by 5 and 3% in control and dr-PBMC respectively, when cells were co-incubated with *L. reuteri* . No modifications on p38 activity neither in control or dr-PBMC were induced by the strain. In contrast, ERK1/2 activity was decreased, indicating that this signaling pathway may be involved in the cellular response to *L. reuteri*. These results provide clues to understand the mechanisms by which *L. reuteri* produces an anti-inflammatory effect on PBMC.

CB-P64.
ADHERENS JUNCTIONS (AJ) INTEGRITY DEPENDS ON DRM-LIPID COMPOSITION IN RAT RENAL PAPILLA

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In epithelial tissues, AJ mediate cell-cell adhesion by using proteins called E-cadherin that span the plasma membrane, contact E-cadherin on other cells and connect with the actin cytoskeleton inside the cell. In previous work we described, in rat renal papilla, a specific DRM, that contained focal adhesion (FA) proteins. Changes in DRM lipid composition provoked by cyclodextrin (CD), neomycin (Neo) and LiCl induced dissipation of FA structures from cultured collecting duct cells. Now, we show that this specific DRM also contains E-cadherin and β-catenin. Western blot analysis showed that changes in DRM lipid composition induced by CD provoke a 10% and 50% decrease in the amount of E-cadherin and β-catenin present in DRM, respectively. Neo induces a more important decrease in β-catenin than in E-cadherin, while no significant changes were induced by LiCl. Immunofluorescence analysis showed that CD and Neo provoked a delocalization of E-cadherin and β-catenin from the plasma membrane, and F-actin was found as a cortical network, typical of isolated cells. After LiCl, both proteins remained in the plasma membrane. These results demonstrate that AJ are located in DRM microdomains, and the preservation of lipid DRM composition is essential to maintain epithelial cell adhesion structures.

CB-P65.
TRYPTOPHAN SEROTONINERGIC PATHWAY AND TRYPTOPHAN HYDROYLASE IN AN ACUTE PORPHYRIA MODEL

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Human acute porphyrias are diseases caused by alterations in heme synthesis. In its clinical expression, neurological disturbances are reported. We study the metabolism of tryptophan in different tissues, to determine the level of the amino acid and its metabolites with the purpose to understand human manifestations and we measure the activity of tryptophan hydroxylase (TRH), that convert tryptophan into 5-hydroxytryptophan the precursor of serotonin. Females Wistar rats were treated with 2-allyl-2-isopropylacetamide; (AIA 100, 300 or 500mg/kg bw) and 3, 5-diethoxycarbonyl 1, 4-dihydrocollidine (DDC 50mg/kg bw). As porphyric parameter, hepatic 5-aminolevulinic-synthase (ALA-S) was measured. Levels of tryptophan, serotonin and activity of TRH were evaluated in brain and liver, by HPLC. For the highest dose, increases of serotonin (21%) in brain tissue and increases of tryptophan (23%) in hepatic one were observed; being the activity of TRH decreased in liver (30%) and in brain (52%). The results suggest that the hepatic increase of tryptophan as well as the diminished serotonin levels could be related to the decrease in the activity of the measured enzyme. In brain increase of serotonin cannot be awarded to the decrease of TRH activity. Possibly alterations of other enzymes such as monoamine oxidase (MAO)-aldehyde dehydrogenase activities could be related with this increase.

CB-P66.
IDENTIFICATION OF VITELLINE ENVELOPE GLYCOPROTEINS WITH SPERM BINDING CAPACITY IN *Bufo arenarum*

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Vitelline envelope (VE) has an active participation in sperm-oocyte interaction in *B. arenarum*. It has been shown that one of the key events by which oocytes could be fertilized is related with the proteolytic cleavage of VE glycoproteins (gp) in the first portion of the oviduct. As a consequence of the action of the protease oviductin, the relative amount of 39 kDa gp and 42 kDa gp increase markedly. In the present study, we investigated whether gp39 or gp42 have sperm binding capacity. Sperm were incubated with total VE proteins coupled to biotin. After washes the sperm were lysated and the pool of proteins obtained was separated by SDS-PAGE and electroblotted onto membrane. The biotin-labeled gp were located by staining with streptavidine-peroxidase. As a result, gp39 and gp42 were detected, suggesting that both gp have sperm binding capacity. Subsequently for analyzing thoroughly the role of gp39 we probed the effect of anti-gp39 antibodies on IVF. The fertilization rate significantly decreased when oocytes were pre-incubated with antibodies indicating that gp39 is involved in sperm binding to oocyte. Furthermore, inmunomarcation studies by electronic microscopy showed gp39 placed throughout the thickness of the VE. In conclusion, gp39 and gp42 were probably involved in sperm-oocyte binding.

CB-P67.
SUBCELLULAR LOCALIZATION OF ADENYLATE KINASES AND NUCLEOSIDE DIPHOSPHATE KINASES

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Cellular energetic homeostasis is maintained by the enzymes, adenylate kinases (Adks) and nucleoside diphosphate kinases (NDPKs). They are the ones involved in nucleotide interconvertion. In typical eukaryotic cells, there are very few isoforms of these enzymes, in trypanosomatids there have been characterized additional isoforms. This unusual number could be explained by the compartmentalized metabolism which characterizes these organisms, each isoforms AdK2 , AdK3 and the nuclear isoform of *T. cruzi*. We measured de activity of these enzymes and generated transgenic parasites which express each variant fused to GFP to find their subcellular localization. With respect to nucleoside diphosphate kinases we have identified two groups in *T. cruzi*, which differ in the presence of an N-terminal domain known as DM10. We studied these isoforms and their role in osmorregulation, nuclease activity and subcellular localization. The identification of the factors that regulate the subcellular localization and expression of this group of enzymes could be a powerful resource in the understanding cellular phosphotransfer network.

CB-P68.
HYDROLYSIS OF β-ALANYL DERIVATIVES OF NEUROTRANSMITTERS IN INSECTS

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We study the metabolism of β-alanyl derivatives of neurotransmitters in insects. The best known of these are N-β-alanyldopamine (NBAD), the main sclerotization precursor of insect brown cuticles, and carcinine (N-β-alanylhistamine), an essential compound of the visual system. In *Drosophila melanogaster*, the gene *tan* codifies the NBAD-Hydrolase (NBAD-H), the enzyme responsible for the hydrolysis of these conjugates. Our aim was to study the biochemical characteristics of the NBAD-H protein in epidermal and nervous tissue: pH dependence, substrate specificity, life cycle dependent expression, etc. We found that the NBAD-H is active in a constitutive manner. Using *in vitro* assays and HPLC, we demonstrated that this enzyme is able to hydrolyze other β-alanyl derivatives. Brain confocal images revealed that the NBAD-H is mainly expressed in the optic lobes. We studied tan^1 and tan^3 mutants and found (by western blots) that tan^1 cannot process the precursor protein. Our results also showed that tan^3 is a complex mutant, with different phenotypes in epidermal and nervous tissues. The NBAD-H, along with the NBAD-synthase, the DDC enzyme and others, are reused in different occasions depending on the life cycle and the physiological requirements.

CB-P69.
MODULATION OF CELL CYCLE REGULATORS mRNA LEVELS ALONG HUMAN EMBRYONIC STEM CELLS DIFFERENTIATION
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Human embryonic stem cells (hESC) exhibit short cell division rates and their division is controlled through unusual mechanisms of cyclin dependent kinases (CDK) regulation. hESC have extraordinary developmental plasticity, illustrated by their unlimited differentiation potential. The cyclin-CDKs complexes are master regulators of cell proliferation. These complexes phosphorylate targets such as pocket proteins (retinoblastoma, p107 and p130) that are essential for cell proliferation and are negatively regulated by small polypeptides, the CDK inhibitors (CKIs) that comprise the INK4 and the Cip/Kip families.To date, little is known about how cell cycle machinery influences hESC properties and their cell fate commitment. In this study we sought to determine the mRNAs levels of key cell cycle regulators, in both stemness and during differentiation. These factors include: CDKs, cyclins, CKIs and pocket proteins.By qRT-PCR we found that mRNA levels of both factors that inhibit proliferation (CKIs and pocket proteins) and those that promote cell division (cyclins and CDKs) are induced along hESCs differentiation. These results suggest that in hESCs cell cycle checkpoints are abbreviated or absent during stemness and established further along differentiation. We propose that these features may be required to allow the high rate of cell division necessary for early embryonic development

CB-P70.
***SUPPRESSOR OF FUSED* IS INVOLVED IN *Xenopus laevis* NEURAL CREST DEVELOPMENT**
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The neural crest (NC) is a migratory and multipotent embryonic cell population specific of vertebrate embryos. It generates many derivatives ranging from neurons to the craniofacial skeleton and melanocytes. A complex gene regulatory network (BMP, Wnt, FGF, Notch and RA) mediates the intricated process of NC formation. However, the participation of other genes and cell signals in NC specification has not been established. The Hedgehog (Hh) pathway presents a high conservation degree in different species. We have previously shown that the expression patterns of *Bhh* and its transcriptional effector *Gli3* overlap with NC markers and both genes participate in the NC induction in *Xenopus laevis* (Xl).The Gli proteins exist as a part of Hh signaling complexes (HSCs) consisting of *Cos2, Fu, Smo*, and *Suppressor of Fused (Sufu)*. In this work we focus our analysis in regulatory aspects of the pathway concerning to HSCs analyzing the role of a novel *N-Sufu* isoform in the NC induction. In silico analysis have shown two regions of high identity between full length *Sufu* and *N-Sufu* isoform. We show that *Sufu* expression overlaps with NC markers and through functional experiments that this gene is implicated in NC formation. Our results demonstrate that Bhh pathway is required for normal development and provide new insights into the possible molecular regulation of Hh pathway in the NC.

CB-P71.
***HAIRY2* GENES CONTROL NEURAL CREST INDUCTION AND CELL MIGRATION IN *Xenopus* EMBRYOS**
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It has been demonstrated that the yuxtacrine cell signaling pathway Notch/Delta is involved in the control of many developmental processes. *Hairy* genes encode transcription factors containing a bHLH DNA binding domain that are direct targets of Notch/Delta signaling in vertebrates and invertebrates. We have previously shown that *Xenopus hairy* genes (*hairy1, hairy2a*, and *hairy2b*) are expressed in the neural fold, the prospective neural crest (NC) region, from late gastrula stage onward suggesting that they have a role in neural crest induction and migration. In order to analyze the role of *hairy* genes during NC development by conditional gain- and loss-of-function, we have prepared chimeric inducible proteins. The overexpression of antisense oligonucleotides or chimeric inducible proteins and their dominant negatives by microinjection of in vitro transcribed mRNAs into developing embryos demonstrated that hairy genes are involved in the induction and migration of NC cells. The overexpression of *hairy2a* mRNA in naive ectodermal tissue showed that this gene is able to induce neural crest markers. The transcriptional activity of the *hairy2b* gene was evaluated for a better understanding of the molecular mechanisms involved in the control of these developmental processes. Our results show that *hairy* genes are key players in the regulatory network controlling *Xenopus* NC development.

CB-P72.
***Rexp52* IS A NEW GENE DIFFERENTIALLY EXPRESSED DURING *Xenopus laevis* EMBRYONIC DEVELOPMENT**
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The development of vertebrate embryos includes the induction of different cell populations. In order to understand how the differences in cell fate are mediated it is important to identify genes which are differentially expressed in all the embryonic territories. The description and analysis of gene expression patterns is crucial to elucidate the physiological functions of genes and to understand the gene network that underlay the processes of normal development. These genes will be useful as molecular markers and are also likely to be involved in controlling the differentiation of different cell types. We identified a novel gene called *rexp52* with a complex expression pattern in *Xenopus* embryos. The temporal and spatial expression of *rexp52* was analyzed by in situ hybridization and RT-PCR and compared with the expression of *FoxD3* and *XK81a*. By neurula stage, the main expression of *rexp52* is located in the inner layer of the ectoderm corresponding to the epidermis and neural crest. At the tailbud stages, is expressed strongly in the CNS, branchial arches, otic vesicle and trunk epidermis. When we modified genes or signals involved in epidermis or neural crest development we found changes in *rexp52* expression. Taken together, these results show that *rexp52* is a new gene with differential expression in the ectoderm that could be involved in the development of this tissue.

CB-P73.
ROLE OF OOCYTE INTEGRINS IN FERTILIZATION-INDUCED SIGNAL TRANSDUCTION IN *Bufo arenarum*
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We previously demonstrated the presence of the integrin β1 subunit in the plasma membrane of *B. arenarum* oocytes and provided evidence for a role of integrins in fertilization. Our aims now were to: a) determine the α subunit that form, together with the β1 subunit, the integrin heterodimer, and b) study the involvement of integrin β1 subunit in the signaling cascade triggered in fertilization.
We performed immunoprecipitation with an anti-β1 integrin antibody in plasma membrane samples from crosslinked oocytes. We evaluated the presence of integrin subunits α5 and α6 among the co-immunoprecipitating proteins. We found that both α5β1 and α6β1 heterodimers are present in *B.arenarum* oocytes. We also analyzed the phosphorylation state of the proteins that co-immunoprecipitated with the integrin β1 subunit before and after fertilization. We found that different proteins, some of them phosphorylated in serine/threonine and others in tyrosine, not only co-immunoprecipitate with this subunit but also change their phosphorylation state due to fertilization. None of the detected bands had the molecular ratio of integrins.
In conclusion, our results suggest that an integrin formed by the β1 subunit (probably as α5β1 and/or α6β1 integrin) is involved in the fertilization-induced signal transduction, even when this protein does not change its phosphorylation state during this process.

CB-P74.
PARAXIS REGULATE THE CELL REARRANGEMENT DURING SOMITE FORMATION IN *Xenopus laevis* EMBRYOGENESIS
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In all vertebrates the Paraxial Mesoderm (PM) segmentation involves the formation of metameric units called somites through a Mesenchymal-Epithelial Transition. However this morphogenetic process is different in the amphibian *Xenopus laevis* because it does not form an epithelial somite, it rather undergoes a complex cell rearrangement. The molecular mechanisms that control these cell behaviours underlying somite formation remain elusive.
Paraxis is a bHLH transcription factor expressed in vertebrate PM and in the somite. To establish the role of *paraxis* in this model organism, we carried out experiments of gain and loss of function by using a hormone-inducible construct.
We found that the knockdown of this gene resulted in a delay in the development timing and morphogenetic movements, defects in the somite formation and a reduction in the expression of somitic molecular markers. These results suggest that *paraxis* could play an important role in Presomitic - Somitic Mesoderm Transition.
We also showed that non-canonical Wnt signalling pathway would be responsible of paraxis expression regulation through CaM Kinase II during *X. laevis* development.

CB-P75.
RETINOIC ACID IN *Xenopus laevis* HEAD MESODERM DIFFERENTIATION
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Retinoic Acid (RA) is known to pattern many tissues during vertebrate embryonic development. There have been many studies analyzing the effects of exogenous Retinoic Acid in neural tube and neural derivatives during *Xenopus laevis* embryonic development. Those studies showed that retinoic acid avoid the formation of anterior neural structures and stated Retinoic Acid as a posteriorising factor, however its role in antero-posterior patterning in others embryonic tissues remains unclear. In this study we aim to clarify whether retinoic acid has any effect on head mesoderm in *Xenopus* embryos.
We saw that the over-expression of the enzyme Cyp26 (Retinoic Acid degrading enzyme) causes alterations in the head mesoderm territory. The expression pattern of *tbx1* and *pitx2* (specific head mesoderm molecular markers) was altered in the RA-free territories.
We also cultured *Xenopus* embryos from stage 11 to 20 in different concentration of RA containing media. Embryos were fixed in glutaraldehyde and the ectoderm (epidermis and neural tissue) was removed with forceps in order to expose the mesoderm layer. Morphological modifications in head mesoderm were analyzed by scanning electron microscopy. We found that the mesoderm tissue was diminished in the anterior region of the treated embryos.
Altogether our results suggest that Retinoic Acid signalling could be involved in the head mesoderm set up.

CB-P76.
SIGNAL MOLECULES IMPLICATED IN *Xenopus laevis* VITELLOGENIN UPTAKE
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The transformation of oogonia into oocytes is commonly described as oogenesis. In many oviparous vertebrates the growth of oocytes from microscopic to macroscopic dimensions is mainly the results of one of the most exciting examples of cell regulation, named vitellogenesis. This process, general to all oviparous vertebrate species is characterized by hepatic production of the glycoprotein vitellogenin (VTG). VTG is transported via the bloodstream to the ovary where enters the oocytes by a receptor-mediated endocytosis.
Previously we demonstrated that in the amphibian *Xenopus laevis* direct gap junctional communication between oocytes and follicular epithelium is a requirement for endocytic VTG uptake. This suggested that a diffusible signal molecule (cAMP or/and Calmodullin) able to pass through fully open gap junctions could be involved in vitellogenic process.
In order to determine the identity of the signal molecule, gap junctions coupling/uncoupling experiments with an endocytic tracer were performed.
Rescue experiments suggest that the cAMP could be the signal molecule involved in vitellogenin uptake during vitellogenesis.

CB-P77.
B-HEXOSAMINIDASE OF *X. laevis*: ISOFORMS AND CHARACTERIZATION IN OOCYTES
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Glycosidases have been proposed to be involved in different fertilization steps as binding, penetration and polyspermy prevention. However, the glycosidase involved in each step remain unclear. Our objective was to characterize *X. laevis* oocytes glycosidases with a potential role in fertilization. Different glycosidase were analyzed. The main activity found was the N-acetyl-β-D-glucosaminidase (Hex), which has been reported to participate both in gamete binding and polyspermy prevention. Our *in-silico* studies showed the existence, so far unknown in amphibians, of two different Hex polypeptides, one corresponding to an α and the other to a β chain. In mammals, they constitute three Hex isoforms: A(αβ), B(ββ) and S(αα) and are codified by two different genes that evolved from a common ancestor. However, in *Xenopus* would be produced by alternative splicing from a single gene. In native gels, oocytes Hex corresponded to an A or S isoform. Western blot experiments indicated that, as humans, their polypeptides appear to be synthesized as prepropolypeptides, further proteolyzed to give the mature chains.
Immunohistochemical assay has shown that Hex is differentially localized in the animal pole of oocytes; the Amphibians sperm entry site. Our evidence suggests the existence of a strategic location of Hex that might directly impact fertilization strategies and polyspermy prevention.

CB-P78.
CADMIUM CHRONIC INTOXICATION: MODULATION OF OXIDATIVE STRESS IN THE RAT HEART BY THE DIET
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The aim of this study was to assess the effect of Cd^{2+} on oxidative stress parameters in heart, and the putative protection of a soy-based diet. Two months old male Wistar rats were separated into six groups: 1, 3 and 5 were fed with a casein-based diet; 2, 4 and 6 were fed with a soy-based diet. Controls (1 and 2) received tap water; groups 3 and 4 received tap water with Cd^{2+} 15ppm; groups 5 and 6, tap water with Cd^{2+} 100ppm. Food and water were available ad libitum, during 4 months. TBARS levels and paraoxonase-1 (PON-1) activity in serum, and antioxidant enzymes activities (SOD, CAT and GPx) in heart were determined spectrophotometrically. TBARS levels and PON-1 activity increased in group3, compared to 1, however TBARS decreased in group 6, compared to 2 ($p<0.05$). CAT activity increased in group 3 in comparison to 1, and it decreased in groups 4 and 6, compared to 2 ($p<0.05$). No differences were found in GPx or SOD activities.
In this study, we observed that Cd^{2+} intoxication increases oxidative stress in serum and heart, modifying at the same time antioxidant enzymes activities. There seems to be an induction of CAT activity in the heart and PON-1 activity in serum with the lower concentration of the metal. This, probably, could be due to a defense mechanism. No increases of lipid peroxidation in serum were observed in the rats fed with the soy-based diet.

CB-P79.
DETERMINATION OF CADMIUM-INDUCED INFLAMMATORY PARAMETERS IN WISTAR RAT CEREBRAL CORTEX
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It has been observed that cadmium causes changes in the level of expression of genes involved in inflammatory disorders in different types cell in cerebral cortex. In this work we measured the concentration of cadmium in samples of cerebral cortex, the expression of inflammatory (TNFa,VCAM1 and iNOS) antiinflammatory parameters (PPARg and MT III) and parameters oxidative stress (TBARS and NO). Our experimental model consisted of adult-male Wistar rats subjected to 15 ppm of cadmium in drinking water (Cd group) and without it (group CO). The total RNA was extracted by the method of TRIzol and levels of RNAm quantified using RT-PCR, the TBARS were quantified and the levels of NO using the reaction of Gries. We observed a significant increase in the concentration of cadmium in cerebral cortex ($p<0.00001$), the expression of inflammatory genes increased significantly in Cd group compared con el control group (TNF-a p: 0.0168; VCAM-1 p:0.0053; iNOS:p: 0.0244) as well as that of MT III p: 0.0356. The expression of PPAR-γ did not change in Cd group. Moreover there was an increase in the levels of TBARS ($p<0.0001$) and a significative decrease in levels of ON ($p< 0.0001$) due possibly to the formation of peroxynitrites. We conclude that cadmium is able to cross the blood-brain barrier, reaching the nervous parenchyma and trigger an inflammatory process at the level of the cerebral cortex.

CB-P80.
EFFECTS OF *IN VIVO* IRON EXPOSURE ON OXIDATIVE STRESS IN THE TEMPERATE BIVALVE *Mya arenaria*
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The aim of this study was to evaluate the effect of *in vivo* Fe exposure over the oxidative metabolism of *Mya arenaria*. Bivalves were placed in aquaria with natural seawater supplemented with 500 μM Fe. Digestive glands (GD) were isolated and total Fe content, measured spectrophotometrically, resulted in a significant increase after 9 to 17 days of exposure. After 2 days of treatment, the content of thiobarbituric acid reactive substances, measured spectrophotometrically, showed a significant increase (3.8-fold), decreasing to control values after 7 days and increasing constantly from day 7 to day 17. Ascorbyl radical content, measured by electronic paramagnetic resonance (EPR), showed a significant increase at day 2 (4-fold) and 9 (25-fold), as compared to controls. The labile Fe pool significantly increased over control values from day 7 until day 17. Nitrate and nitrite content, measured by Griess reaction; and nitric oxide (NO) content, assessed by EPR, decreased on day 2 with no further alteration until day 17 suggesting a role for NO in the Fe mediated effect. These data showed that at early stages of Fe exposure, Fe uptake is limited and an initial phase of damage could be adequately controlled by the cells. However, after 7 days of treatment, endogenous Fe increased continuously, severely damaging the cells leading to injury and death.

CB-P81.
EFFECTS OF THALLIUM ON PC12 CELLS PROLIFERATION. MODULATION BY EGF
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The mechanisms involved in the cytotoxicity of the heavy metal thallium (Tl) are still under elucidation. We previously demonstrated that Tl(I) and Tl(III) alter PC12 cells redox state and cause apoptosis. In the current work we investigated whether Tl(I) and/or Tl(III) (1-100 μM) could affect PC12 cells proliferation either in the absence or presence of epidermal growth factor (EGF). After 24 h, Tl(I) caused the accumulation of cells in S phase while Tl(III)-treated samples had higher percentage of cells in G2/M phase. Both Tl(I) and Tl(III) increased the number of apoptotic cells. These effects were partially prevented by EGF only in Tl(III)-treated cells. Tl(III) but not Tl(I) increased PC12 cells plasma membrane fluidity at the water-lipid interface, without affecting lipids hydration or the fluidity of the hydrophobic portion of the bilayer. Again, EGF protected cells from Tl(III) effects. Cells pre-loading with Tl had no effect on PC12 cells cycle, either in the absence or in the presence of EGF. Together, present results indicate that in addition to promoting cell apoptosis, Tl alters cell cycle and membrane properties. The observation that EGF could modulate Tl(III)- but not Tl(I)-mediated effects suggests different mechanisms could be operative in the cytotoxic effects of both cations.
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CB-P82.
CHARACTERIZATION OF TL(III)-PRODUCTION OF LUMINOL CHEMILUMINESCENCE IN JURKAT T CELLS
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Trivalent thallium (Tl(III)) is highly toxic to humans through not well understood mechanisms. Among other effects, Tl(III) promotes an oxidative stress status in cultured cells. In the present work we investigated the possible origin of luminol chemiluminescence (CL) in Tl(III)-treated Jurkat T cells. Cells preincubation with free radical scavenger Trolox® did not affect the magnitude of the effect. In a cell free system, Tl(III) per se did not induce CL at pH 7.4. In the presence of H_2O_2, Tl(III) enhanced CL, in a Tl(III) (1-100 μM)- and H_2O_2 (0.1-1 mM)- dependent fashion. In the absence of agitation, the reaction followed Belousov-Zhabotinski behavior, feature that disappeared under continuous agitation. Superoxide anion generation was evidenced, and the addition of superoxide dismutase decreased the magnitude of Tl(III)-H_2O_2 CL production. Only 40-60 % of added Tl(III) was recovered as Tl(I), suggesting that either Tl(III) acts as a catalyst of the reaction, or Tl(I) generated remains bound to luminol and could not be detected by the current methodology. Together, experimental results show that Tl(III) caused both in cultured cells and in a cell free system luminol CL, effect that depended on H_2O_2 presence. The rapid source of H_2O_2 in Tl(III)-treated Jurkat cells remains to be elucidated.
Supported by grants of UBA (B086), ANPCyT (PICT 32273) and CONICET (PIP 112-200801-01977).

CB-P83.
THE *Drosophila* INSULIN DEGRADING ENZYME. ROLE IN INSULIN SIGNALING AND GROWTH REGULATION
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The Insulin degrading enzyme (IDE) is a metalloprotease which in mammals is known to degrade Insulin, amongst other small peptides, and hence *ide* knockout mice show hyperinsulinemia and glucose intolerance. In *Drosophila*, Insulin-like peptides (dILPs) control not only circulating levels of sugar and lipids, but also growth through the activation of the PI3K pathway. We have explored the role of *Drosophila* IDE homolog (dIDE) in growth control of the organism. Ubiquitous over-expression of dIDE resulted in pupae that were 6% ($t=2.13$; $p<0.05$) smaller than the controls, and adults with wings 4.5% smaller ($t=2.13$; $p<0.001$). We then investigated if this effect was organ-autonomous by over-expressing dIDE in the dorsal compartment of the wing anlage. This treatment brought about dorsally-curved wings supporting the notion that dIDE regulates dILPs levels. dIDE loss-of-function was induced through the expression of double stranded RNA in a transgenic line. This RNAi expressed through an MS1096-Gal4 driver produced, dorsally-curved wings, showing that dIDE is normally found in this imaginal disc and may be necessary for normal wing development. We are currently generating null-mutants to gain further insights on the role of dIDE in *Drosophila melanogaster*.

CB-P84.
TGF-β SIGNALING IN DIABETIC INTESTINAL MUCOSA
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Long-term diabetes is associated with morphological, functional, and metabolic alterations in the intestine. Using an experimental model of diabetes in rodents, we explored the hypothesis that diabetic intestinal disfunction, could be consequence in part of a defect in the mucosa TGF-β system. The principal change in diabetes was an up-regulated TGF-β1/Smad signaling in the small intestine. TGF-β1 and TGF-β receptors RII were increased in the diabetic mucosa at mRNA and protein level. p-Smad2/3 protein was distributed throughout the mucosa, but the highest levels of active protein were associated with mesenchymal cells. This cellular population, predominantly SMA^+/$vimentin^+$ myofibroblasts , were incresed in the mucosa of diabetic animals. We also observed that diabetes environment upregulates extracellular matrix (ECM) deposition in the intestinal mucosa. Proteins such as type IV collagen and laminin were accumulated throughout the basal lamina, whereas fibronectin was restricted to lamina propia. An increased synthesis of type III collagen in mesenchymal cells was also observed.
Collectively all the data showed that the TGF-β1 deregulation in diabetic mucosa is associated with the emergence of cells with myofibroblast phenotype and ECM accumulation. Diabetes causes an imbalance in the normal mucosa remodeling, leading to a fibrotic process at early stage of the disease.

CB-P85.

BIOCHEMICAL ALTERATIONS INDUCED BY AZINPHOSMETHYL AND CARBARYL IN *Rhinella arenarum* LARVAE

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Organophosphate (OP) and carbamate pesticides act by inhibiting cholinesterase (ChE) and also they are able to induce oxidative stress and alter antioxidant enzymes. We analyze the effect of carbaryl (CB) and the OP azinphosmethyl (AM) on reduced glutathione (GSH) levels and enzymatic activities of ChE, carboxylesterases (CabE), catalase (CAT), GSH peroxidase (GPx), GSH reductase (GR), GSH transferase (GST), PKC and PKA of *Rhinella arearum* larvae. The larvae (stage 25) were exposed 48 h to 3 mg/L and 6 mg/L AM or 10 mg/L and 20 mg/L CB. Enzyme activities and GSH were determined spectrophotometrically. PKC and PKA activities were measured with 32PγATP and histone. ChE and CabE were significantly inhibited by CB (78%, 64%) and AM (33%, 44%). Both pesticides increased GST (39%) and GR (72%-93%), while GSH levels and GPx were not significantly affected. CAT was significantly inhibited by pesticide exposure (20%-48%). PKC and PKA activities showed a tendency to increase in exposed larvae, differing with the response observed in early embryos in our previous works. Beside the strong inhibition of esterases as classical targets, both pesticides affect antioxidant enzymes. Larvae show an adaptive response increasing GST and GR enzymes, thus activating the GSH-dependent pathway, while CAT may be directly downregulated by the reactive oxygen species produced by pesticide metabolism

CB-P86.

SLIM AND FLY: CONTROLLING *Drosophila* SIZE WITH MITOCHONDRIAL 2-OXOGLUTARATE TRANSPORTER PAMPE

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Exogenous factors, such as nutrient and oxygen availability modulate *Drosophila* growth and developmental timing through Insulin and TOR pathways. However it is still unclear which molecular sensor reports the nutritional status of the organism. In this regard, one of the molecules whose levels are known to correlate with the nutritional state of the cell is the TCA intermediate 2-Oxoglutarate. This mitochondrial keto acid is a precursor for amino acid synthesis, and is exported to the cytoplasm by specific mitochondrial inner membrane transporters. In this work, we have experimentally decreased cytosolic 2-oxoglutarate levels through RNAi mediated silencing of one of such transporters, encoded by a gene that we have named pampero (pmp). Ubiquitous RNAi-mediated silencing of Pmp led to consistent reduction of body size and delayed pupariation as well as to increased size of fat body lipid droplets. Since the *Drosophila* HIF-prolyl-4-hydroxylase Fatiga (Fga) utilizes 2-oxoglutarate as a co-substrate for catalysis, it is possible that Fga mediates the effect of 2-oxoglutarate in growth regulation. Consistent with this possibility, fga loss of function mutants are smaller than their wild type siblings and pupariation is delayed. We are currently dissecting the genetic pathways involved in pmp dependent growth control with the aim of determining the mechanism of nutrient sensing.

EN-P01.
β-D-GLUCOSE-1P AS A SUBSTRATE OF NDP-GLC PYROPHOSPHORYLASES OF TWO ACTINOBACTERIA
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It is well known that the á-anomer of glucose-1P (áGlc1P) is a common metabolite in different cells. However, only a few organisms were reported to use the ß-anomer (ßGlc1P) in their metabolisms. Maltose and trehalose phosphorylase, together with ß-phosphoglucomutase from certain organisms are the only enzymes identified as metabolizing ßGlc1P. Although the presence of ßGlc1P in both bacteria and algae was reported, information about the metabolic role of this intermediate is scarce. ßGlc1P can be used as a precursor for cell wall material in *Lactococcus lactis* and also it could be a component of a surface glycan in *Enterococcus faecalis*. Our group is focused in the characterization of several NDP-GlcPPases and we analyzed ßGlc1P as substrate of these enzymes. In our hands, certain ADP-GlcPPases and UDP-GlcPPases utilized this alternative substrate. Interestingly, these enzymes belong to two actinobacteria: *Mycobacterium tuberculosis* H37Rv and *Streptomyces coelicolor* A3(2). The mycobacterial ADP-GlcPPase used ßGlc1P with about 3-fold lower affinity than the á-anomer, to also reach a 3-fold lower V_{max}. Also, the enzyme activity with the ß-anomer was not affected by allosteric regulators. Results suggest that after the promiscuity to use anomeric forms of Glc1P certain NDP-GlcPPases may be differentially involved in divergent metabolic routes of carbohydrates in specific organisms.

EN-P02.
DITHIOL GLUTAREDOXIN FROM *Trypanosoma cruzi*: CLONING, EXPRESSION AND CHARACTERIZATION
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Trypanosoma cruzi is the causative agent of Chagas' disease. Similar to that found for other aerobic parasites, *T. cruzi* is exposed to several reactive oxygen species, which are highly toxic for the parasite. These chemical species are generated both during the host defense reaction and also as byproducts of the aerobic metabolism. The metabolic pathways used by this organism to cope with such environmental changes and redox homeostasis are matter of our work. Glutaredoxins are ubiquitous oxidoreductases that play an important role in the control of cellular function. Despite a putative dithiol glutaredoxin (*Tc*Grx) is encoded in the genome of *T. cruzi*, it was not yet characterized. In this work we present the cloning, expression and functional characterization of recombinant *Tc*Grx. The protein was able to catalyze the reduction of oxidized glutathione and glutathionylated compounds using trypanothione as electron donor, demonstrating the functional relevance of this enzyme for the parasite. We present experimental assays (western blotting and enzymatic activity) that strongly support the occurrence of a dithiol glutaredoxin in *T. cruzi* epimastigote, being necessary to consider this protein as a new molecular component of the redox metabolism in trypanosomatids.
This work was supported by grants from ANPCyT (PICT0´05 13129, PICT´07 668), CONICET (PIP6358), UNL (CAI+D 2006).

EN-P03.
A NEW METALLOCARBOXYPEPTIDASE INHIBITOR DETECTED BY INTENSITY FADING, A PROTEOMIC TECHNIQUE
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Biological actions of many proteases are regulated by their interaction with specific proteinaceous inhibitors. Unlike endoproteases, for which several examples of protein inhibitors have been reported, naturally occurring metallocarboxypeptidase inhibitors are rather limited and, so far, they have only been identified in *Solanaceae*, tomato and potato. Intensity Fading is an Interactomic technique (belonging to proteomics) use to the identification of biomolecules and their ligands by perturbation of the signals of mass spectrometry applied to crude biological extracts or complex. In this study, a new inhibitor of metallocarboxypeptidase A was detected, isolated and characterized biochemically and electrophorectically from tubers of *Solanum tuberosum* Desirée variety. The extracts obtained from potato tubers were subjected to Intensity Fading MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight). The detection and confirmation of the inhibitory activity was found by testing kinetic inhibition. The crude extract was partially purified by heat treatment at 80 °C and then the sample was purified using affinity matrices specially designed by immobilization of carboxypeptidase A in glyoxyl agarose gel. The inhibitor showed a molecular weight of 4.2 kDa (mass spectrometry MALDI TOF). The crude extract and the isolated inhibitor were characterized by SDS PAGE and IEF.

EN-P04.
ANALYSES OF THE INTERACTION OF SUBUNITS OF NADP MALIC ENZYMES OF MAIZE
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Maize NADP-ME protein family has long being studied due to the essential role of one of the isoforms in carbon fixation (ZmME1). Even though the sequences in maize are well conserved, the photosynthetic isoform (ZmME1) is more closely related to a plastidic enzyme associated with housekeeping functions (ZmME2). Both isoforms have been extensively compared with regard to their kinetics and structural features. Besides the kinetic parameters, the oligomeric states also differ, while ZmME1 aggregates in the form of tetramers, ZmME2 remains as dimers in solution. Artificially generated chimerical structures allowed us to identify a region that strongly affects the quaternary structure of these enzymes. Moreover, we identified in silico some residues candidates to be involved in the assembly and stabilization of the tetramer. The aim of this work was to study the contribution of these residues to the basic kinetic parameters and the quaternary structure. In this regard, we replaced five residues in ZmME2 by the analogs found in ZmME1 by site-directed mutagenesis. The modified proteins were characterized by kinetic analysis and migration in non-denaturing PAGE. The results show that some single replacements had little or no effects on the structure, while two residues had major effects which indicate their involvement in the dimer assembly.

EN-P05.
STRUCTURAL DETERMINANTS FOR THE HIGH CATALYTIC EFFICIENCY OF PLASTIDIC FERREDOXIN-NADP$^+$ REDUCTASES

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The goal of this work was to search for the structural characteristic that endows high catalytic efficiency to the plastidic ferredoxin-NADP$^+$ reductases (FNRs). This features may be absents in the low catalytic efficiency bacterial FNRs (i.e. *E. coli*). The conformation adopted for the FAD prosthetic group (extended in plastidic and contracted in bacterial FNRs) and the movement of C-terminal tyrosine both may be responsible for the high catalytic efficiency. Thus, we engineered FNR chimeras of pea FNR containing structural features of the bacterial enzymes, and mutants *E. coli* FNRs that emulate the plastidic ones, in a effort to exchange the kinetic behavior between both FNRs. Surprisingly, only small variations of the kinetic parameters were observe for all chimeric enzymes. Our results indicated that the analyzed structural features are involved in enzyme stability, FAD and NADP$^+$ affinity and, related to the discrimination between NAD$^+$ and natural substrate NADP$^+$. *Ab initio* calculations and crystallographic studies suggest that the strong interaction between FAD and a C-terminal tyrosine in *E. coli* FNR may limit its catalytic efficiency. Moreover, we found that *E. coli* FNR tightly bound NADP$^+$ probably contributing to the enzyme stability and decreasing the catalytic efficiency of the bacterial enzyme.

EN-P06.
A NEW PROTEIN WITH PROBABLE CAPACITY OF BINDING OF CALCIUM

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The "mejillón", *Mytilus edulis platensis*, is a mollusk that inhabits the Argentinean coast. The gonads are extended in a diffuse way in the mantle lobes, responsible for the shell secretion.
In this work we studied the proteins involved in the mechanism of formation of the shells.
Small gland pieces were resuspended (1:1) in 50 mM sodium phosphate (pH 7.4). The cytosolic fraction of the gonad and mantle lobes were fractionated on a Sephadex G-75 and DEAE-cellulose equilibrated with a 30 mM Tris-HCl buffer (pH 8.5).
We purified a protein which was analyzed by HPLC-MS (ESI) and digested with trypsine and Glu-C. Peptides resulting from each digestion were separated by RP-HPLC and then submitted to Edman degradation.
The calcium binding capacity was analyzed from the technique of "Stains all", coloring that allows to identify the proteins that calcium binding.
The results show a protein with a molecular mass of 11,900 Da. We have sequenced approximately 62% of the amino acid residues. This sequence determination no shows identity with proteins description in the databases. The analysis by means of the technique of "Stains all" it showed a probable binding of calcium.
These results suggest a new protein with probable capacity of binding of calcium that could be involved in the formation of the shell.

EN-P07.
THE AUTOGALACTOSYLATION OF GLYCOGENIN

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We have described that glycogenin (GN), at the concentration at which it exists as monomer, has the ability to autoglucosylate with a first order kinetics (Bazán, S. *et al*., Biochem, Biophys. Res. Commun. 371, 328-332, 2008). In order to understand this intramonomeric mechanism of reaction, a molecular dynamics study will be done, as performed before (Bazán, S. *et al*., Biocell 32, 2008), to analyze the distances separating the glucosylable Tyr-194 from Asp-162 (required for activity) and the bound UDPGlc. These distances can not be accurately determined in the reported crystallographic structure of the autoglucosylated GN, because the mobility of the linked maltosaccharide makes "invisible" the Tyr-194 residue. Furthermore, no monoglucosylated GN can be isolated in order to determine such distances to the first incorporated glucose. Here we describe a way to avoid the mobility of the autoglucosylated Tyr-194, and produce a homogeneous monoglycosylated GN species for X-ray diffraction studies, by using UDPGal as donor substrate. The results demonstrate that GN self-incorporates a unique galactose unit into its tyrosine residue, being incapable to continue the O-glycosylation of the linked galactose from UDPGal or UDPGlc. X-ray diffraction analysis of the obtained galactosylated GN crystals might allow us to determine also the anomeric configuration of the Tyr-O-galactose linkage.

EN-P08.
GLYCEROL-3-PHOSPHATE DEHYDROGENASE ISOFORMS EXPRESSION IN *Triatoma infestans* FLIGHT MUSCLES

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Triatoma infestans (*T. infestans*), Chagas' disease vector, acquires wings and the ability to fly after the last molt from fifth instar nymph to adult. The ability to fly is important for insect dispersion. Flight dispersal is the most important mechanism for re-infestation of houses at a village scale after insecticide spraying. Glycerol-3-phosphate dehydrogenase (GPDH) plays a central role in insects' intermediate metabolism and glycerophosphate cycle in thoracic flight muscles. The enzyme is usually present in several dimeric isozymic forms with different properties and specific tissue and developmental distribution. The aim of this work is to study the correlation in the expression of cDNAs and isozymes activities. With RACE-PCR we have characterized from *T. infestans* two cDNAs encoding for GPDH-1 and GPDH-2 and we have demonstrated that the cDNAs are differentially expressed during flight muscles development. GPDH transcripts expression was studied by semi-quantified RT-PCR. The isoforms activities were analyzed by native PAGE revealed by specific enzyme activity. Electrophoretic results showed two principal isoforms with mobility according with their theoretical isoelectric point (Ip). Isozymes activities were related with transcripts expression change. The isoforms expression may responds to a biochemical adaption required for flight activity development.

EN-P09.
CAN A CHEMOLITHOTROFIC ORGANISM SYNTHESIZE GLYCOGEN AS A CARBON-ENERGY STORAGE?

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Glycogen and starch serve as important energy and carbon storage compounds for nearly all living organisms. In bacteria and plants, ADP-glucose pyrophosphorylase (ADPGlc PPase, the product of the *glgC* gene) catalyzes the rate-limiting step of the glucan biosynthesis pathway. The regulation of this enzyme is mediated by various metabolites serving as allosteric activators and inhibitiors according with the carbon utilization pathways. We co-expressed in *Escherichia coli* cells a putative *glgC* gene from de chemolithotrofic organism *Nitrosomonas europaea* using the GroEL-GroES chaperone system. This resulted in a solube and active enzyme which was purified following ammonium sulphate precipitation, DEAE-Sepharose and Phenyl-Sepharose chromatographies. The purified enzyme was kinetically characterized fitting the experimental data to the *Hill* equation. $S_{0.5}$ for Glc-1P, ATP and Mg^{2+} were calculated in 0.1 mM, 0.64 mM and 13 mM, respectively. The enzyme was activated 10-fold by pyruvate while phosphoenolpyruvate only activated 2-fold. The AMP inhibited the enzyme and this effect is more notable in presence of the pyruvate activator. This behaviour shows allosteric regulation of *N. europaea* ADPGlc PPase, making it interesting for structure/function relationships studies. Results support the occurrence of glycogen metabolism in chemolithotrophes at least for carbon storage functions.

EN-P10.
REDOX CHARACTERIZATION OF *Phaeodactylum tricornutum´*s CHLOROPLASITIC PHOSPHOGLYCERATE KINASE

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Diatoms are key constituents of aquatic phytoplankton communities. Because of its small genome and the possibility of being routinely transformed, *Phaeodactylum tricornutum* has emerged as a model species for dissecting diatom biology. Our interest is to characterize enzymes involved in carbon metabolism in the diatom. Chloroplastic phosphoglycerate kinase-1 (E.C. 2.7.2.3; PGKase-1) catalyses the ATP-dependent phosphorylation of 3-phospho-glycerate to 1,3-bis-phospho-glycerate, in a reversible reaction needing Mg^{2+} as essential cofactor, a key step in the Calvin cycle. Expression of the gene encoding *P. tricornutum* PGKase-1 in *Escherichia coli* rendered a soluble and active enzyme. After purification, kinetic, regulatory and structural properties of the recombinant enzyme were characterized. The purified enzyme showed inactivation by thiol-modifying reagents, property observed in some animal enzymes but never described for diatoms. Inhibition constants were obtained for oxidants such as diamide, sodium nitroprusside and hydrogen peroxide. Reversion of the oxidation was tested with DTT and *E. coli* thioredoxin and the activation constants calculated and compared. *P tricornutum* PGKase-1 has 6 cystein residues. A homology modelled structure served to predict those cysteines involved in redox regulation and mutants were constructed in order to elucidate the regulatory mechanism.

EN-P11.
PURIFICATION OF PHBV DEPOLYMERASE FROM *S. omiyaensis* AND PHYTOTOXIC PROPERTIES OF ISOLATED MONOMERS

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A bacterial strain capable of degrading poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) was isolated from a soil sample. This organism, was identified as *Streptomyces omiyaensis* SSM5670, secreted PHBV depolymerase into the culture minimum medium when PHBV was added as the only carbon source.
The poly (3-hydroxybutyrate-co-hydroxyvalerate) depolymerase from S.omiyaensis SSM5670 was purificated from culture supernatant. This enzyme was concentrated 90-fold by high pressure ultrafiltration (Stirred Ultrafiltration Cells Modes 8200 Device, Milllipore) through YM 10 membrane then was purified to electrophoretic homogeneity by ion exchange column chromatography on DEAE-Sephadex A-50 and gel filtration on Sephadex G-150. The molecular mass of the PHBV depolymerase was estimated to be 24.0 kDa by 12% SDS-PAGE. The maximum activity was observed near pH 7.0 and 32ºC, the activity was lost at temperatures above 45ºC. The enzyme, released into the medium butyric and valeric monomers, 1mM of these compounds inhibited the bacterial growth up to 30%. The same inhibition effects were observed on Lactuca sativa when stabilized compost with PHBV was inoculated with this actinomycete. Concentrations of 5 mM of butyric and valeric acid released from PHBV samples showed full inhibition of seed germination. The phytotoxicity of these monomers increased with time contact and monomer concentration.

EN-P12.
KINETIC AND STRUCTURAL STUDY OF THE FERREDOXIN-NADP REDUCTASE FROM *Xanthonomas citri*

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Ferredoxin-NADP(H) reductases (FNRs) are FAD-containing monomeric enzymes that deliver NADPH or low potential one-electron donors (ferredoxin, flavodoxin, adrenodoxin) to redox-based metabolisms in plastids, mitochondria and bacteria. The FNR variants present in most prokaryotes (FPRs) can be phylogenetically classified into two subclasses represented by the *Azotobacter vinelandii* (subclass I) and the *Escherichia coli* (subclass II) FPR prototypes. *Xanthomonas axonopodis* pv. *citri* (Xac) is a Gram-negative, aerobic bacteria that possess a single *fpr* gene with the characteristic features of subclass I bacterial reductases. We have expressed in *E. coli* and purified to homogeneity the recombinant Xac FPR protein, rendering a monomeric product with an absorption spectrum characteristic of flavin-containing proteins. Then, we further characterized Xac FPR protein by analyzing its kinetic behavior and interaction with the substrates NADPH, NADH, pea ferredoxin and *E. coli* flavodoxin. The accessibility of the prosthetic group FAD was also evaluated. Comparative analysis of the collected results with those reported for typical plastidic (i.e. pea) and bacterial (i.e. *E. coli*) FNRs suggest that Xac FPR resembles plastidic reductases regarding the interaction with nucleotidic substrates and the prosthetic group, but displays kinetic parameters typical of bacterial flavoenzymes.

EN-P13.
PURIFICATION AND PARTIAL CHARACTERIZATION OF A CYTOSOLIC NADH DEHYDROGENASE-2 FROM *E. coli*
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Respiratory NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a membrane-bound flavoprotein with cupric-reductase activity. Preliminary studies suggested that the C-terminal 43 aminoacids region is involved in the enzyme anchorage to the membrane. The aim of this work was to obtain a water soluble protein that allows a detergent-free purification. To achieve this goal, we generated a NDH-2 truncated version, Trun-3, where the mentioned C-terminal fragment was deleted. The mutant gene was cloned in frame into the expression vector pETG-30A immediately downstream from 6xHis residues. By ultracentrifugation and SDS-PAGE, we localized Trun-3 in the cytosolic fraction. Afterwards, it was purified by inmobilized metal affinity chromatography (IMAC) in the absence of detergents throughout the entire process. The purified protein showed NADH:duroquinone, NADH:MTT and NADH:Cu(II) oxidorreductase activities, always in the presence of 80 µM FAD. Spectroscopic measurements suggest the absence of FAD bound to the enzyme. The purified water soluble version of NDH-2 will be useful for further characterization of its functional domains.

EN-P14.
COPROPORPHYRINOGEN OXIDASE LOCATION IN HEXACHLOROBENZENE (HCB) INTOXICATED RAT´S LIVER MITOCHONDRIA
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Why isocoproporphyrin (IC) appears in HCB poisoned rats´ feces (experimental model for human porphyria cutanea tarda)? After 8 weeks, rats showed high porphyrin excreta and 50% liver uroporphyrinogen decarboxylase activity inhibition. Uroporphyrin plus heptaporphyrin exceeded coproporphyrin in urine, in feces, IC, from abnormal pentacarboxylic porphyrinogen-III (Pentagen-III) oxidative-decarboxylation by liver coproporphyrinogen oxidase (CPO), became the main porphyrin. Trypsin-treated mitochondria showed their membrane permeability barriers highly conserved after HCB intoxication and CPO and marker enzyme activities were not affected. After mitochondrial Digitonin treatment nearly 100% CPO, adenylate kinase (AK), and sulfite oxidase (SO) activities were recovered in the supernatant. In sediment and supernatant Trypsin treatments, no CPO, AK and SO activities were detected in any preparation and citrate synthase and malate dehydrogenase (matrix enzyme) activities were not modified.
In digitonin-treated HCB mitochondria, CPO was free in the mitochondrial intermembrane space, whereas in normal mitochondria, 30 to 50% remained anchored to the inner membrane. The free state, makes the enzyme more flexible and would allow Pentagen-III (increased levels), to compete with coproporphyrinogen-III and being transformed into dehydroisocoprogen, liver forerunner of fecal IC.

LI-P01.
CITOTOXICITY AND BIODISTRIBUTION OF CATIONIC LIPOPOLYMER/PLASMIDIC DNA COMPLEXES

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Liposomes have been widely used as drug delivery systems. In particular, polymeric liposomes have shown interesting membrane properties and enhanced stability**.** The aim of this study was to evaluate cell interaction of polymeric liposomes with and without DNA complexing *in vitro* and *in vivo*.
Lipopolymers were formulated with 1,2-bis(10,12-tricosadiynoyl)-*sn*-Glycero-3-Phosphocholine ($DC_{8,9}PC$) and 1,2-dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) in a 1:1 molar ratio and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), octadecylamine (SA) or trimethyl(2-myristoyloxyethyl) ammonium (MCl) in a 0.2 mol ratio. Size, Z potential and cytotoxicity was evaluated for each system, complexed or not with plasmidic pDsRed2-N1 DNA. Cell interaction, transfection and biodistribution in Balb-c mice, were analized by flow cytometry.
Results showed higher cell toxicity in those systems containing SA wich was reduced when complexed with DNA. Though no signifficant transfection was found for described systems, an important cell interaction was observed related to Z potential and size. Furthermore, after intravenous injection of the systems (elapsed time, one hour) lipopolymer fluorescence was found in spleen and intestines cells for $DC_{8,9}PC$:DMPC:DOTAP (1:1:0,2)/pDsRed (16:1). No differences respect to control were found for $DC_{8,9}PC$:DMPC:MCl (1:1:0,2)/pDsRed (16:1) complexes nor with the systems alone. In this sence cationic lipid influenced cell interaction and biodistribution of lipopolymer/DNA system.

LI-P02.
***Opuntia salagria* CLADODES AND SEEDS MODIFY KIDNEY LIPIDS IN DIABETIC RATS**

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Cactus *Opuntia* has been reported to display hypoglycemic and hypolipidemic effects but the mechanisms involved have not been elucidated to date. Our previous studies evidenced that *O. salagria* reduces plasmatic glucose, cholesterol, triacylglycerides (TAG) and LDL-c in streptozotocin (STZ)-induced diabetic rats. The purpose of this study was to determine the effect of *O. salagria* administration on kidney lipids in diabetic rats. In addition, some parameters of kidney function were analyzed. Cladodes and fruits were harvested, dried and milled. Diabetes was induced in male Wistar rats by intraperitoneal injection of STZ. The rats received a daily dietary supplementation with cladodes and seeds over one month. Kidney lipids were extracted, isolated by thin-layer chromatography and derivatized by methanolysis. On the other hand, 24 hours urine and blood samples were collected to determine uremia and creatinine clearance as a measure of glomerular filtration rate. Phospholipid and cholesterol kidney levels were not affected by *Opuntia* administration while significant changes in TAG and free fatty acid content and composition were observed. The creatinine clearance and serum urea levels in diabetic rats recovered control values after cladodes and seeds consumption. Altogether, these results suggest that *Opuntia salagria* may have a potential use in diabetes mellitus treatment.

LI-P03.
TRANSCRIPTIONAL REGULATION OF CKα AND CCTα DURING NEURONAL DIFFERENTIATION

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The morphological hallmark of neuronal differentiation is characterized by sprouting and elongation of neurites. Acceleration of membrane phospholipids synthesis is associated with neurite elongation and phosphatidylcholine (PtdCho) is the major membrane phospholipids in mammalian cells. PtdCho biosynthesis occurs via the Kennedy pathway in which CTP:phosphocholine cytidyltransferase (CCTα) and Choline Kinase (CKα) catalyze rate limiting steps.
We used Neuro2a cells as an in vitro differentiation model. Microarray and qPCR analysis revealed that the transcription of genes encoding key enzymes in the CDP-choline pathway of phospholipids biosynthesis is stimulated following retinoic acid (RA)-induced differentiation; including the Chkα gene for CKα and the Pcyt1a gene for CCTα. By promoter reporter construct, 5´ deletions and gel shift assays we identified region of the promoters of these genes that contains DNA sequences which are proposed to be binding sites for transcription factors (TFs) that activate the expression in a RA-dependent manner. Furthermore, we delineated the mechanism that coordinately activates gene expression during neuronal differentiation.

LI-P04.
GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE 2 EXPRESSION CORRELATES WITH TRIACYLGLYCEROL MASS IN RAT TESTIS

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Polyunsaturated fatty acids (PUFA) play an important role in testicular metabolism. It has been suggested that PUFA-rich triacylglycerols (TAG) play a special role in the traffic and metabolism of PUFA in testis. Glycerol-3-P acyltransferases (GPAT) catalyze the first and committed step in *de novo* glycerolipid synthesis. The mitochondrial isoform GPAT2 is highly expressed in rat testis and has no preference for saturated acyl-CoA substrates. We have previously shown by Oil-red-O staining that GPAT2 overexpression in CHOK1 cells increased the TAG content compared to GPAT1-overexpressing and control cells. To correlate gpat2 expression with TAG synthesis in testis, we measured TAG mass and gpat2 mRNA and protein expression during postnatal development. Testicular 30-day old rat TAG mass was 2-fold higher than 19, 40 and 60-day old rats. Interestingly, gpat2 mRNA expression followed the same pattern, reaching a peak at 30 days of age, consistent with GPAT2 protein expression. NEM sensitive GPAT activity was measured in purified mitochondria with palmitoylCoA and oleoylCoA substrates. This activity did not change at different stages of development when palmitoylCoA was used as a substrate but reached a peak at 40 days of age when oleoylCoA was used.Our results suggest that GPAT2 is involved in TAG synthesis in testis and that its activity is important for the onset of spermatogenesis.

LI-P05.
ACCRETION OF LIPIDS WITH LONG AND VERY LONG CHAIN POLYENOIC FATTY ACIDS IN RAT DEVELOPING TESTIS

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The aim of this work was to survey developmental changes in the lipids that contain long-chain (C_{18}-C_{22}) and very long-chain (VLC) (C_{24}-C_{32}) polyunsaturated fatty acids (PUFA) in rat testis. Glycerophospholipids, cholesterol esters and triacylglycerols (TAG) had 20:4n-6 as their longest PUFA and no VLCPUFA altogether in sexually immature testis (P14). At that age, a minor triglyceride subclass, typical of the adult testis, mainly 1-*O*-alkyl-2,3-diacylglycerols (A-DAG), was not produced. The three neutral lipids accumulated important percentages of long and very long chain PUFA once the first round of spermatogenesis was completed and throughout adulthood. The major VLCPUFA of TAG, A-DAG and glycerophospholipids (24:4n-6 and 24:5n-6) increased at the same pace as their major PUFA, 22:5n-6. The latter was the fatty acid that increased the most with development, since it was the main PUFA of all testicular lipids. The data are consistent with specific PUFA elongations, followed by a VLCPUFA desaturation and chain shortening, being activated with spermatogenesis in germ cells: 20:4n-6 › 22:4n-6 › 24:4n-6 › 24:5n-6 › 22:5n-6. This is supported by the expression (mRNA) in these cells of Elovl5 and Elovl2, two members of the *Elovl* (elongation-of-very-long-chain-fatty-acids) gene family involved in the elongation of $C_{18\text{-}20}$ and $C_{20\text{-}22}$ PUFA, respectively.

LI-P06.
EFFECT OF A CHEMOTYPE OF *Lippia alba* FROM COSTA RICA ON CELLULAR GROWTH AND LIPID METABOLISM

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Lippia alba (Miller) N.E. Brown (Verbenaceae) is a widely spread shrub used in traditional medicine. *L. alba* essential oil (EO) has a great chemical variability related to the geographical origin. In Costa Rica a chemotype presenting mainly ocimenone, mircenone, 1,8-cineol and mircene, was identified. Monoterpenes in EOs show chemotherapeutic activities and may represent anti-cancer drugs. We studied the effects of this *L. alba* chemotype EO on the cell growth and lipid metabolism of pulmonary adenocarcinoma A549 cells. Cellular viability was analyzed by MTT test (IC50=130 µg/ml) and trypan-blue-dye-exclusion cell count, [50 µg/ml] of EO inhibited cell proliferation by 35%. Lipid extraction was made from cells incubated with [50µg/ml] EO and ^{14}C-acetate (5µCi). The incorporation of ^{14}C-acetate into total lipids, nonsaponifiable lipids and fatty acid fraction was determined; a decrease of radioactivity was found in treated cells. Cholesterol and its precursors were separated using TLC. ^{14}C-acetate incorporated into cholesterol was decreased and the incorporation into dolichol and ubiquinone was increased. Fatty acids were separated by $AgNO_3$-TLC, observing a change in the unsaturated/saturated ratio (30%/70%) in treated cells. We concluded that *L. alba* EO has a cytotoxic effect on A549 cell line and that this may be related with the effects on lipid metabolism of these tumoral cells.

LI-P07.
EVALUATION OF GERANIOL AS HYPOCHOLESTEROLEMIC AND ANTIPROLIFERATIVE AGENT

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Epidemiological studies have revealed that a high intake of fruits and vegetables correlates with a reduction in cancer incidence. Among the phytochemicals that could have antitumor activity, the isoprenoids have multiple effects on mevalonate metabolism; some of them may account for their effects on diverse tumor cells. We have reported that geraniol (G), a natural monoterpene, inhibits cholesterol synthesis and cell growth at concentration greater than 10µM and 100µM respectively in HepG2 cells.The aim of this work was to study at which level of mevalonate pathway geraniol exert these effects. Cells were treated with 50 and 200µM G. Cellular proliferation, HMGCoA reductase, cholesterogenesis, protein prenilation, cholesterol and P content, exogenous cholesterol uptake and ^{3}H-choline incorporation into PC were determined.Low concentration of G (LCG) decreased cholesterol synthesis by inhibiting the conversion of lanosterol into cholesterol, increased the incorporation of exogenous cholesterol but it did not affect cell proliferation. High concentration of G (HCG) inhibited cell proliferation, cholesterol and PC biosynthesis and protein prenilation. LCG could affect cholesterolemia by inhibiting cholesterol synthesis at a point beyond the branch of the mevalonate pathway. While with HCG the inhibition in protein prenilation and PC synthesis could result in an antiproliferative effect.

LI-P08.
REGULATION OF CHOLESTEROL METABOLISM BY HIPOTHYROIDISM IN POLYMORPHONUCLEAR LEUKOCYTES

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The phagocytes are the first line of defense against infectious agents and their lipid composition is very important for their live and functions. The aim of this study was to assess the hypothyroidism effect on the cholesterol content in polymorphonuclear leukocytes (PMN). Methods: Female Wistar rats were separated in control (Co) and hypothyroid (HT) groups. The hypothyroidism was induced by administration of 0.1g/L PTU in the drinking water. Food and water were administrated ad libitum. HT and Co rats were killed by decapitation after 30 days treatment for blood recollection. In serum were assessed by enzymatic methods total (TC) and HDL cholesterol. LDLcholesterol was calculated. PMN were isolated from blood by gradient density to determine in them phospholipids (Fisk, 1929), total, free (FC) and esterified (EC) cholesterol (Zack, 1954) separated by thin-layer chromatography. LDL–R and HMGCoaR mRNA expression were measured in PMN. Results: LDL–col and TC were increased in HT group .The HDL–Col levels did not differ. The phospholipids content showed no differences, but the cholesterol levels (TC, FC and EC) were significantly low in PMN of HT group. The HMG–CoAR and LDL–R mRNA levels were diminished and not modificates, respectively, in PMN of HT. According to our results, the hypothyroidism modifies cholesterol composition of PMN leukocytes, by regulation of its metabolism.

LI-P09.
BINDING OF RECONSTITUTED DISCOIDAL HIGH DENSITY LIPOPROTEINS IN TWO DIFFERENT LINE CELLS. INFLUENCE

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Discoidal high density lipoproteins (dHDL) of apolipoprotein A-I (apoAI) are very important intermediates in the antiatherogenic reverse cholesterol transport mechanism and the genesis of mature HDL in circulation. We have previously shown with reconstituted dHDL that the conformation of apoAI in these lipoprotein complexes is highly dependent on their size and cholesterol content, and also that the ability to interact and exchange cholesterol with artificial membranes decreases as the size and cholesterol content of dHDL increase. More recent results from our lab showing that cholesterol efflux from CHOK1 cells is also inversely correlated with dHDL size and cholesterol content. We have used here an enzyme-linked immunoassay directly on adsorbed cells (ELISA test) to measure the ability of dHDL for binding to murine RAW 264.7 macrophages and CHOK1 epithelial cells in comparison with lipid-free apoAI. In RAW cells small sized dHDL (78 Å diameter) binds to these cells with the same efficiency as lipid-free apoAI. For cholesterol-free dHDL, binding efficiency is higher for larger complexes (96 and 120 Å). The presence of cholesterol in dHDL has no influence on the binding efficiency of the small 78 Å complexes, but it was decreased in the large complexes. In CHOK1 cells binding efficiency was found to be higher than that in lipid-free apoAI.

LI-P10.
ROFECOXIB PREVENTS DIMETHOATE-INDUCED INHIBITION OF TESTOSTERONE BIOSYNTHESIS IN RAT LEYDIG CELLS

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Previous work from our lab demonstrated that administration of dimethoate (D) to rats increased COX-2 expression, decreased transcription and translation of StAR protein, and increased the level of PGE2 and PGF2α. Concomitantly, plasma LH and FSH was increased while testosterone was diminished compared to control. These alterations were paralleled by an oxidative stress condition (OS) induced by D. In order to study the contribution of prostaglandin level and the OS in the mechanism(s) involved in testosterone biosynthesis inhibition, we treated rats simultaneously with D and either a-tocoferyldiacetate (T) or rofecoxib (R) at doses that neutralized lipid peroxidation and COX-2 activity, respectively. T injection reduced OS; however, hormonal changes induced by D persisted. The decreased level of arachidonate (A) observed under D treatment was not normalized by T association. In contrast, injection of R raised the level of A, decreased PGF2α production, and normalized hormonal level although the production of lipoperoxides still persisted elevated. When D, T and R were administered in combination all the parameters returned to control values. The main conclusion was that there was a compensation of the opposite effects caused by D and R, being the contribution of prostaglandin biosynthesis the main target of the alteration evoked by D on the androgenic status.

LI-P11.
PURIFICATION AND CHARACTERIZATION OF SPIDER VITELLINS FROM THE EGGS OF *Polybetes pythagoricus*

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Vitellins are essential ovigerous species for embryogenesis and viability of progeny, but there virtually no information in arachnids. The present work aims to purify and characterize the vitellins from *P. pythagoricus* spiders. Lipoproteins were isolated by ultracentrifugation and their lipids, proteins and carbohydrates moieties analyzed by a combination of chromatography, electrophoresis and immunology. Two vitellins of high and very high density, with 36.0 and 15.6 % total lipids, respectively were isolated, the HDL named lipovitellin1 (LV1) and the VHDL, LV2. Phospholipids were the major components in both, representing 90.7 and 45.5% of total lipids, respectively. Major fatty acids were 18:1>18:2>16:0>18:0 in LV1, and 16:0>18:1>18:2>18:0 in LV2. Native LV1 has a MW of 680 KDa, and 2 apolipoproteins of 74 and 25 kDa. Both LVs are glycosylated with 1.48 and 0.69 % by wt total carbohydrates, in LV1 and LV2, respectively. Native LV2 is more heterogeneous with 3 particles of MW 571, 400 and 270 kDa. The 3 have the same apolipoproteín composition with 3 subunits of 181, 93 and 60 KDa, though in different ratios. Espectrophotometric and Dot blot analysis discarded the presence of the respiratory pigment hemocyanin in both, an important component in adult spiders circulating lipoproteins. LV1 and LV2 are the first egg lipoproteins described in Arachnids other than mites.

LI-P12.
CHARACTERIZATION OF A TRIGLYCERIDE-LIPASE AND ITS NATURAL SUBSTRATES FROM CRUSTACEAN HEPATOPANCREAS

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The aim of this work was to purify and characterize a citosolic triglyceride-lipase (lipase) activity and its natural substrates from the hepatopancreas of *Macrobrachium borellii*. Purification was followed by a zymographic assay and the esterase activity was confirmed by the hydrolysis of ^{14}C-TG. The optimal temperature and pH values were 36°C and 8.0, respectively. The kinetic characterization using pNPP as substrate showed a Michaelis-Menten behavior with a Vmax=3.4x10^{-4} erization of a lipase and its natural substrates in crustaceans.umol.min-1.mg-1 and a Km=1.63 mM. A MW of 72 KDa was estimated by PAGE. The TGs species from *M. borellii* hepatopancreas were studied by silver nitrate chromatography allowing the separation of several groups of increasing R*f* hereafter named group I-IV. Fatty acid (FA) composition of each TG group was analyzed by capillary GC. Group I was the major PUFA-containing one (48.5%) followed by group II (45.5%). Group III was dominated by monounsaturated FA (58.3%), while group IV had both monounsaturated and saturated FA (50.0% and 45.6%, respectively).
Finally substrate specificity of lipase was determined by competence of Group I and different non-radiolabeled TGs on the rate of hydrolysis of labeled triolein. The enzyme showed higher specificity toward TG PUFA-containing species than toward saturated TGs. This is the first purification and characterization of a lipase and its natural substrates in crustaceans.

LI-P13.
EFFECTS OF COPPER OVERLOAD ON THE SURVIVAL OF HUMAN DERIVED CELLS IN CULTURE (HEPG2 AND A549)

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Copper (Cu) may cause important alterations in the antioxidant defence system on human cell lines. In this work we reported differential responses by Cu overload (0-180 nM/24 h) in HepG2 and A549 evaluated through various parameters involved in apoptosis or necrosis. For both lines, the rate of cellular growing was depressed in a concentration-dependent way which was more evident in HepG2. However, it showed shorter estimated mitosis times than A549. The changes were associated to an increased mili- and microcalpaine activity which was higher for HepG2. Caspase-3 activity was raised at different doses (40 and 80 µM Cu, for A459 and HepG2 respectively). LDH was measured as a function of Cu overload and demonstrated that necrosis is linear in the case of HepG2, while for A549 the response seems to be sigmoid. Cu was accumulated in both cells, being more important in the case of A549. These cells also exhibited a slow elevation of ceruloplasmine. The increase of metallothionein was more evident in HepG2. Results suggest that Cu overload induces both apoptosis and necrosis; however, in HepG2 the necrotic mechanism should be more prevalent. Modifications in cell survival induced by Cu may be important for the understanding of damage produced by involuntary exposition to this cation, and also in the ethiology of illnesses associated to Cu overload such as neurodegenerative disorders.

LI-P14.
DIFFERENTIAL RESPONSE OF CULTURED HUMAN CELLS EXPOSED TO COPPER OVERLOAD AND NATURAL POLYPHENOLS

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The overload of copper (Cu) induces a failure in the antioxidant defence system. We studied the effect caused by the association of Cu with natural polyphenols. HepG2 (liver) and A549 (lung) derived human cells were treated with Cu 100 µM/24-48 h and simultaneous or differed supplementation with 100cafeic acid (CA), resveratrol (RES), or curcumin (CR) (100 µM). All the treatments reduced the total cellular protein and the cellular viability, except for the CR-treated cells. Cells were homogeinized and analyzed for nitrates + nitrites, TBARS, and protein carbonyls as biomarkers for oxidative stress. All the parameters were elevated with the time of treatment under Cu supplementation, being more pronounced in HepG2 than A549. Association with CA or RES produced an increment in the level of damage observed on both type of cells. Again, the degree of damage was more evident in HepG2. In contrast, inclusion of CR ameliorates the oxidative stress condition induced by Cu, especially in A549. Glutathione was increased only in HepG2 in response to the damages evoked by the association of Cu with CA or RES. Results suggest that it should be crucial to consider the chemical structure of polyphenols as antioxidants to prevent the damages associated to Cu overload. CR might be protective, while RES or CA could be pro-oxidants in association with non-physiological Cu levels.

LI-P15.
FRECUENTELY-USED AGROCHEMICALS LEAD TO FUNCTIONAL AND MORPHOLOGICAL SPERM ALTERATIONS IN RAT

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A reproductive health decline has been incremented in the last two decades. It seems to be a multi-factorial event that involves genetic and environmental factors. Nowadays, it is well established the relationship between male infertility and oxidative-nitrative stress induced by pollutants. Previous works of our lab demonstrated that low-doses of widely used pesticides seriously impaired the testicular antioxidant defense system. In this work we treated male Wistar rats sub-chronically (5 weeks) with low doses (1/250 LD50) of a mixture of zineb (Z), glyphosate (G) and dimethoate (D). Alterations in mature sperm vitality and morphology were evaluated. In the group treated with the mixture of Z, G and D the percentage of vital mature sperm decreased. Concomitantly, agonic and dead sperm increased compared with controls. The treated group showed a 20 % decrease in acrosomal reaction ability while fructose and free tiols were increased by 30 and 40 %, respectively. The number of morphologic alterations at sperm head, body and tail were also higher in the treated group. Taking into account the low doses that provoked these sperm alterations, environmental pollution with agrochemicals may play a key role, increasing fertility abnormalities since 1980's.

LI-P16.
OXIDATIVE STRESS IN WOMEN USING INTRAUTERINE DEVICE TCU380A

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Copper intrauterine device (Cu-IUD) is a highly effective contraceptive method in women, considered the most effective contraceptive strategy now available. Copper ions (Cu) participate in the Häber-Weiss reaction to produce ROS. It is known that an excess of ROS can cause oxidation of crucial biological macromolecules (proteins, lipids, DNA), leading to metabolic damage. Some authors have demonstrated an elevation of Cu plasma concentration in women using Cu-IUD.The purpose of this study was to measure Cu concentration in plasma of women using Cu-IUD and test (i) the effect on the oxidative stress biomarkers, (ii) the levels of Cu transport proteins in plasma - ceruloplasmine (CRP), metallothioneine (MTs)-, and (iii) the status of some liver damage markers, in relation with the time of using the Cu-IUD. Cu concentration was increased in women using Cu-IUD, with respect to the controls, concomitantly with main oxidative stress biomarkers (TBARs, protein carbonyls, glutathione and nitrates+nitrites), hepatic enzymes (LDH, transaminases), MTs and CRP, in a time-dependent way. It is known that the increase of Cu may be in direct relationship with the increase of atherogenic risk and neurodegenerative disease. Thus, the present results show the importance of going into studies about the use of Cu-IUD in order to elucidate the possible risk of employing this contraceptive strategy.

LI-P17.
ENZIMATIC ACTIVITIES RELATED TO PHOSPHATIDIC ACID METABOLIZATION IN RAT LIVER NUCLEI

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Lipid phosphate phosphatases (LPP), diacylglyceride lipase (DAGL) and monoacylglyceride lipase (MAGL) activities which are capable of generating diacylglycerol (DAG), monoacylglycerol (MAG) and glycerol have been demonstrated in liver nuclei. The aim of the present work was to characterize the phosphatidic acid (PA) metabolization in liver nuclear fraction. To this end, adult (4 mo) rat liver was homogenized, and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Nuclear preparations were checked for purity by electron microscopy and DAPI stain. Our results indicate that triton X-100 (0.2mM) increased the generation of MAG and water soluble products (WSP) by 74% and 91%, respectively. An increase of 41% in DAG production was observed at 1mM of the detergent. Ca^{2+} (1mM) and Mg^{2+} (4mM) ions stimulated MAG formation by 60%. These ions also produced an increase in the formation of WSP. Furthermore, NaF (50mM) diminished DAG and MAG generation by a similar percentage (67%), with an increase of WSP (142%). However, when ^{3}H-MAG was used as substrate, MAGL activity showed to be inhibited not only by NaF, but also by oleate and RHC 80267. These results demonstrate the presence of important signaling pathways generated from PA in rat liver nuclei.

LI-P18.
MODULATION OF PHOSPHATIDIC ACID METABOLIC PATHWAYS IN CEREBELAR NUCLEI

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We have demonstrated the existence of lipid phosphate phosphatases (LPP), diacylglyceride lipase (DAGL) and monoacylglyceride lipase (MAGL) activities which generate second messengers implicated in intranuclear signaling. The present work evaluates the modulation of the nuclear enzymatic activities related to the metabolization of phosphatidic acid (PA). To this end, adult (4 mo) rat cerebellum was homogenized, and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Nuclear preparations were checked for purity by electron microscopy and DAPI stain. Our results show that 0.2 mM triton X-100 increased monoacylglyceride (MAG) production by 219% without modifying diacylglyceride (DAG) production. However, 1 mM triton X-100 caused an increase of 100% in DAG and MAG production. Ca^{2+} and Mg^{2+} ions stimulated MAG formation by 190%. Furthermore, 50 mM NaF diminished DAG and MAG generation by a similar percentage (95%). On the other hand, vanadate decreased DAG production by 42% and produced a 30% inhibition in MAG generation. Additionally, we observed MAGL, LPA-phosphohydrolase (LPAPase) and LPA-phospholipase (LPAase) activities by using 2-arachidonoyl[^{3}H]glycerol and 1-oleoyl[^{3}H]-lysophosphatidic acid as substrates. These results demonstrate the presence of important signaling pathways in rat cerebelar nuclei.

LI-P19.
CHANGES IN THE LIPID PATTERN WITH FUNCTIONAL SENESCENCE IN *Ceratitis capitata* (DIPTERA, TEPHRITIDAE)

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We postulate that the physiological state of an insect could be determined independently of its chronological age, through evaluation of behavioural, cellular and molecular indicators. The main orchard pest, *Ceratitis capitata*, was used as biological model to study functional senescence. As general indicator of the normal state of tissues we determined the corresponding profile of structural, storage and signal lipids. Thus, the demographic parameters for single-sex flies populations maintained under standard laboratory conditions (23ºC, 60% RH, L:D 16:8 h), were correlated with age-dependent changes in lipid composition that may reflect the physiological decline. The components of main lipid categories of fly populations of different ages were analyzed by quantitative and semi-quantitative methods. Using multivariate analysis we found significant changes in the proportion of lipid categories with age, which were condensed in a reduced variable. At the same time, we performed behavioural experiments to correlate the decline in locomotor activity with these studies and with age. We determined an age-dependent reduction in negative geotaxis behaviour, as well as a trend to spontaneously remain at the bottom of the flasks. Differences between sexes in demographic, biochemical and behavioural parameters were found.

LI-P20.
ACTIVATION OF PHOSPHOLIPASE A_2 IN A RETINAL MODEL OF NEURODEGENERATION

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Transitions metals, considered as neurotoxic agents, generate an unbalance in cellular redox mechanisms, producing lipid peroxidation, mitochondrial damage, and cell death. In this work, we characterized the participation of phospholipase A_2 (PLA_2) in an experimental model of age-related macular degeneration (AMD). For this purpose, we used bovine retinas exposed to increasing Fe^{2+} concentrations (25, 200 and 800 µM), and we determined mitochondrial function (measured as MTT reduction) and lipid peroxidation levels (determined by TBARS). The activity of $cPLA_2$ and $iPLA_2$ was measured using palmitoyl-[^{14}C]arachidonoyl- and [^{14}C]dipalmitoyl-phosphatidylcholine as exogenous substrates, respectively. Fe^{2+} induced a concentration-dependent decrease in mitochondrial function after 1 h of Fe^{2+}-exposure (36% and 45% of MTT reduction for 200 and 800 µM, respectively). TBARS generation was significantly increased at all the concentrations assayed both in membrane and soluble fractions. This was associated with the activation of ERK1/2 and tyrosine kinases. Microsomal and cytosolic $cPLA_2$ activities were stimulated by 30% and 200% after 1 h of metal exposure. $iPLA_2$ activity was stimulated by 30% with respect to controls. Results suggest that this is an appropriate model to study the role of iron-induced neurotoxicity and to evaluate the role of PLA_2 in an experimental model of AMD.

LI-P21.
AGE-ASSOCIATED CHANGES IN THE LIPID COMPOSITION OF SYNAPTIC MEMBRANE RAFTS
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Membrane rafts act as signal transduction platforms in many physiological processes. In this study we determined the lipid composition of cerebral cortex synaptic membrane rafts (SMR) obtained from adult (4 month old) and aged (28 month old) rats. SMR showed an enrichment in cholesterol and sphingomyelin (SM) content with respect to isolated synaptic endings (Syn). Additionally, SM content increased by 36% in SMR obtained from aged animals with respect to adults. Both SMR preparations showed an enrichment in phosphatidylcholine (PC), and a decrease in phosphatidylethanolamine and phosphatidylserine content (by 28%, 25% and 25% respectively) with respect to Syn. Phospholipid-fatty acid composition from SMR presented an increase in 16:0 (35%), and a decrease in 20:4n-6 (67%) and 22:6n-3 (68%) content in adults. The most striking changes in fatty acid composition were mainly due to changes in PC. Similar changes in fatty acid composition were observed between SMR and Syn obtained from aged rats. Nevertheless, the 20:4n-6 and 22:6n-3 content significantly decreased (80%) with respect to SMR from adults. SMR displayed a more saturated fatty acid composition than Syn and this effect was even more pronounced in aged animals. Our results suggest that the distinct fatty acyl composition in SMR could be responsible for the signaling impairment observed in nervous system during aging.

LI-P22.
GENERATION OF CHIMERICAL FATTY ACID DESATURASES WITH BIFUNCTIONAL Δ12/Δ15 REGIOSELECTIVITY
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We have previously characterized Δ12 and Δ15 desaturases from *Leishmania major*, respectively involved in conversion of oleoyl- to linoleoyl- and linoleoyl- to linolenyl-moieties of parasite´s phospholipids. Both proteins share 76% of identity and 88% of similarity. Analysis of primary structure revealed both a conserved theoretical membrane topology and consensus sequences for the three clusters of histidines, presumed to be part of the active site. However, both desaturases show some significant differences in certain regions, for example in the luminal domain located between the first and second transmembrane helixes or in some parts of the catalytic site. Construction of chimerical desaturases between both enzymes allowed us to generate hybrids that showed a bifunctional activity, catalyzing the conversion of 16:1 to 16:2, 18:1 to 18:2 and 18:2 to 18:3. On the other hand, we constructed some chimeras that totally lack desaturase activity. These results allowed us to identify structural determinants involved in substrate discrimination and (or) regioselectivity. A small N-terminal domain from Δ15 desaturase is sufficient to create a bifunctional enzyme when replacing the equivalent region in Δ12 desaturase. These findings are relevant from academic and biotechnological perspectives.
This work is dedicated to the memory of Dr. Rodolfo A. UGALDE

LI-P23.
MODIFICATION OF SPHINGOBASES IN TRYPANOSOMATIDS
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Sphingolipids (Spl) are important class of lipids and essential components of eukaryotic membranes. In addition to their structural role, they are involved in cellular processes such as growth regulation, apoptosis and stress response. Previously, we cloned a δ4-Spl Des from *Trypanosoma brucei* and characterized it as a pure desaturase, in contrast to bifunctional desaturases/hydroxylases. In this work, we cloned the orthologous enzyme of *T. cruzi* in pYES2, and analyzed the enzymatic activity in a yeast mutant for long chain base (LCB) hydroxylase (sur2), being typified also as a desaturase. Since parasites are subjected to changes throughout their lives cycles, it is possible that Spl are implicated. After the examination of the LCB content of parasite membranes, we found an important increase in the levels of unsaturated versus saturated 18 C LCB in bloodstream *T. brucei*, in comparison with procyclic form. This level is intermediate in epimastigotes of *T. cruzi*. These results may indicate some type of regulation of the δ4-SplDes. Finally, we investigated the effect of GT11, a strong inhibitor of δ4-SplDes activity, on different cultures of trypanosomes. Interestingly, whereas epimastigotes of *T. cruzi* remained growing albeit the activity of δ4-SplDes droped to the 20% of untreated cultures, bloodstream *T. brucei* arrested their growth after 24 hs in presence of 10 μM Gt11.

MI-P01.

A COMBINED *IN SILICO-IN VIVO* SCREENING STRATEGY TO IDENTIFY *Brucella abortus* EFFECTOR PROTEINS

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Brucella abortus is a facultative intracellular pathogen that replicates inside mammalian phagocytes. A type IV secretion system is known to be essential to subvert lysosome fusion and to create an organelle that supports replication, probably by means of effector protein translocation.
Our main goal is to identify and characterize these proteins using an *in silico* approach. We searched *B. abortus 2308* genome for proteins with certain properties that could make them good candidates for secretion, including: shared homology with eukaryotic domains, conserved genes in γ-proteobacteria, special location in the genome, shared homology with known effectors in related species and presence of certain secondary structures known for their role in protein-protein interaction. As a result, we found 85 proteins, each containing at least one of the mentioned properties. These proteins were cloned in a pBBR4 derived vector specially designed to make fusions to the catalytic domain of the *Bordetella pertussis* Adenilate Cyclase, a reporter system that proved to be successful for our goal. These constructs are currently under screening to determine those proteins that are actually translocated into the host cell. Fusion proteins positive for translocation under wild type genetic background that result negative under an isogenic VirB null background, will be identified as potential VirB effectors.

MI-P02.

BEP-1, A *Brucella* VirB EFFECTOR THAT PROMOTES CORTICAL ACTIN REORGANIZATION

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Brucella abortus is an intracellular pathogen that replicates inside mammalian cells. A type IV secretion system (VirB) is essential to subvert lysosome fusion and to create an organelle that supports *Brucella* replication inside host cells. Translocation of effector proteins via VirB likely modulates host vesicular traffic, allowing the biogenesis of the endoplasmic reticulum-derived replicative organelle.
Translocation of potential substrates into host cells was assayed using the *Bordetella pertussis* Adenylate Cyclase (CyaA) fusion approach. A *B. abortus* protein that is translocated to the host cell via VirB was identified and called BEP-1. We could also determine that substrate recognition by VirB machinery involves an N-terminal translocation signal.
BEP-1 shows similarity to the helix domain of the eukaryotic protein cortactin. Cortactin is an actin-binding protein and a central regulator of the actin cytoskeleton. It has been involved in many cellular processes including cortical actin assembly, endocytosis, and cell migration. Importantly, cortactin is also a common target exploited by many microbes during infection. Interestingly, BEP-1 ectopically expressed in HeLa cells promotes lamellipodia formation and colocalizes with cortical actin and cortactin, suggesting that the identified VirB substrate might be interacting with the host cell actin cytoskeleton.

MI-P03.

THE ORNITHINE LIPID OF *Brucella abortus* AND ITS ROLE IN PHYSIOLOGY AND VIRULENCE

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The main cell envelope (CE) lipids from *Brucella abortus* are PC, PE and the aminolipid OL (25-30% of each one). The genes *olsB* and *olsA* are involved in the two-step reaction that converts ornithine in OL. In order to understand the role that OL plays in the physiology and virulence of this pathogen, the *B. abortus* genes were identified and disrupted. *B. abortus olsB* (*Ba olsB*) and *Ba olsA* mutants are unable to synthesize OL whereas the latter accumulate the intermediate lyso-OL (LOL).
Absence of OL does not affect viability, but the mutants display several phenotypic traits such as an increased bacterial growth rate under nitrogen deprived medium, membrane proteome alterations and reduced virulence in an intragastric mice infection model. Remarkably, the *Ba olsB* strain over-expressed a response regulator belonging to the NtrXY two-component system, which is involved in nitrogen metabolism. Some of these features could be explained by the increased polyamine synthesis observed in the absence of OL, which is a consequence of the augmented supply of ornithine.
The structural similarity of OL with the Lipid A suggests that it could exert some immunomodulatory effect on the immune system. Indeed, mice splenocytes treated with OL were unable to proliferate upon stimulation with *E. coli* LPS.
Altogether, these results suggest that OL is implicated in the virulence performance of *Brucella*.

MI-P04.

Mce2R REGULATES EXCLUSIVELY THE TRANSCRIPTION OF Mce2 OPERON AMONG *mce* GENES

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The mce2 operon is one of the four mce operons present in Mycobacterium tuberculosis that encode exported proteins with a probable role in the virulence mechanisms of this bacterium. In a previous study we demonstrated that Rv0586, which encodes a putative GntR-like regulator, is part of the mce2 operon. Moreover, we found that the product of this gene represses the expression of Mce2 proteins (Santangelo et al. 2009). For this reason, we have re-named the repressor protein Mce2R. In order to find other proteins regulated by Mce2R, in the present study we performed microarrays experiments comparing the expression profile of an M. tuberculosis mutant strain in which the Rv0586 gene was disrupted with the one from the parental strain. The results obtained from this analysis were also validated by real time PCR. We found that Mce2R represses only the expression of the mce2 operon, but not the one from the other highly homologous mce1, mce3 and mce4 operons. These results add novel evidence of the regulation of mce operon expression. In addition, the comparative lipid profile of cell extract from the mutant, the complemented and the wild type strains did not show any differences, indicating that genes involved in lipid metabolism are not co regulated by Mce2R as it has been previously observed for Mce3R, the regulator of mce3 operon.

MI-P05.
IL-8 LEVELS IN GASTRIC MUCOSA: EFFECT OF *H. pylori* VIRULENCE FACTORS AND HOST POLYMORPHISM -251A/T

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This study was aimed to investigate the correlation of IL-8 level in gastric mucosa and *H. pylori* (Hp) putative virulence factors (cag-PAI, oipA and dupA genes) status, in addition to the -251 A/T polymorphism of host IL-8 gene promoter analysis. A total of 113 patients were studied, 42 were Hp-positive and antral-corpus multiple-biopsies were analysed (224 samples). DNA was extracted from host biopsies and Hp isolates. cag-PAI status was determined by PCR, oipA and dupA genes status were determined by PCR and sequencing analysis. IL-8 levels in each biopsy's supernatants were measured by ELISA, including patients Hp-positive and Hp-negative. IL-8 promoter polymorphism was analysed by RFLP-PCR. For statistical analysis, SPSS program version 16 was used. Gastric mucosa IL-8 level seemed to increase with the presence of isolates showing complete cag island and oipA "on" status ($p<0.05$). However, IL-8 did not seem to be affected by dupA status. Considering host IL-8 promoter polymorphism in patients colonized with isolates carrying complete cagPAI and oipA "on", those exhibiting -251 A/A and A/T showed antral mucosa IL-8 level 40% higher that individuals -251 T/T. No difference in IL-8 level was observed in corpus biopsies. These findings highlight the importance of host factors status and Hp virulence factors in the inflammatory response in mucosa, leading to gastric disease.

MI-P06.
ORAL SUBLETHAL INFECTION WITH *Salmonella* AS A MODEL TO STUDY REACTIVE ARTHRITIS

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We have recently described the sequelae of a single, low dose of virulent *S.* enteritidis in a murine model. BALB/c mice inoculated intragastrically with a sublethal dose of 10^3 cfu of *S.* Enteritidis showed changes in the gut that persist for at least 21 days. Histological studies revealed diffuse mild enteritis showing an intestinal epithelium diminished in height. Q-PCR amplifications demonstrated that, compared to control mice, the expression of intestinal COX-2 is 3-fold increased whereas IL-17 is 2 times higher. Intestinal inflammation induced by *Salmonella* led to joint tissues changes (moderate hyperplasia of the synovial membrane). Based on these findings we tested the effect of probiotic treatment (*L.* delbrueckii, *S.* thermophilus and *L*. casei) on the changes produced by *Salmonella* in our model. Four groups of mice were studied: i) mice fed with probiotic strains ad libitum for 7 days and challenged at day 8 with *Salmonella*, ii) mice fed with *Lactobacillus* without bacteria, iii) mice infected without probiotic treatment, and iv) untreated animals. All mice were sacrificed at day 21 post infection. Results showed that previous treatment with probiotic ameliorates the *Salmonella* induced intestinal mucosal oedema and the overall diffuse enteritis. Interestingly, the damaging effect of *Salmonella* infection in the joints was significantly reduced in probiotic-fed animals.

MI-P07.
AUXOTOPHIC MUTANT OF *Staphylococcus aureus* AS A POTENTIAL IMMUNOPROPHYLACTIC TOOL IN THE NASAL CARRIAGE

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S. aureus nasal carriage is a risk factor for infection in humans, particularly in the hospital environment. The active colonization with a strain of *S. aureus* which possess minimal pathogenic properties is able to prevent colonization by more virulent strains. In this work auxotophic mutant of *S. aureus* Newman strain was constructed by genetic replace. The selected working mutant was called NK41 and showed to be attenuated and stable. In a murine model, previous NK41 nasal colonization significantly reduces the implantation of the most prevalent clones of *S. aureus* in Argentina [pediatric (Hde288/1743) and cordobes (HU-71/1743) clones]. In the same way, NK41 was evaluated in a human nasal in vitro environment on A549 cell line. The mutant showed the same level of internalization that its parental Newman strain. However, inside the cells, the effect of NK41 differs from Newman causing not cellular damage and inability to trigger the production of the pro-inflammatory interleukins IL-6 and IL-8. Moreover, NK41 was able to interfere the invasion on A549 cell line of Newman, Hde288/1743 and HU-71/1743 strains of *S. aureus*. These results encourage the possibility of local topical applications with the mutant NK41 to avoid, through bacterial interference, the entrance of other pathogenic strains of *S. aureus* in hospitalized patients, without affect the nasal microenvironment.

MI-P08.
CHARACTERIZATION OF *Yersinia* OMPs AS IMMUNOGENIC COMPONENTS IN *Yersinia*-INDUCED ARTHRITIS

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Reactive arthritis (ReA) is an aseptic synovitis that follows an extra-articular infection. *Yersinia enterocolitica* causes intestinal infection which could be complicated by ReA. Since the pathogenesis of ReA is incompletely understood, appropriate treatments are not available. We previously demonstrate that *TNFRp55*$^{-/-}$ mice develop *Yersinia*-induced arthritis, and *Yersinia* outer membrane (OM) is the relevant bacterial fraction triggering ReA. The purpose of the present work was to characterize the bacterial proteins that induce antibody response in the joint. *TNFRp55*$^{-/-}$ mice were orally infected with *Y. enterocolitica* O:3. At 21 days after infection, antibody response to OM proteins was studied by Western blot of the joint extracts. OM proteins were separated by SDS-polyacrylamide 12% gel and stained with Coomasie blue. The protein bands were excised and sent for MALDI-TOF mass spectrometry analysis. Mass data collected during MALDI-TOF analysis were processed and submitted to the search software MASCOT. By Western blot, reactions to 35 and 40 kDa OM proteins were detected. Database searches of peptide mass fingerprinting indicated *Yersinia* OmpA and OmpC proteins as the 35 and 40 kDa immunoreactive proteins, respectively. We concluded that OmpA and OmpC correspond to the immunogenic OM in the joint of arthritic *TNFRp55*$^{-/-}$ mice.

MI-P09.
IDENTIFICATION AND CHARACTERIZATION OF VIRULENCE FACTORS IN *Brucella abortus*
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Brucella abortus is a gram-negative, intracellular bacterium, responsible of brucellosis, a worldwide zoonosis of veterinary and human concern. Virulence of this pathogen is associated with the ability to survive and replicate within professional and non-professional phagocytes. With the aim of finding and characterizing virulence factors, we used a bioinformatic approach to search for potential proteins that might play a role in the intracellular survival. The candidates were fused to an antigenic epitope, expressed in *B. abortus* and its expression and localization analyzed by western-blotting, and indirect immunofluorescence in infection assays. We found that ORF Bab1_1492, that codes for a hypothetical protein with a molecular weight of 24 Kda, is secreted during the intracellular life cycle and that it localizes to the *Brucella* containing vacuole. Sequence analysis showed that it has no potential transmembrane domains or a canonical signal peptide. Analysis of the promoter allowed us to identify a potential secondary structure that could play a role in the transcriptional/translational regulation. We cloned the ORF with and without a 250 bp upstream region in a replicative plasmid and analyzed expression in *E. coli* and *B. abortus*. Our results suggest that this upstream region may play a role in the transcriptional regulation of this hypothetical protein.

MI-P10.
IDENTIFICATION OF A PATHOGENICITY ISLAND OF *Brucella abortus* INVOLVED IN THE INVASION OF HOST CELLS
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Brucella abortus is a facultative intracellular pathogen that belongs to the alpha proteobacteria group and causes brucellosis, a worldwide zoonosis important for animal breading and of human health impact in endemic areas. The virulence of *Brucella* is dependent on the capacity of the bacteria to invade, persist and replicate within host cells, a task that depends on the presence of specialized virulence factors. With the aim of finding and characterizing novel virulence factors, we used a bioinformatic approach to search for potential proteins that might play a role in the intracellular survival. Our search identified a pathogenicity island that harbours four open reading frames, one of which (Bab1_2009) has a domain with homology to the intimin of *E. coli* EPEC, a receptor essential for attachment of this pathogen to the host cell. The other three ORFs are unique to *Brucella* and have no significant homology to any proteins in the database. Deletion of the entire island resulted in 10 times less invasion than the wild type parental strain measured by a standard antibiotic protection assay. Moreover, over-expression of Bab1_2009 in the wild type strain rendered a strain that is at least 100 times more efficient in invading either phagocytic as well as non-phagocytic cells. Our results indicate that this novel pathogenicity island is involved in the invasion process of host cells.

MI-P11.
ROLE OF SIGNAL TRANSDUCTION SYSTEMS IN BIOFILM FORMATION OF *Streptococcus pneumoniae*
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Biofilm formation plays an important role in bacterial pathogenesis. In Streptococcus pneumoniae, it has been demonstrated that biofilm development is associated with nasopharyngeal carriage and otitis media pathogenesis. In the present work, we studied the contribution of signal transduction systems to biofilm development of *S. pneumoniae*. For this purpose, we constructed knock-out mutants of the 14 two-component systems and the unique serine/threonine kinase (StkP) and its cognate phosphatase (PhpP). Biofilm formation was evaluated by crystal violet staining of cells growing statically in polystyrene plates. The ritR and the ciaH mutants showed significantly less biofilm formation than the wild-type strain. On the other hand, the php mutant also showed decreased biofilm formation, while the stkP mutant displayed a wild-type strain phenotype. These results indicated that RitR, an orphan response regulator involved in iron metabolism, and CiaH, a histidine kinase, together with the phosphatase PhpP, are required for biofilm development. Because it has been recently reported that PhpP can interact with RitR and positively regulate ritR transcription, we suggest a connection between PhpP, RitR and iron metabolism for the control of biofilm development in *S. Pneumoniae*.

MI-P12.
ROLE OF SRC FAMILY OF TYROSINE KINASES IN *Pseudomonas aeruginosa* INFECTION OF EPITHELIAL CELLS
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Pseudomonas aeruginosa (PA) is an opportunistic pathogen that can cause urinary and respiratory acute infections as well as pulmonary damage and consequent death in patients with cystic fibrosis. More than half of clinical isolates can get internalized into non-phagocytic cells. This suggests an important role of internalization in PA infection. We approached the internalization mechanism using a model of epithelial cells (MDCK) infected with a human isolate of PA. We have shown that PA aggregates are found at MDCK apical surface associated to large host membrane protrusions. After longer infection periods, bacterial aggregates can be seen inside epithelial cells. We have also shown that the PI3K/AKT pathway is important for both protrusion formation and internalization. It has been reported that PI3K is regulated by tyrosine kinases and that PA infection induces a raise of host tyrosine phosphorylation. In the present work we address the role of Src family of tyrosine kinases (SFKs) in PA epithelial cell infection. Our results show that SFKs strongly activates upon PA infection. Inhibition of SFKs diminishes PA internalization while it partially reduces PI3K/AKT activation. Moreover, it profoundly alters protrusion formation. These findings point to an important role of SFKs in PA infection. Current work is aimed at the identification of the SFKs members involved in this process.

MI-P13.
***Bordetella pertussis* SURVIVES AND REPLICATES IN NON ACIDIC COMPARTMENTS IN HUMAN MACROPHAGES**
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Although *B. pertussis* has been observed to survive inside macrophages, its ability to resist or evade degradation in phagolysosomes has not. We here investigated the trafficking of *B. pertussis* upon entry into human macrophages by confocal microscopy. Intracellular bacteria survival was evaluated by Polymyxin B protection assays. During the first hours following phagocytosis a high percentage of bacteria were destroyed within acidic compartments positive for the lysosome-associated membrane proteins (LAMP). However, roughly 1/4 of the uptaken bacteria evade this initial killing event, remaining in non acidic compartments. Forty eight hours after infection the number of intracellular bacteria per cell increased, suggesting that *B. pertussis* is capable of replicating in this type of compartment. Viable bacteria accumulated within phagosomal compartments positive for the early endosomal maker Rab5 but not the late endosomal marker LAMP. Moreover, *B. pertussis*-containing phagosomes acquired exogenously added transferrin, indicating that intracellular bacteria have access to extracellular components and essential nutrients via the host cell recycling pathway. Overall these results suggest that *B. pertussis* survives and replicates in compartments with early-endosomes characteristics, potentially contributing to its extraordinary ability to persist within hosts and populations.

MI-P14.
GENOTYPIC AND PHENOTYPIC TYPING METHODS OF COAGULASE NEGATIVE *Staphylococci* ISOLATED FROM BOVINE MILK
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Coagulase-negative staphylococci (CNS) have begun to be the predominant pathogens causing bovine mastitis. These consequences impose economic losses for the farmers and the dairy industry. The aims of this study were to identify the most common staphylococcal pathogens involved in bovine mastitis using PCR-restriction fragment length polymorphism (RFLP) analysis of a partial *groEL* gene sequence and to compare our results with the identification carried out by the phenotypic test, Staph-Zym. A total of 50 isolates of CNS from bovine milk were analyzed by both methods. The size and number of the fragments obtained by either *AluI* or *HindIII/PvuII* digestions made possible to form clear patterns differentiating, among the isolates. Most of the isolates shown patterns coincident with the reference strains of *S. chromogenes* (15) and of *S. haemolyticus* (11). Compared to the genotypic method, the Staph-Zym test correctly identified 20% of the CNS species. The low sensitivity of the Staph-Zym for most species in our study was mainly caused because this test was developed to identify CNS isolates from human origin. Despite additional testing, many isolates could not be identified to the species level by the Staph-Zym test. Thus, the PCR-RFLP of the *groEL* gene constitutes a reliable and reproducible molecular method for identification of CNS species responsible for bovine mastitis.

MI-P15.
RELATIVE QUANTIFICATION OF *BglA* AND *Bgl* IN *Shewanella* sp. G5 BY REAL TIME PCR
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Shewanella sp. G5 was isolate from the intestinal content of *Munida subrrugosa* in the Beagle Channel (Argentina), and characterized as ß-glucosidases (ßGs) producers using cellobiose as carbon source inductor. ßGs (EC.3.2.1.21) are a heterogeneous group of enzymes classified into of the families 1 and 3 of the glycosyl hydrolases and their study present biotechnological interest. *bglA* and *bgl* genes which encoding two ßGs, were characterized molecularly in previous studies.
The aim of this study was to determine the growth conditions where *bglA* and *bgl* increased its expression level. *Shewanella* sp. G5 was grown in different medium (LB and MMB), temperatures (10 and 30 ºC) and carbon sources (cellobiose and glucose). *bglA*, *bgl* and *gyrB* genes were quantified as relative genetic expression using $2^{-\Delta\Delta Ct}$ method. The amplifications products were verified by standard and dissociation curves and amplot analysis (reporter of Ct). Normalization assays using $2^{-\Delta\Delta Ct}$ method was carried out with *gyrB* housekeeping gene. The best values of $2^{-\Delta\Delta Ct}$ to relative expression for *bglA* and *bgl* were 13, 14, 27 and 15, 19, 30 respectively; obtained in MMB incubated at 10 and 30 ºC using cellobiose as carbon source. Thus, these increased levels of expression in both genes allowed us to establish the optimum growth conditions for the best induction of ßGs in relation to other tested conditions.

MI-P16.
ROLE OF EFFLUX SYSTEMS IN THE PHYSIOLOGY OF *S. epidermidis* MULTIRESISTANT
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The biofilm formation, operon icaADBC expression and the growth speed were analysed in 7 clinical isolates epidemiologically non-related. This isolates showed expression of any efflux system to quinolones (NorA, SepA and/or MdeA). Biofilm formation was determined through the crystal violet quantitative technique in 96 wells plates, the presence of the ica operon was determined by conventional PCR and its expression by Reverse-Transcriptase PCR. Then the growth speed with and without ciprofloxacin was determined in three strains: 2 (expressed MdeA and SepA), 51 (expressed NorA) and 59 (expressed NorA, MdeA and SepA). It was observed that in those isolates that came from the same anatomic site, tose that harbored more that one efflux system, demonstrated by RT-PCR, produced more biofilm. The biofilm production was not related to the expression of the ica operon. Regarding the growth cinetics, it was observed that the isolates that expressed more than two efflux systems reached 108 UFC in less time that those that expressed only one system. It is concluded that in certain situations the expression of an efflux system would give some advantages to survive in the environment where the bacteria inhabits.

MI-P17.
VANCOMYCIN (VAN) AND CIPROFLOXACINE (CIP) ENHANCE BIOFILM FORMATION IN *Staphylococcus aureus*
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Staphylococcus aureus is the causative agent of a diverse array of acute and chronic infections owing to its ability to attach to a surface and form a biofilm. Biofilm is a very important virulence factor and it is involved in the development of device-related infection and chronic infections of tissues. Biofilm in *S. aureus* may be produced in an ica-dependent or ica-independent way. Glycopeptide VAN has been used to treat serious infections caused by methicillin-resistant *S. aureus* strains due to the emergency of resistance to CIP. The aim of this work was to evaluate whether subinhibitory concentrations (SubMIC) of VAN and CIP affect the expression of *S. aureus* virulence factors. Biofilm formation in the Newman strain was enhanced after subMIC of VAN ($p=0.0017$) and CIP ($p<0.0001$) exposures as assessed by spectrophotometric measures. However, the same treatments reduced the polysaccharide capsular production in the Newman strain. Furthermore, after subMIC exposure with those antibiotics we did not observe changes in ica expression. Moreover, subMIC of VAN and CIP were shown to enhance biofilm formation even in an ica-deficient strain. Altogether, our results demonstrate that subMIC of VAN and CIP enhance biofilm formation in an ica-independent way in the Newman strain of *S. Aureus*.

MI-P18.
MOLECULAR MECHANISMS OF CIPROFLOXACIN RESISTANCE IN HYPERMUTATOR STRAINS OF *P. aeruginosa*
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Although ciprofloxacin is one of the most effective antibiotics against *P. aeruginosa* infections, resistance to ciprofloxacin emerge quickly in the clinical setting, and is often acompaigned with cross-resistance to other antibiotics. Two major resistance pathways were described: the disruption of drug-target interaction (by mutations in GyrA and ParC subunits of the target topoisomerases), and the expression of multidrug efflux pumps (by mutations in the transcriptional represor NfxB).
In this work we analyze the molecular nature of ciprofloxacin resistance for the PAO1 wild type strain and the hypermutators *mutS* and *mutT*, defective in the mismatch repair and 8-oxoguanine repair systems respectively. To this purpuse, we sequenced *gyrA, parC* and *nfxB* genes for groups of resistant cells selected at different ciprofloxacin concentrations.
We report the main resistance pathway and mutational spectrum for each strain, and show that *gyrA* is predominantly mutated in cells selected at high ciprofloxacin concentrations, while *nfxB* mutations are dominant al low drug concentration. These *nfxB* mutants showed cross-resistance to other antibiotics such as erythromycin and trimethoprim.
In this context, we discuss the influence of the exposure to low and high doses of ciprofloxacin on the emergence of multidrug resistant strains, both in a wild type and hypermutator background.

MI-P19.
CLONING AND PURIFICATION OF A HYBRID BACTERIOCIN
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Bacteriocins are ribosomally-synthesized antimicrobial peptides produced by Gram (-) and (+) bacteria with antibacterial spectra generally limited to phylogenetically related microbial strains. In order to obtain a broad-spectrum antimicrobial peptide, we carried out a transcriptional fusion between enterocin CRL35 (a bacteriocin produced by *Enterococcus mundtii* CRL35) and colicin V (a microcin produced by *Escherichia coli*) structural genes (*munA-cvaC*). The engineered gene was cloned into the *E.coli* expression vectors, pET28b+ and pET43.1a, the last one carrying a NusA tag and a factor Xa-cleavage site-encoding sequence upstream from the chimeric gene. Both constructions were expressed into *E. coli* BL21 (pLysS) after IPTG induction. MunA-CvaC and NusA-MunA-CvaC resulted in biologically-active fusion proteins. MunA-CvaC was purified by ionic exchange chromatography (SP-sepharose) followed by reversed phase chromatography. NusA-MunA-CvaC was purified by affinity chromatography by means a HiTrap FF Column. To confirm purity and identity, collected active fraction was resolved by SDS-PAGE and revealed with Coomassie stain, western blot (using anti-poly His primary antibody) and antimicrobial activity. The proteolytic cleavage with Factor Xa allowed us to obtain MunA-CvaC protein without extra amino acids in the N-terminal of the molecule.

MI-P20.
***Acinetobacter baylyi* CarO IS A CHANNEL FOR BASIC AMINO ACIDS AND POSITIVELY CHARGED SUBSTRATES**
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CarO, an outer membrane protein (OMP) member of a family only present in the family Moraxellaceae, is related to imipenem (IPM) resistance among clinical Acinetobacter baumannii strains. However, the physiological function of members of the family is still unknown. *Acinetobacter baylyi* represents a valuable genetic tool and useful model to study the role of genus-specific proteins. To obtain clues on a general role of CarO in the genus we previously constructed carO deletion mutants (ÄcarO) and evaluated their growth capabilities on different carbon sources. The mutants exhibited reduced rates of growth on L-arginine and increased resistance to IPM as judged by comparisons of minimal inhibitory concentration (MIC) values towards this antibiotic. MIC values determined for A. baylyi in medium supplemented with different substrates indicated that positively charged amino acids and amines acted as the most effective competitors of IPM uptake. We further characterized A. bayly CarO roles in IPM uptake by kinetic studies using wt and ÄcarO bacteria transformed with a plasmid directing production of a periplasmatic (VIM-2) imipenemase. These studies indicated that A. baylyi CarO provides a saturable, specific OM channel for IPM, and competition for IPM permeation by L-arginine supported the above notion that CarO may serve physiological channel roles for positively charged substrates.

MI-P21.
IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE-NEGATIVE *Staphylococci* FROM BOVINE MILK

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Coagulase-negative staphylococci (CNS) as a group are increasingly seen as important mastitis pathogens. In several recent prevalence studies, CNS were the most frequently isolated udder pathogens from bovine milk samples with high somatic cell count (SCC). To assess the pathogenic significance of individual CNS species and to develop species-specific management practices, accurate species identification is needed. The aims of this study were to identify the most common CNS involved in bovine mastitis and to analize their susceptibility to antimicrobials commonly used for mastitis therapy. A total of 131 CNS isolated from bovine milk in dairy basin of Villa María, Argentine, were identified using a conventional phenotypic method. Sensitivity was determined by Kirby-Bauer method. Results indicated that *S. chromogenes* was the predominant specie (41.7%), followed by *S. haemolyticus* (15.7%). In this study we demonstrated that milk from cows with intramammary infection (IMI) cause by CNS had significantly high SCC. Moreover the high resistance to penicillin and ampicillin associated to most prevalent species found in this study emphasize the importance of identification of CNS when IMI are present.

MI-P22.
FINALS STEPS OF MEGALOMICIN BIOSYNTHESIS. PRODUCTION OF NEW ANALOGUES

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Megalomicins consist of a 14-membered macrolide which have shown antibacterial, antiparasitic and antiviral activities. The biosynthesis of megalomicin A was proposed to be similar to erithromycin D biosynthesis were the finals steps include hydroxylation of erithromycin D catalyzed by Meg K to generate erithromycin C and incorporation of a third sugar, megosamine, by a glycosyltransferase in order to generate megalomicin A. However this pathway was never validated
In this work we have demonstrated that MegDI/MegDVI, the sugar transferases, catalyze the transfer of L-megosamine to either erythromycin C or erythromycin D, establishing two possible routes for the production of megalomicin A.
Flexibility of these enzymes towards the macrolide substrate allowed producing two new C-6-megosaminyl containing macrolides. The antibacterial activity of these new macrolides were tested on gram positive, gram negative bacteria and gram positive bacteria resistant to macrolides, we observed that these compounds have less potency compare to the original molecules.
On the other hand, we have evaluated the activity of these new compounds against P. falsiparum Preliminary data showed that the new compounds are active against P. falsiparum resistant to macrolides which suggest a different mechanism of action of the new compounds in comparison to the original macrolides.

MI-P23.
FIRST REPORT OF PLASMID-MEDIATED AmpC ß-LACTAMASES OF ENTEROBACTERIAS FROM TUCUMÁN. MULTICENTER STUDY

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Introduction: Plasmid-mediated AmpC β-lactamases in enterobacterias is increasing worldwide. Between March and July 2009 a monitoring of AmpC β-lactamases in enterobacterias was implemented in Tucuman; this search was conducted by a multicenter study involving two hospitals and two outpatient centers. The objetive of this work was to detect plasmid AmpC enzymes in extended-spectrum-cephalosporins (ESC) resistant enterobacterias. Materials and methods: of 98 (ESC) resistant strains, those without extended-spectrum β-lactamases (ESBL) and cefoxitin resistant were selected. Synergy test was implemented by aminophenylboronic acid (APB), cefoxitin and cefotaxime disks; enzymatic activity against cefoxitin was confirmed by both, microbiological assay and polymerase chain reaction (PCR) for the detection of plasmid-mediated AMPc genes of different groups. Results: a *P. mirabilis* and three *E. coli* strains presented the right criteria for selection; the APB showed positive synergy, the microbiological assay was positive, and a 462-bp amplicon was obtained when using primers directed against the CIT group in the four strains. Conclusions: the emergency of plasmid-mediated AmpC β-lactamases indicated the importance to implement the sistematic monitoring of these enzymes to optimize the antimicrobial therapy in Tucumán.

MI-P24.
A PERIPLASMIC PROTEIN INFLUENCING GOB METALLO-BETA-LACTAMASE BIOGENESIS IN GRAM NEGATIVE BACTERIA

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Metallo-b-lactamases (MbL) can hydrolyze most clinically employed b-lactam antibiotics, representing the main mechanism of resistance in Gram-negative pathogens. We have recently reported that GOB MbL is secreted in *E. coli* by the Sec machinery in an extended conformation, adopting the proper folding and Zn (II) ion in the bacterial periplasm. The aim of our work is the identification and cooperation of proteins influencing GOB biogenesis in the periplasm of Gram-negative bacteria. For this purpose we expressed the *gob-18* gene in *Salmonella enterica* and used MudJ random mutagenesis, searching for insertion mutants displaying decreased cefotaxime (CTX) resistance. One of these mutants was affected in the expression of dacD, which codes for a periplasmic protein proposed to act as a penicillin-binding protein with poor DD-carboxypeptidase activity. The mutation produced no effect on the intrinsic CTX resistance of the bacteria. The *Escherichia coli* DdacD mutant expressing gob-18 also showed decreased CTX resistance. Analysis of periplasmic fractions from wild type and DdacD cells by SDS-PAGE indicated several changes in the periplasmatic protein profile of the mutant, and immunoblots with anti-GOB revealed lower quantities of this protein in DdacD cells. The overall results suggest a new function for DacD in either the secretion or stability of MbLs in the bacterial periplasm.

MI-P25.
FUNCTIONAL CHARACTERIZATION OF A NOVEL *Acinetobacter baumanii* INSERTION SEQUENCE, ISAba825
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We previously showed that *A. baumannii* CarO functions as an outer membrane channel for basic amino acids and carbapenem b-lactam antibiotics, and that its loss is correlated with carbapenem resistance. The natural inactivation of carO by insertion elements such as ISAba825, was identified as responsible for CarO loss indicating that the element is involved in *A. baumannii* genome plasticity. We evaluated here by PCR the distribution of ISAba825 among the genus Acinetobacter and other clinical bacterial species sharing habitats with *A. baumannii*. Among different strains analyzed, including members of the *Acinetobacter* genus as well as enterobacterial clinical strains and *P. aeruginosa*, we detected ISAba825 only in Acinetobacter lwoffii. For further studies, we tagged ISAba825 with a kanamycin resistance gene cassette and cloned this construction into a conditionally suicide plasmid (AmpR) for *A. baumannii* and *E. coli*. The selection of kanamycin-resistant, ampicillin-sensitive colonies of these bacteria after transformation and plating was used as a criterion for identifying transposition events. We could demonstrate by this approach transposition of ISAba825::Kn in *A. baumannii* but not in *E. coli*. The overall results indicated that ISAba825 could transpose only in some members of the genus Acinetobacter and that either *A. baumannii* or *A. lwoffii* could act as reservoir of the element.

MI-P26.
PHYSIOLOGICAL ROLE OF BlaB AND GOB METALLO-ÂETA-LACTAMASES IN *Elizabethkingia meningoseptica*
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Elizabethkingia meningoseptica is an opportunistic pathogen associated with meningitis. This organism is resistant to all β-lactams antibiotics, apparently because of three cromosomically encoded β-lactamases: a serine-β-lactamase CME, and two distantly related metallo-β-lactamases (mβls), BlaB and GOB. All mβl-producing bacteria so far investigated produce a single mβl accompanying a serine-β-lactamase. Given that the substrate profile exhibited by BlaB and GOB is similar, we decided to investigate the involvement of each of these mβls in carbapenem resistance in *E. Meningoseptica*.
Phenotypic assays suggested that resistance to carbapenems is exclusively associated with mβl production. RNA expression levels of bla_{CME}, bla_{BlaB} and bla_{GOB} genes were similar, as assayed by qRT-PCR. Immunoprecipitation of GOB from an *E. meningoseptica* crude extract showed no diminution in imipenemase activity, indicating that resistance to imipenem depends primarily on BlaB. Expression analysis revealed an increase in levels of BlaB RNA as *E. meningoseptica* enters stationary phase. Intact cells activity against imipenem, however, showed a marked diminution in that stage. These results suggest that *E. meningoseptica*'s resistance to carbapenemes is due only to BlaB, being its β-lactamase activity a secondary effect of some other cellular process. BlaB would be implicated in stationary phase metabolism.

MI-P27.
THE IMPLICATION OF A TYROSIL RADICAL IN THE MICROCIN J25 MOLECULE
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Our previous results showed that MccJ25 targets the bacterial membrane respiratory chain enzymes (NADH and lactate dehydrogenase activities), inhibiting cell oxygen consumption. This effect is mediated by an increase in superoxide production by the respiratory processes. We also demonstrated that Tyr9 of MccJ25 is essential for the action on the respiratory chain. In this work, we investigated the tyrosil radical formation using Electronic Paramagnetic Resonance (EPR) and Fourier transform infrared spectroscopy (2D-FTIR). The tyrosil radical was detected by EPR when 2.4 mM MccJ25 was incubated in the presence of 2.5 mM H2O2 and 1 mM DBNBS (trapping of the tyrosil radical). Moreover, FTIR experiments showed an increment of tyrosil radical band located at 1,478 cm^{-1} in the spectrum. To confirm that only Tyr9 is the responsible of tyrosil radical formation, we carried out the reaction with the MccJ25 (Y9F) mutant and no tyrosil radical was detected. On other hand, MccJ25 was unable to inhibited enzymes activities when the experiment was performed with membrane from an *E. coli cyd*AB. These results confirm the presence of a tyrosil radical in MccJ25 molecule and suggest that it could be involved in the superoxide overproduction induced by the antibiotic. Moreover, we proposed that cytochrome bd is essential for the MccJ25 effect on respiratory chain enzymes.

MI-P28.
INTERACTION BETWEEN PURINE METABOLISM AND POLYKETIDE PRODUCTION IN *Streptomyces coelicolor*
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We have previously identified a cluster of genes *sco6243, sco6247* and *sco6248* from *S. coelicolor* encoding putative proteins involved in allantoin metabolism which are negatively regulated by Sco6246 (AllRStr), a protein homologue to the allantoin regulator protein AllR from *E. coli*. Genetic and transcriptional analysis studies have demonstrated that AllRStr encode a transcriptional repressor of this gene cluster. The inactivation of this protein strongly impairs antibiotic production. In order to check if increases of expression of the cognate genes of this pathway (i.e. *sco6243, sco6247* or *sco6248*) are the reason for the impaired antibiotic production we have constructed mutant strain for each of these genes on an allRStr mutant background.
In addition, we have made proteomic studies in order to identify other putative proteins that can be under control of AllRStr. In this way we have found several proteins, from purine metabolism and others involved or related to the acid tricarboxylic cycle, with an increase expression in the allRStr mutant strain compared to the *S. coelicolor* M145. This experiment also allowed verifying that Sco6243 were overexpressed in the allRStr mutant strain.
These results could suggest that availability of precursors for synthesis of ppGpp or acetyl-CoA could be a check point for the production of secondary metabolites in *Streptomyces*.

MI-P29.
Dam CONTROLS O-Ag CHAIN LENGHT IN *Salmonella enterica* BY REGULATING THE EXPRESSION OF Wzz PROTEIN

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Dam protein is a global regulator of bacterial gene expression. We have previously observed that a *S.* Enteritidis *dam* mutant (Δ*dam*) has a partial defect in the length distribution of O Ag polysaccharide. Since Wzz protein is implicated in the regulation of LPS O Ag chain length, we speculate that Dam modulates *wzz* expression. The amount of Wzz, determined by Western blot, was 3-fold lower in Δ*dam* than in the wt. Δ*dam wzz* promoter activity showed a reduction compared to the wt (results further confirmed by RT-PCR). RcsB and PmrA proteins regulate the expression of *wzz* gene. The ΔrcsB mutant of *S.* Enteritidis shows an LPS pattern similar to that of the Δ*dam*. For that reason we investigated whether LPS pattern is affected in a Δ*dam* mutant with an RcsB and PmrA overproducing background. To this purpose, *rcsB* and *pmrA* genes were cloned into pUC18 and electroporated in Δ*dam* and wt. We observed that the O Ag pattern inΔ*dam* RcsB overproducer was similar to that shown by the wt. On the contrary, the overproduction of PmrA did not modify the LPS chain length in Δ*dam*. The expression of both *pmrA* and *rcsB* genes measured by RT-PCR was reduced in the Δ*dam* mutant background. Altogether, our results suggest that Dam methylation modulates LPS O Ag synthesis in *S.* Enteritidis by the regulation of the Wzz production, probably in an RcsB-dependent fashion.

MI-P30.
THE RcsC/RcsD/RcsB SYSTEM IN *Salmonella typhimurium*: A BACTERIAL TWO-HYBRID ANALYSIS.

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The Rcs phosphorelay is an adaptive response system composed of three proteins: the sensor RcsC, the cognate response regulator RcsB; and the intermediary in the phosphoryl transfer RcsD. Has been described that the flow of phosphoryl groups through the Rcs phosphorelay can occur in following way RcsC→RcsD→RcsB. At present, the signal that activates the Rcs system has not been determined. We have demonstrated in a previous study that the overexpression of *rcsB* modulates the RcsB-dependent genes in an *rcsC* or *rcsD* mutant but not in the doble mutant. These results suggest that only RcsB-P, the RcsB phosphorylated form, is able to induce this modulation, and we postulated that the RcsB-P would occur by two or more different pathways. The goal of present work was study the possible phosphorelay pathways. In this work we used the two-hybrid assay (BACTH), consisting of the reconstitution of the adenylate cyclase activity in *E. coli* when two target proteins interacts *in vivo* in bacteria, measured by β-galactosidase activity. In this assay we probe the RcsC, RcsD and RcsB interaction to define the Rcs phosphorelay transduction mechanism.

MI-P31.
PBP2b PARTICIPATES IN SHAPE DETERMINATION AND CONTROL OF CELL DIVISION IN *Streptococcus pneumoniae*

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β-Lactam resistance (βLR) in *Streptococcus pneumoniae* is due to mutations in penicillin-binding proteins (PBPs) PBP1a, PBP2x, and PBP2b, enzymes involved in cell wall synthesis and division. We have previously described a relationship between pbp2b mutations and morphological alterations, growth retardation, a high fitness cost, unusual simultaneous septa and FtsZ delocalization. The clinical βLR strains harboring these pbp2b mutations showed no morphological abnormalities, but in a laboratory strain, the association of pbp mutated genes in double and triple pbp mutants caused partial and complete compensatory effects on cell division and fitness. By optical microscopy, subpopulations of pbp2b cells showed bacillar and coccoid (as wild-type strain) shapes. However, by electron microscopy, these coccoid-shape cells exhibited different defects, such as incomplete and atypical septum formation and positioning, intracellular structures and frequent asymmetrical divisions. The double pbp2b/2x mutant showed inner cell wall accumulation, but the triple pbp mutant compensated these morphological alterations. In addition, the localization pattern of the PBP2b*-GFP fusion protein in wild-type and pbp2b mutant cells was consistent with a cell cycle deregulation. These results suggested that PBP2b is involved in essential processes related to cell shape determination and cell division.

MI-P32.
FabPR GOVERNS TRANSCRIPTIONAL ACTIVATION OF FATTY ACID BIOSYNTHESIS GENES IN *Streptomyces coelicolor*

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Membrane lipid homeostasis is of critical importance to bacterial physiology. Biophysical properties of membranes are determined largely by the *de novo* produced fatty acids. Thus bacteria have evolved mechanisms to control fatty acid biosynthesis. *Streptomyces coelicolor* fatty acid biosynthesis genes are clustered at a single location within its genome. Bioinformatics analysis of this region revealed the presence of a highly conserved open reading frame upstream of *fabD*, named *fabPR*. The *fabPR* disruption mutant strain, AM6, showed an evident reduction in the growth rate and a delay in the timing of morphological and physiological cell differentiation. AM6 also presented diminished FAS activity, altered FA composition and reduced triacylgliceride content. Transcriptional analysis of fab operon by XylTE transcriptional fusions showed an 8-fold reduction on its expression in AM6 cells. *In vivo* and *in vitro* studies demonstrated that FabPR binds specifically to the *fabPR-fabD* intergenic region which contains the *fab* promoter (P*fab*). *In silico* analysis of this DNA sequence showed two highly conserved inverted repeats (IR). The relevance of this sequence was demonstrated by mutation the IR motif, which severely comprised the binding of FabPR to the DNA and prevented the *in vivo* activation of the P*fab*. These data provides the first example of positive transcriptional regulation of FAS.

MI-P33.
ROLE OF ACCD6 IN FATTY ACID AND MYCOLIC ACID BIOSYNTHESIS
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Mycolic acids are essential for survival, virulence and antibiotic resistance of the human pathogen *Mycobacterium tuberculosis*. Their structure and biosynthetic pathways are well studied, however, little information is available regarding the biosynthesis of the precursors for these complex molecules. In Mycobacteria, Acyl-CoA Carboxylases (ACCase) are meant to provide the building blocks for de novo fatty acid biosynthesis. Unlike other organisms, Mycobacteria contain several ACCase complexes, whose specific roles are not well defined. It has been proposed that *M. smegmatis* ACCase 6 complex is the essential ACCase that provides malonyl-CoA for both fatty acid and mycolic acid biosynthesis. By generating a conditional mutant in the *accD6* gene, we demonstrated that AccD6 is the essential carboxyltransferase component of the ACCase 6 complex, and that this complex is implicated in the biosynthesis of malonyl-CoA. We have also characterized a potent AccD6 inhibitor with anti-mycobacterial properties, highlighting the relevance of AccD6 as a target of novel anti-mycobacterial agents. Finally, we studied the effect of malonate supplementation in the *accD6* conditional mutant and found that it could help to overcome the lack of ACCase 6 and therefore of malonyl-CoA deficiency. These experiments constitute the first evidence of a link between malonate metabolism and fatty acid biosynthesis.

MI-P34.
ROLE OF LIPOIC ACID METABOLISM IN REGULATION OF MEMBRANE FLUIDITY IN *Bacillus subtilis*
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Lipoic acid is an essential cofactor of several key enzymes involved in oxidative metabolism. The current model for protein lipoylation involves two pathways: one in which exogenous lipoate is transferred to apoproteins by lipoate ligase (LplA), and an endogenous one, that involves LipB, which transfers octanoate to target proteins. These octanoylated domains are converted into lipoylated derivatives by lipoyl synthase (LipA).
We have previously constructed a *Bacillus subtilis* mutant deficient in *lipA*. In this work we report its use as a tool to study the interplay between branched-chain and unsaturated fatty acids (FA) metabolism in this organism. Interrupting lipoate-dependent metabolism strongly impairs the generation of branched chain FA and leads to the accumulation of copious amounts of straight-chain saturated FA into *B. subtilis* membranes. Although LipA depletion induces the expression of theΔ5-desaturase, the synthesis of unsaturated FA is insufficient to support growth in the absence of precursors for branched chain FA. However, deregulated overexpression of the Δ5-desaturase functionally replaces the deficiency in the lipoylated enzyme branched-chain 2-oxoacid dehydrogenase. This work reports the first characterization of a mutant deficient in lipoate synthesis in a Gram-positive organism and the role of this important cofactor in FA metabolism in *B. Subtilis*.

MI-P35.
ROLE OF THE *Bacillus anthracis* TWO COMPONENT SYSTEM BA5597-BA5598 IN TEMPERATURE SENSING
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Anthrax, a potentially fatal disease of animals and humans, is caused by the Gram-positive endospore-forming bacterium *Bacillus anthracis*. The anthrax toxin is one of its major virulence determinants. The toxin genes are coordinately regulated at the level of transcription and are induced by bicarbonate and increase in temperature, but the mechanism of thermal regulation remains unsolved.
In this study we examined two genes located in *B. anthracis* chromosome, BA5597-BA5598, as they present high homology with *B. subtilis* DesK-DesR two component system, which regulates transcription of its sole desaturase in response to environmental temperature changes. According to this, we carried out *in vitro* experiments to asses the autokinase activity of a cytoplasmic N-terminal truncated form of the potential histidine-kinase, BA5598C, and its ability to phosphorilate its cognate response regulator, BA5597. We also observed crosstalk capability between DesKC and BA5597. Currently, we are analyzing *B. subtilis* strains that lack DesK to evaluate if expression of BA5598 restores the ability of the mutant to transcribe the des gene at low temperature.This will mean that the *B. anthracis* kinase is responding to the same signal sensed by DesK, and would support a more general role of these TCSs in G+ bacteria.

MI-P36.
LIGHT REGULATES PUTATIVE BLUF GENE EXPRESSION IN *Acinetobacter baylyi* STRAIN ADP1
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Light is an essential environmental factor, and both phototrophic and chemotrophic organisms have evolved photosensory proteins to perceive information carried by it.
A BLAST search revealed four putative Blue Light Using FAD (BLUF)-domains in the sequenced genome of *Acinetobacter baylyi* (ADP1). This strain produces peritrichous thin pili, implicated in adhesion, and polar thick pili for twitching motility. Interestingly, it was demonstrated that light suppresses twitching motility in this organism (1).
With the aim to analyze light regulation of the expression of these putative BLUF genes, total RNA was isolated with Trizol reagent from cells incubated in the light or in the dark on twitching plates. After DnaseI treatment, RT and its negative control were performed with and without M-MLV reverse transcriptase, respectively. Two putative BLUF domains (ACIAD1499 and ACIAD2129) and the housekeeping gene (ACIADrRNA16S) were analysed by Real-time PCR using specific primers for each gene and SYBRGreenI as fluorescent dye. Results showed that genes ACIAD1499 and ACIAD2129 are expressed 2.61 and 3.11 fold times more highly in the dark than in light, respectively. In addition, ACIAD2129 is more highly expressed than ACIAD1499 in light and in darkness.
Light reduces the expression level of two putative BLUF genes in *Acinetobacter baylyi* (ADP1).
(1) *Bitrian et al.* MI-P80 SAIB2008

MI-P37.
DYNAMIC NADPH HOMEOSTASIS IS ESSENTIAL TO OBTAIN MAXIMAL ANTIOXIDANT PROTECTION IN *Escherichia coli*
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Bacteria have evolved sophisticated molecular mechanisms to monitor oxidant levels and to activate antioxidant defence genes in response to specific signals. SoxR serves in *Escherichia coli* as a redox sensing protein activated upon exposure to superoxide-generating agents such as methyl viologen (MV), and becomes active when the 2Fe-2S cluster is oxidized. During aerobic growth, SoxR is maintained in the inactive reduced form by a reducing activity dependent on NADPH. When oxidized, SoxR activates the transcription of the *soxS* gene. The SoxS protein induces transcription of the *soxRS* regulon, whose products act collectively to avoid and repair oxidative damage.
The aim of this work was to investigate how directed alterations in NADP(H) levels could affect both deployment of the SoxRS response and tolerance to MV-driven oxidative stress. *E. coli* transformation with a plasmid encoding a strong NADPH producer, the non-phosphorylating plant glyceraldehyde 3-phosphate dehydrogenase, led to build-up of NADPH contents, down-regulation of the SoxRS response and decreased tolerance to MV. Conversely, incorporation of a NADPH consumer, chloroplast ferredoxin, resulted in the opposite behaviour. These observations indicate that NADP(H) homeostasis plays a critical role in proper execution of the SoxRS response.

MI-P38.
ISOLATION OF SIGNAL MOLECULES THAT INDUCE PROTEASE PRODUCTION IN HALOALKALIPHILIC ARCHAEA
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Bacteria can communicate through a signaling pathway mediated by molecules secreted during growth, denoted as *quorum sensing* (QS). Little is known on the role of QS in extreme environments, and this mechanism has not been confirmed in Archaea. The alkaliphilic haloarchaeon *Natrialba magadii* secretes a serine protease (NEP) as the cells enter the stationary phase. We have shown that early production of NEP is induced by cell-free spent medium from stationary phase cultures suggesting the occurrence of a QS-like mechanism in *N. magadii*. To further explore this possibility, the aim of this work was to isolate the "signal molecule" present in stationary phase cultures of *N. magadii*. Signal molecules were purified by: 1. Ethyl acetate extraction (EA); 2. Reverse phase chromatography. Flow-through (FL) and fractions eluted with 5, 30, 60 and 100 % (v/v) methanol were assayed. These fractions were added to low density cultures of *N. magadii* and then samples were removed to measure cell growth and protease activity. Fresh medium was used as control. Early induction of protease activity was observed in cultures assayed with EA extracts, and FL/30% methanol relative to control cultures while the duplication times were similar. These data suggest a hydrophobic nature of the signal molecule; however, conditions need to be optimized to improve reproducibility.
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MI-P39.
DIFFERENT MECHANISMS OF BACTERIAL SURVIVAL/RECOVERY FROM SEVERE AND SUBLETHAL HEAT STRESS
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The roles and cooperation of the major folding chaperone of the *Escherichia coli* cytoplasm DnaK and its J protein cochaperones DnaJ, DjlA and CbpA in cell survival from severe heat stress at 50 ºC (thermotolerance) were investigated by genetic procedures. As opposed to the isogenic parental strain, ΔdnaK and ΔdnaJΔcbpA mutants exhibited the most pronounced rate of bacterial inactivation at 50ºC. Measurements of intracellular protein aggregation indicated that only ΔdnaK and ΔdnaJΔcbpA mutants showed increased initial rate of aggregate formation at 50ºC as compared to the parental strain, although no differences in the final aggregated protein amount were found after 1 h of heat treatment. This suggests that bacterial death from severe thermal challenge is not due to protein aggregation as has been suggested, but to other processes occurring after stress has abated, i. e., the recovery phase. Moreover, and contrary to the dissolution of aggregates observed in bacteria exposed to sublethal temperatures, protein disaggregation was not necessary after a 50ºC heat shock to resume growth when physiological temperatures were reinstated. The overall results point to different mechanisms operating in response to moderate and severe heat stress, with DnaK along with DnaJ and CbpA being crucial for the recovery period.

MI-P40.
STUDY OF THE REGULATION OF THE INNER MEMBRANE PROTEIN SbmA IN AN *Escherichia coli T*
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SbmA transports MccB17, MccJ25, bleomycin and proline rich peptides into the *E. coli* cytoplasm. *sbmA* gene homologous were found in a variety of bacteria suggesting that an important physiological role might exist for these genes, which remains unknown. We studied the *sbmA* expression in a *tolC* mutant background, resulting in strong increases of β-galactosidase activity levels, indicating a *sbmA* positive regulation control by the absence of *tolC* gene. The goal of this work was determinate the mechanism of this *sbmA* regulation. It is know that *tolC* mutation is involved in the regulation of porins, OmpC and OmpF, through MicF. Our results indicated that MicF is not involved in the *sbmA* regulation. *sbma* was also regulated in response to the temperature of the growth medium. We observed that the regulation in a *tolC* mutant was reverted when *ompR* or *lrP* genes were mutated. *E. coli* responds to the osmolarity changes by OmpR phosphorylation state regulation, which controls the porins expression levels. We analyzed the effects of the osmolarity shift on *sbmA* expression in different genetics backgrounds. OmpR overexpression increased the σ^E activity, so we tested *rybB* expression, a σ^E dependent gene, in *sbmA*, *tolC* and in *sbmA-tolC* double mutants to determinate if these mutations are able to enhance the σ^E activity. Our results suggest a possible function of SbmA in the stress responses.

MI-P41.
MODIFICATIONS OF THE S-LAYER OF *Bacillus sphaericus* DUE TO AN OSMOTIC STRESS
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Crystalline surface layers proteins (S-layers) are present in Archeae and Bacteria,have the ability to assemble into crystalline arrays wrapping the cells. S-layers would modify in order to allow a better adaptation to environmental stresses, glycosylation being the most frequent postranslational modification.
B sphaericus 2362 possess an S-layer and when expose to high NaCl the cell envelope were thinner and less diffuse than those of control condition. We suspected the S-layer was modified. In order to check if this S-layer plays a role in the osmoadaptation, the growth of cells depleted or not of the S-layer in media containing or not a high salt concentration was compared. Mild treatment with LiCl depletes the S-layer without affecting cell viability. However, these cells showed slower growth rates than cells with intact S-layer in all conditions tested, but particularly in high salt medium. Western blot analysis showed that the synthesis of the S-layer of treated cells, started very early during growth resume. Moreover, the S-layers from cells grown in hypersaline medium presented different mobility and glycosylation patterns compared to those from control medium.
Since we have previously observed a pathogenic activity against mosquito larvae with an S-layer from *B sphaericus*, we are investigating the role of these modifications in such process.

MI-P42.
NEW PROTOCOL FOR TRANSFORMING *Lactobacillus casei* USING GROWTH IN HIGH SALT
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Because of their widespread industrial and medical importance, there is considerable interest in the manipulation and improvement of *Lactobacillus* strains using modern genetic engineering techniques.
Limited transformability is in part consequence of their cell wall composition that is highly resistant to disruption by mechanical or cell-wall hydrolases treatments.
We have described that *L. casei* BL23 grown in high salt shows modifications of their cell-wall that increases their sensitivity to lysis (Piuri et al. 2005).
Here we report a new method based on the cell wall weakening by incorporation of high salt into the culture medium that enhanced the transformation frequency. For this purpose, *L. casei* was grown overnight in media containing 0.9 M of NaCl, carefully washed and kept at -70ºC before submitting to different electroporation conditions. Consistent frequencies of >105 transformants per µg plasmid DNA have been obtained using an electroporation voltage of 12.5 kV cm^{-1}. Various plasmids have been assayed and been successfully transferred: pNZ235, pGK13, pIL253, pRV610, pRV620.
Comparison of this method to others using lysozyme, glycine or penicillin as cell wall weakening agents are discussed. At the moment we are trying to extend this novel method to other less transformable *Lactobacillus* strains such as *L. plantarum, sakei* and *curvatus*.

MI-P43.
PHENOTYPICAL CHARACTERIZATION OF AN *Escherichia coli K12* DOUBLE MUTANT *sbmA tolC*
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E. coli inner-membrane protein SbmA transports the peptide antibiotics microcin B17, J25 and bleomycin to the cell cytoplasm. In addition, the outer membrane-protein TolC is an essential component of several efflux pumps in *E coli*. We have previously demonstrated that an *E coli* K12 double mutant *sbmA tolC* carrying a Tn10 transposon, which should confer high resistance to tetracycline, is hypersusceptible to that antibiotic. Several studies of this double mutant showed that it presents a deficient growth in solid medium even in the absence of tetracycline, forming small colonies compared to the parental control strain MC4100. In addition, a *sbmA tolC* double mutant rapidly loses viability four to five days after entry in stationary phase at 37ºC. Curiously, this phenotype was not observed when the cultures were grown at 30ºC but was enhanced at 42ºC, suggesting that the growth temperature markedly affects the double mutant physiology.
Interestingly, this death phenotype in stationary phase is reversed by a diffusible peptide factor, which is present in the stationary-phase supernatants from liquid cultures of laboratory strains of *E. coli* K-12. Ours present efforts are aimed at clarifying the mechanism by which the high temperature affects the ability of the double mutant to form colonies, as well as purifying and characterizing the putative *E. coli* quorum sensing factor.

MI-P44.
IDENTIFICATION AND CHARACTERIZATION OF A TOXIN-ANTITOXIN SYSTEM ON PLASMID pRC18
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A plasmid-borne toxin-antitoxin (TA) system is composed of a stable toxin protein and an unstable antitoxin component and helps in plasmid maintenance by killing of plasmid-free segregants. The lactic acid bacterium *Lactobacillus curvatus* CRL 705 harbors plasmid pRC18, which is associated with the production of lactocin Lac705, a two-component bacteriocin. Although several plasmid-curing protocols have been applied to strain CRL705, no pRC18-cured derivative has been yet isolated; in some lactocin Lac705-negative mutants, plasmid pRC18 seems to undergo structural rearrangements and be in low copy-number (i.e., strain Sac7). In this study, we describe the presence of a putative TA module (partitioning locus) on plasmid pRC18, which is organized in a single operon that produces a 92-amino-acids antitoxin (*orf4*) and a 118-amino-acids toxin (*orf3*); these proteins belong, respectively, to the superfamilies PhdYeFM and COG3041. Plasmid maintenance functions of the putative TA locus on pRC18 may explain the remarkable stability of this plasmid.

MI-P45.
PELLETS FORMATION IN *Aspergillus niger* MYA 135: ROL OF HYPHAL MORPHOLOGY AND CONIDIA ADHESION
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The morphology of filamentous fungi in submerged culture affects the broth rheology and in this turn may affect product yield. Thus, results of quantification of *A niger* MYA135 conidia adhesion capacity during the first hours of cultivation and its relationship with pellets formation were described. Modifications in the environmental conditions were tested by changing either the incubation temperature, the initial pH or by the addition of either $CaCl_2$ or $FeCl_3$ to medium. Conidia adhesion was quantify in polystirene microtiter plates by using sulforhodamine B staining. The adhesion unit was defined as the amount of adherent substance that produces an increase in absorbance at 570 nm of 0.01 per min per ml of culture. The hyphal growth unit length (l_{HGU}) was calculated by dividing the total hyphal length by the number of tips.This study suggested that conidia adhesion units per ml higher than 0.50 could be necessary to afford pellets formation. Thus, conidia showing 0.43 adhesion units per ml were followed by a dispersed mycelial growth. It was also observed that once the pellet was formed the l_{HGU} had an important influence on its final diameter. Multiple linear regression with pellets diameters versus conidia adhesion (6h) and l_{HGU} explains 82.6 % of the variability of the data ($R2=0.826$; $R2_{(adj)}=0.777$; $P=0.002$).
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MI-P46.
THE *eutD* GENE OF *Escherichia coli* ENCODES A PROTEIN WITH PHOSPHOTRANSACETYLASE ENZYME ACTIVITY
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Phosphotransacetylases (PTA) reversibly converts acetyl-CoA and inorganic phosphate to acetyl-P and CoASH. Distinct isoforms of PTA are expressed in prokaryotic organisms, where they play diverse metabolic roles. Surveys of sequenced microbial genomes reveal two classes of Pta enzymes. Class I enzymes (PtaI) are ~350 residues in length, whereas class II enzymes (PtaII) are twice as long as PtaI (~700 residues). PtaI enzymes share end-to-end homology with the C-terminal domain of PtaII enzymes; hence, it is inferred that the active site of PtaII enzymes is located within their C-terminal domain. Two PTA genes have been identified by sequence homology in E. coli: pta and eutD. eutD belongs to the PtaI class and it is codified within the 17-gene eut operon. The aim of the present work was the cloning and expression of EutD. The protein was purified and kinetically characterized. The product of eutD displays PTA activity, although the properties of this enzyme were different from that of the Pta protein. The reverse activity was tested and the Km for acetyl-CoA was obtained. Finally, the metabolic regulation and oligomeric state of the recombinant EutD were analyzed. All in all, these results indicates that EutD and Pta fulfil different roles *in vivo*

MI-P47.
CHARACTERIZATION OF TWO MEMBERS OF MALIC ENZYMES FROM *Enterococcus faecalis*
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Enterococci are an ubiquitous microorganisms that compose the natural flora of a variety of fermented foods and also are capable of causing disease as opportunistic pathogens. *E. faecalis* contains two different members of the malic enzyme family, EF3316 (*Ef*CitM, a putative oxaloacetate (OAA) decarboxylase) and EF1206 (*Ef*MaeE, a putative malate oxidoreductase (OAA-decarboxylating)). *citM* is transcribed from the citrate fermentation operon while *maeE* is located near *maeP* implicated in malate utilization. It is widely accepted that both fermentative pathways contribute to pH homeostasis in bacteria. In this work, we described the purification of the recombinant enzymes expressed in *E. coli*. Our *in vitro* studies showed that i) *Ef*CitM is an OAA decarboxylase with maximal activity at pH 4.4, a $K_{m,OAA}$ of 0.47 ±0.066 mM, and a K_{cat} of 5 ±1.0 s^{-1}. No malate activity was detected. ii) *Ef*MaeE catalyzed the oxidative decarboxylation of malate at an optimum pH of 8.5 with a $K_{m,malate}$ of 0.56 ±0.035 mM, and a K_{cat} of 0.29 ±0.03 min^{-1}. Interesting, we found OAA decarboxylase activity for *Ef*MaeE at low pH. Considering the high optimum pH for malic activity of *Ef*MaeE, this enzyme would not have a role in pH homeostasis in *E. faecalis*. Nevertheless, the contribution of the OAA decarboxylase activity of both enzymes to the intracellular alkalinization of *E. faecalis* will be analyzed in future works.

MI-P48.
EFFECTS OF INTRAVAGINAL INOCULATION OF A POTENTIALLY PROBIOTIC *Lactobacillus* STRAIN IN MICE
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The aims of this work were to evaluate the capability of vaginal colonization and the effects on the host of a potentially probiotic *Lactobacillus* strain, by employing an experimental murine model. 2-months-old female BALB/c mice were estrogenized through intramuscular injection of estradiol. At day 2 post-injection, *Lactobacillus* spp. CRL 1263 (from the CERELA Culture Collection) was intravaginally inoculated to mice, in four doses of 10^7 or 10^8 colony forming units per dose (CFU/dose). Animals were sacrificed by cervical dislocation at days 2, 5 and 7 post-inoculation. The injection of estradiol produced an appropriate hormonal synchronization of mice, because most of animals reached the estrous stage of the cycle at day 2 post-injection of hormone. Only at 2 days post-inoculation, *Lactobacillus* spp. CRL 1263 was recovered from vaginal samples (10^2-10^3 CFU/50ul of vaginal exudates and 10^2-10^4 CFU/g of vagina). Administration of lactobacilli did not produce structural modifications in vaginal mucosa, determined by histological evaluation of vagina. T lymphoid and myeloid populations of vaginal fluid were not altered by administration of lactobacilli, which were determined by flow cytometry analyzing the expression of CD3 and Gr-1. The results suggest that the strain evaluated would be a novel, safe candidate for a probiotic formula to prevent urogenital infections in women.

MI-P49.
OADH FROM *Enterococcus faecalis*, A NOVEL SUBUNIT AMONG THE MEMBRANE-BOUND OXALOACETATE DECARBOXYLASES
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Citrate metabolism in *E. faecalis* involves the conversion of citrate to pyruvate, by the sequential action of citrate lyase and oxaloacetate decarboxylase (OAD) enzymes. Our previous reports indicated that the gene cluster involved in citrate degradation in *E. faecalis* encodes an OAD membrane-bound complex homologous to the one present in *Klebsiella pneumoniae*. In *K. pneumoniae* the complex is composed of three subunits (Kpn-α, β and γ), while in *E. faecalis* a four subunit complex was found (Ef-α, β, δ and η). Interestingly, we found that the EfoadH gene encodes a new subunit (Ef-η), which may have functional properties analogous to the Kpn-γ subunit. Analysis of the subunits localization, revealed that those that in principle were soluble (α and δ) were found in the cytoplasm as well as attached to the membrane of *E. faecalis*, suggesting that α, δ and ηsubunits form a soluble complex that is able to interact with the membrane-bound β subunit in a dynamic way. In this work, the nature of the interaction between the subunits was analyzed. We observed that the complex was stable at acidic pH suggesting that it´s integrity could be maintained in a different manner than the one reported for Kpn-OAD. Furthermore, different subunits were cloned and expressed in *E. coli*. In summary, we propose that the new η subunit mediates the interaction between the remaining subunits of the complex.

MI-P50.
ANALYSIS OF MEMBRANE-BINDING REGION OF A BACTERIAL GLUCURONOSYLTRANSFERASE
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Current classification of membrane proteins describes proteins that cross the membrane at least twice (polytopics), once (bitopics), and not at all (monotopics). The last one is a very particular group and has not been fully described yet. GumK is a monotopic glycosyltransferase involved in xanthan biosynthesis, transferring a glucuronic acid residue to a lipidic acceptor. Recently, GumK crystal structure was solved in our laboratory. Structural data show a patch of basic and hydrophobic aminoacids that would be involved in both membrane and acceptor substrate binding. Bionformatic simulation supports this hypothesis. To study the role of this region, four GumK mutants were constructed: B1 (R55N, R58N, K60Q), B3 (R86N), H1 (L56S, M59S), and H2 (L99S, V102T, M106S). Analysis of subcellular localization show that all mutants are membrane-associated. The activity of these mutants was studied in a gumK$^-$ strain (XcK) by complementation assays. B1 and B3 produce similar amounts of xanthan compared to wild type, H1 drastically reduces xanthan production, and H2 produces 50% less xanthan than wild type. These results are the first evidence that biological activity of this monotopic protein depends deeply on its partial membrane insertion.

MI-P51.
SALT TOLERANCE EFFECT IN ALFALFA NODULATED WITH DIFFERENT STRAINS OF *Sinorhizobium meliloti*
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Under nitrogen-limiting conditions legumes interact with symbiotic rhizobia to produce nitrogen-fixing root nodules. Rhizobial symbiont *Sinorhizobium meliloti* is able to invade and differentiate inside its host plant alfalfa (Medicago sativa). Formation of effective nodules required the presence of cyclic beta(1,2)glucans (CGs) in the periplasm of the bacteria. Cyclic beta(1,2) synthase (Cgs) is the enzyme responsible for the synthesis of CGs. High osmolarity was found to inhibit the *in vivo* accumulation of cellular CGs in *Sinorhizobium meliloti* 1021 strain, however exceptions have been reported with respect to osmoregulation in Rhizobiaceae. *Brucella abortus* also accumulates periplasmic CGs, but unlike *S. meliloti* 1021 strain its synthesis is not osmoregulated at high osmolarity. *In vitro* studies revealed that *B. abortus* Cgs is active at 0.25 M NaCl. We analyzed nodulation of several *S. meliloti* strains in the presence of different NaCl concentrations in Monarca alfalfa cultivar. We found effective nodulation up to 0.125 M NaCl using *S. meliloti* wild type strains 102F34, GR4 and its cgs mutant complemented with *B. abortus cgs* wild type. Thin-layer chromatography (TLC) analysis confirmed that in these strains the synthesis of CGs is not osmoregulated at the NaCl concentration used.

MI-P52.
TYPE III SECRETION SYSTEM IN THE *Mesorhizobium loti-lotus japonicus* INTERACTION
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Type III secretion system (T3SS) has a relevant role in the simbiosis of rhizobia with legumes. The effectors translocated by this system were described to favor the nodulation efficiency in some legumes or conversely affect negatively the symbiosis in others. *M. loti* possesses a functional T3SS, and putative effectors are been now in study. Effectors secreted by *M. loti* MAFF303099 T3SS favor the nodulation efficency on Lotus. We have determined that mutation of the T3SS (*rhcN*) affects its competitiveness on *L. tenuis* negatively.
L. japonicus is the model legume used for molecular analysis of the changes that occur in the plant during nodulation. Analysis in its roots of the expression levels of NIN, an inducible transcription factor necessary for the development of effective nodules, indicates that the process induced by the *rhcN* mutant suffers an inhibition in an intermediate phase. This result correlates with the obsevation that the mutant induces on *L. japonicus* minor number of nodules than the wild type strain.
It was described that NIN expression is induced by Nod factors. The expression of the bacterial genes involved in the synthesis of Nod factors is related with the expression of the T3SS effectors. *In vitro* we have not found for the mutant strain an alteration in the activity of the promoters that contain the *nod*-box, but an *in vivo* effect could not be discarded.

MI-P53.
INVOLVEMENT OF A SDR ENZYME ON THE SURVIVAL OF *Ochrobactrum* sp. 11A UNDER IONIC AND THERMAL STRESS

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The plant growth-promoting rhizobacterium *Ochrobactrum* sp. 11a is able to use a variety of substrates, including sugars, phenolic compounds and heavy metals. This ability to utilize a wide spectrum of substrates probably contributes to cell survival and proliferation in the nutritionally complex soil and rhizosphere environments.
In this study, we have analyzed the function of a short chain dehydrogenase/reductase (SDR) from an *Ochrobactrum* sp. 11a-Tn5-B21 mutant. This mutant appeared to be sensitive to saline (300 mM NaCl) and thermal (40 ºC) stress. The introduction of plasmid pJN105 containing a copy of the gene encoding the SDR protein fully restored the ability of the mutant strain to tolerate both saline and thermal stresses, indicating that these observed changes were caused by the inactivation of this gene.
Multiple alignment analysis of the predicted protein sequence showed a high similarity to SDR proteins from different symbiotic bacteria such as *Sinorhizobium meliloti* 1021 (84 %) and *Mesorhizobium loti* MAFF303099 (64 %). In addition, this protein showed a conserved domain corresponding to 3-ketoacyl-(acyl-carrier-protein) reductase, an enzyme of fatty acid biosynthesis found in many bacterial species. Therefore, it is likely that this SDR protein may contribute to the catabolic ability and stress survival in *Ochrobactrum*.

MI-P54.
CHARACTERIZATION OF A CATALASE-DEFICIENT MUTANT OF *Xanthomonas axonopodis* pv. *citri*

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Xanthomonas axonopodis pv. *citri* (Xac) is a Gram-negative obligate aerobic bacterium that infects citrus plants. Pathogenic bacteria are usually exposed to H_2O_2 produced either by normal aerobic metabolism or as a part of the plant defense response against microbial invasion. In order to survive and colonize plant tissues Xac must overcome H_2O_2 toxicity, and catalases are enzymes employed for its detoxification. We have previously shown that KatE is the major catalase induced in Xac during the stationary phase of growth and in the XVM2 medium, suspected to mimic the environment of plant intercellular spaces. In this study, a Xac*katE* mutant strain was generated by insertional mutagenesis and was genetically verified. The effect of *katE* disruption on the catalase pattern was assessed by native gel electrophoresis and catalase staining, demonstrating the absence of an upper, slow-migrating band present in wild-type cells. In contrast to wild-type bacteria, no induction of total catalase activity was observed in the stationary phase cultures of the Xac*katE* mutant. Furthermore, the mutant strain was more sensitive to H_2O_2 treatment, the difference of survival being more pronounced during the stationary phase. Finally, the *katE* mutant was assayed in planta during host and non-host interactions, revealing a novel role for KatE during the establishment of plant disease.

MI-P55.
QUANTITATIVE PROTEOMIC ANALYSIS OF *Gluconacetobacter diazotrophicus* INTERACTION WITH SUGARCANE

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Gluconacetobacter diazotrophicus is a N_2-fixing bacterium found in plants such as sugarcane. Its contributions to a profitable association with the host-plants include several mechanisms. To characterize the response of *G. diazotrophicus* to the presence of sugarcane plants two genotypes, SP70 and Chunee, with distinct biological nitrogen fixation (BNF) abilities were used. A quantitative analysis of protein expression by the plants and bacterium was undertaken by using stable isotope ^{15}N protein labeling. Sugarcane roots, bacterial cells and secreted molecules were collected after 1 day of interaction. Proteins were separated by SDS-PAGE. Each gel was sliced into 10 segments and proteins in all samples were trypsin digested and then analyzed by Q-Tof mass spectrometry. *G. diazotrophicus* differentially expressed regulatory proteins during interaction with SP70 (high BNF contribution), as an effort to adapt to the host environment and interact with the host, and proteins involved in energy metabolism, upon interaction with the Chunee plant (low BNF contribution). Proteins up-regulated in SP70 plant in the presence of the bacterium are involved in growth and hormone-induced growth; those differentially expressed in Chunee have roles in bacterial recognition. These results showed the great potential of proteomics to describe events in the bacterium-plant interaction.

MI-P56.
EVALUATION OF PHOSPHATE SOLUBILIZING BACTERIA ACTIVITY UNDER PHOSPHATE STARVATION

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Mineral phosphate (P) solubilization is an important plant growth-promoting ability via which P solubilizing bacteria (PSB) have found extensive applications as inoculants. The P solubilizing ability of bacteria is attributed to the secretion of organic acids, and the best characterized mechanism of P solubilization involves secretion of gluconic acid, which is produced by direct extracellular oxidation of glucose by a glucose dehydrogenase (GDH).
The aims of this work were isolate and characterize PSB from the rizosphere of Lotus tenuis. All the isolates were characterized by antibiotic resistance and 16S rRNA gene sequencing. The analysis of this gene showed that isolates grouped with the genus Erwinia, Pantoea and Pseudomonas. One isolate related with Pantoea ananatis was selected for analysis of GDH activity, based on its high capacity of P-solubilization and L. tenuis growth promotion in “in vitro” assays.
GDH activity was measured in cells grown with Ca3(PO4)2 as the insoluble P source and different levels of soluble P. The activity of GDH was increased 4-fold by starvation of soluble P and it was not repressed by the presence of high levels of soluble P.
These preliminary results suggest that the P solubilizing ability of this isolate occurs due to the release of organic acids produced by glucose oxidation via GDH activity, as has been reported for other microorganisms

MI-P57.
PYOCHELIN HAS ANTIBIOTIC ACTIVITY AGAINST *Xanthomonas axonopodis* pv.citri
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Using a screening method for finding active antibiotics against *Xanthomonas axonopodis* pv.citri (citrus canker agent), we isolated a bacterial strain capable of inhibiting this plant pathogen growth. After sequencing 16S rDNA and analyzing it using BLAST, we were able to determine genus and speciation. The producing strain, identified as *Pseudomonas aeruginosa*, secreted the active compound to the supernatant during stationary phase, and its production was prevented if iron was added into the culture medium. In order to purify the compound, the supernatant of a stationary phase culture was run through a C18 cartridge and fractions with different methanol/water solutions were collected. The 40% fraction containing the active compound was analyzed with a LC/MS. The UV spectrum and mass data (324Da) corresponded with the siderophore pyochelin. After HPLC purification using a 40-100% Acetonitrile/water 0.1% formic acid, NMR analysis confirmed the chemical nature of the siderophore. Even though there is literature evidence of siderophore interchangeability, pyochelin is acting in our assays as an antimicrobial in an iron-independent manner. The action mechanism, as an antimicrobial agent, and uptake-efflux transporters involved are part of our research goals. This represents an important finding due to the potential use of pyochelin producing bacteria in citrus canker biocontrol.

MI-P58.
CHARACTERIZATION OF BIOFILM FORMATION-DEFECTIVE MUTANTS OF X*anthomonas axonopodis* pv.citri (XAC)
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Xanthomonas axonopodis pv. *citri* (Xac) is the causative agent of citrus canker, an important threat to the citrus industry worldwide. Previous work from our laboratory demonstrated that Xac is able to form biofilm communities in biotic and abiotic surfaces where the extracellular polysaccharide xanthan (EPS) plays a key role in the biofilm maturation. To obtain new genes involved in Xac biofilm formation we performed a screening using a library of 6000 Tn5 Xac mutants. We used a 96-well microtiter polyestirene plate as the abiotic attachment surface and the crystal violet staining technique to evaluate attached cells. We found 25 mutants defective in adhesion that also shows impairment in the infective process. Some of them are also impaired in flagellar independent motility and swimming motility. The genes disrupted were identified by using inverse PCR. The genes can be grouped in different functional categories: 1-transcriptional regulators, 2-membrane proteins, 3-structural proteins, 4-hypotetical proteins. The identification of these new genes related with biofilm formation in Xac and infection is a great step towards the understanding of plant-pathogen interactions and could allow the development of strategies to control citrus canker.

MI-P59.
GROWTH INHIBITION OF FRUIT POSTHARVEST PATHOGENS BY AN OXIDATIVE TREATMENT
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Oxidizing compounds such as sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2) are widely used in food sanitization because of their antimicrobial effects. In our laboratory, an oxidative treatment (OT) able to inhibit the growth of the *Penicillium digitatum* conidia was developed. The OT consists in a preincubation with 10 ppm NaClO followed by an incubation with 6 mM $CuSO_4$ and several H_2O_2 concentrations. Here, we analyze the effects of the OT on the mycelium growth of pathogens causing postharvest diseases in a variety of fruits, including *Penicillium italicum*, *Geotrichum candidum* and *Cladosporium cladosporioides*. The sensitivity of fungi to OT was dependent on the pathogen species. The growth of *P. italicum* was inhibited with OT with 150 mM H_2O_2 , while for *G. candidum* similar inhibition was obtained at 10 mM H2O2. When C. cladosporioides conidia were submitted to OT with up to H_2O_2 300 mM, only a slight inhibition occurred. However, increasing the NaClO concentration to 50 ppm in the preincubation produced the complete inhibition of mycelium growth with 50 mM H_2O_2 . Under these conditions, lethality for the other fungi was obtained. These results indicate that an adaptation of OT could be useful for postharvest control of fruit diseases.

MI-P60.
MEMBRANE DAMAGE INDUCED BY AN OXIDATIVE TREATMENT AGAINST *P. digitatum, AGENT OF CITRUS GREEN MOLD*
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Peroxides produces free radicals through the Fenton reaction, catalyzed by metals like copper or iron. These radicals lead to the damage of lipids, carbohydrates, proteins, and nucleic acids, affecting the cellular integrity. In our laboratory, an oxidative treatment (OT) to inhibit the growth of *Penicillium digitatum* conidia has been developed. The OT consists in a preincubation with 10 ppm NaClO followed by incubation with 6 mM $CuSO_4$ and several H_2O_2 concentrations. Here, we analyze the effects of OT on conidia germination, mycelium growth and membrane integrity of fungi. Conidia germination was totally inhibited by OT using 100 mM H_2O_2. In addition, mycelium growth was more sensitive to the treatment than conidia, resulting inhibited at 50 mM H_2O_2. We determined the degree of membrane damage by Sytox Green dye, which only enters into cells with altered membranes. The assay was applied on conidia and mycelium samples after exposure to the OT. The percentages of fluorescent and non-viable conidia were proportional increased with H_2O_2 concentration. When *P. digitatum* mycelium was treated with lethal OT, there was broad dye incorporation. Our results suggest that the OT based on the combination of NaClO, $CuSO_4$ and H_2O_2 alters integrity of fungal membrane, which could be related with the lethality of the treatment.

MI-P61.
COMPARISON OF DEHALOGENASE ACTIVITY BETWEEN PURE AND MIXED CULTURES OF ACTINOMYCETES
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Halogenated compounds are important environmental pollutants. Lindane is a halogenated insecticide prohibited in countries. Its bacterial degradation liberates chlorine by dehalogenases, playing an important role in this process.
Lindane microbial degradation has been studied using pure and mixed microbial cultures. Mixed cultures are suitable for bioremediation because its biodiversity increase the catabolic pathways available for contaminant biodegradation. The aims of this work were to compare dehalogenase activities between pure and mixed cultures of actinomycetes isolated from pesticides contaminated soil, and its identification.
Streptomyces sp. M7, S. coelicolor A3 and four actinomycetes isolates were cultivated alone in minimal medium (MM) with lindane for acclimation. These strains, as pure and mixed cultures, were cultivated in MM with lindane (1.66 mg L-1). Microbial cells were used to obtain cell-free extracts for dechlorinase activity assays. Enzyme activities ranged between 5.14 to 82.39 µmolCl-/h/mg protein. The mixed culture A5-M7 showed the best activity and it was higher than the sum of each activity of pure culture.
The isolates were characterized by 16S rDNA amplifications and sequenced. Actinomycetes were mostly identified as members of *Streptomyces genus*. These native streptomycetes present better ability for lindane bioremediation in mixed cultures.

MI-P62.
KEY ROLE OF Spo0A OF *B. subtilis* PROBIOTIC ON THE INNATE IMMUNE RESPONSE & PATHOGEN EXCLUSION
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Antibiotic resistance has become a major clinical problem. One of the causes has been attributed to their use as growth promoters in animal feed. Probiotics could replace them by generating a protective barrier against harmful microorganisms after colonization of the intestinal mucosa and by stimulating the innate immune response. We studied the effect of probiotic *B subtilis* spores on weight gain, immune response, and pathogens exclusion on chickens. Weight gain resulted to be 5% higher than control group in chickens fed daily with supplements of spores. We also found that Spo0A the master regulator of sporulation is involved in the activation of complement system via classical, alternative and lectin pathways. In contrast, a mutant strain lacking active Spo0A was unable to activate complement system. Spo0A-dependent activation of complement systems by B subtilis reached C5 convertase and binding of C9 was not detected. This could indicate that bacteria are opsonized and then phagosytated by macrophages. Macrophages would then serve as APC rising a specific immune response and thus protection of the host against bacterial pathogens. Moreover, Spo0A was also found to be responsible of exclusion of binding to extracellular matrix proteins of several bacterial pathogens such as *Staph. aureus, Salm. enterica, Vibrio cholerae, Pseud. aeruginosa* and *Clostriidum perfringens*.

MI-P63.
PRESENCE OF REACTIVE PROTEINS AGAINST ANTI-UBIQUITIN ANTIBODY IN HALOPHILIC MICROORGANISMS
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Ubiquitin is a highly conserved protein that takes part in important processes within eukaryotes, but is not present in prokaryotes. However, both eukaryotic and prokaryotic cells contain ubiquitin like proteins (Ubls), which share similar folding and role with ubiquitin.
We previously found proteins recognized by anti-ubiquitin antibodies plus a PCR-product codifying for a peptide with ubiquitin-like structure in haloalkaliphilic archaea. Based on these results, a search for proteins that react with the antibody against ubiquitin was performed in several halophilic archaea and bacteria. For this, microorganisms growth was followed by estimation of OD_{600}, and DNA, RNA, and protein contents. Protein extracts were separated by 1D SDS-PAGE and subjected to Western blot test using a polyclonal anti-ubiquitin antibody. All samples displayed immuno-reactive bands and these results were confirmed by 2D gel electrophoresis. Four most characteristic reactive spots (Mr 50-55 kDa and Ip 3-4) were excised from the gel and subjected to protein sequence determination. The analysis of a 53 kDa spot from *Halobacterium halobium* showed that it corresponds to the elongation factor EF-1α, which is not an Ubl but takes part in misfolded protein degradation mediated by ubiquitin. Its relationship with Ubls is being analyzed.
Supported by CONICET and UNMdP.

MI-P64.
DETECTION OF δ-PCCH AND 1,4-TCDN IN Streptomyces CELL-FREE EXTRACTS BY THE DECHLORINASE ON δ-HCH
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The synthesis of dechlorinase in Streptomyces sp. M7 was induced when the microorganism was grown in the presence of lindane (δ-HCH) as the only carbon source. It was grown in MM medium containing 100 µg ml-1 δ-HCH and incubated at 30 ºC for 48 and 96 h. After incubation, pellets were aseptically harvested by centrifugation.
The cell-free extract was used for extraction of δ-HCH, δ-pentachlorocyclohexene (δ-PCCH) and 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN). They were extracted by solid phase extraction using C18 columns and the residue was resuspended in hexane. Routine quantitative determinations of lindane catabolism were carried out with gas chromatograph- electron capture detection-mass spectrometry analysis.
The δ-PCCH and 1,4-TCDN were detected, both being products of the dechlorinase activity from the cell-free extract at 48 and 96 h of growth of *Streptomyces sp*. M7 in MM with lindane. The appearance of δ-PCCH (Rt 6.26 min) and 1,4-TCDN, (Rt 5.29 min), the first and second product of the lindane catabolism by a specific dechlorinase was proposed by other researchers in *Sphingomonas*. The relative abundance of both increased one and half times, at 96 h compared to 48 h of growth. Were not found in MM with lindane. This is the first time that an enzyme with dechlorinase activity has been demonstrated in an actinomycete strain isolated in Tucumán, Argentina.

MI-P65.
BALANCE OF NEUTRAL AND DETERMINISTIC DYNAMICS DURING ACTIVATED SLUDGE FLOC ASSEMBLY

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Understanding the processes that generate patterns of community structure is a central focus of ecological research. With that aim, we investigated the dynamics of bacterial community assembly in the course of the development of the activated sludge floc, a highly diverse bacterial community involved in wastewater treatment. We manipulated the diversity of bacteria to test the influence of the size and composition of the inocula on the dynamics and reproducibility of the community structure of 12 identically operated lab-scale bioreactors. Bacterial diversity was analyzed using denaturing gradient gel electrophoresis (DGGE) of RT-PCR amplified 16S rRNA, a culture-independent fingerprinting technique. High rates of change in DGGE profiles from consecutive sampling points suggested a high level of dynamics in all reactors. The application of the Raup and Crick probability-based similarity index indicated that bacterial communities in replicates were more similar than expected by chance, suggesting determinism in floc development. On the other hand, the rate of replacement of species (bacterial taxa-time relationships) was strongly dependent on the number of available species in the source community. The results demonstrate that both neutral and niche-oriented dynamics operate together in the assembly of the bacterial floc.

MI-P66.
NOVEL LINEAR MEGAPLASMID FROM *Brevibacterium* sp. ISOLATED FROM EXTREME ENVIRONMENT

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Brevibacterium sp. Ap13, isolated from flamingo's feces in Laguna Aparejos, a lake located at approx. 4,200 m in the northwest of Argentina was previously found to be resistant to multiple antibiotics, and was therefore screened for plasmids that may be implicated in antibiotic resistance. *Brevibacterium* sp. Ap13 was found to contain two megaplasmids of ca., 87 and 436 kb, designated pAp13 and pAp13c, respectively. Using different pulsed-field gel electrophoresis (PFGE) running conditions, no changes in the electrophoretic mobility of pAp13 were observed, indicating that the plasmid is linear rather than circular. However, a considerably altered electrophoretic mobility became evident for pAp13c, suggesting that this molecule is circular. pAp13 was isolated and subjected to exonuclease treatment. Exonuclease III completely degraded the plasmid, whereas λ exonuclease did not, indicating that the DNA 5' ends are protected, presumably by covalently attached terminal proteins. In conclusion, we have detected a linear megaplasmid in a *Brevibacterium* sp., which to our knowledge is the first report of a plasmid greater than 70 kb found in *Brevibacterium*, and the first description of a linear replicon for that genus. In light of the important role of *Brevibacterium* in the dairy industry, pAp13 may provide a new tool for genetic manipulation of this commercially important genus.

MI-P67.
QUANTIFICATION OF A NOVEL *narG* COMMUNITY IN SEDIMENTS OF SUQUIA RIVER BASIN ALONG A NITRATE GRADIENT

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We evaluated the molecular diversity of *narG* gene from sediments of Suquía River basin, which is affected by anthropogenic pollution, to assess the impact of nitrate concentration on the composition and structure of the nitrate-reducing bacterial community. To this aim, a library of one of the six monitoring stations corresponding to the highest nitrate concentration was constructed and 118 *narG* clones were screened. Nucleotide sequences were associated to *narG* gene from α-, β-, δ-, γ-proteobacteria and Thermus thermophilus. Remarkably, 18% of analyzed clones encode NarG sequences with less than 69% similarity to NarG sequences available in databases. Thus, pointing out the presence of nitrate-reducing bacteria with novel sequences, which were quantified by qPCR. Results show a variable number of *narG* copies, ranging from undetectable to 5.0 x 104 copies per ng DNA or to 0.05 *narG*/16S rDNA ratio, in correspondence with the nitrate concentration and water quality index monitored along the basin at different times. Thus, bacterial biodiversity is also affected by the nitrate gradient produced by anthropogenic activities.
Supported by CONICET, FONCyT and SECyT-UNC.

MI-P68.
THE HALOARCHAEAL ATP-DEPENDENT LON PROTEASE IS A MEMBRANE-BOUND DNA BINDING PROTEIN

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Haloarchaea encode membrane-bound homologs of the ATP-dependent Lon protease, however, this enzyme has yet to be characterized and the functional role of Lon in *Archaea* is poorly understood. Previously, we cloned and sequenced the *lon* gene of the alkaliphilic haloarchaeoan *Natrialba magadii*. To determine the biochemical properties of *Nm*Lon, the enzyme was synthesised in *E.coli* (*Ec-Nm*Lon), it was purified and specific antibodies were generated. This allowed the demonstration of the membrane-bound localization of *Nm*Lon in *E.coli* and *N. magadii* cells. *Ec-Nm*Lon displayed ATPase activity, however, it was unable to degrade large proteins, in the presence or absence of ATP, or hydrolyse synthetic peptides. Interestingly, the *Nm*LonB bound DNA molecules, a feature in common with the LonA subfamily but which has not been reported for archaeal LonB proteases. To understand the physiological role of the LonB protease, the expression pattern of *Nm*Lon was analysed throughout the growth curve in *N. magadii* cultures growing with different concentrations of yeast extract by Western blotting and protease activity determination. The membrane-associated ATP-dependent caseinolytic activity increased in nutrient limited cultures as the cells approached the stationary phase, suggesting that *Nm*Lon may participate in protein turnover during starvation.
Supported by UNMDP, ANPCyT y CONICET.

MI-P69.
PHAS ACCUMULATION AFFECTS SURFACTANT PRODUCTION AND BIOFILMS DEVELOPMENT IN PRESENCE OF HYDROCARBONS
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Biofilms are complex bacterial structures with high stress and antibiotic resistance, and the prevalent way of living in the nature. We used three polyhydroxyalkanoate (PHA) producers: KA-08, isolated from a contaminated sludge, *Pseudomonas extremaustralis* and *P. extremaustralis* / pGEc4 (transformed with a plasmid containing *alk* genes), to test the capability to develop static biofilms in presence of diesel as sole carbon source. Two types of bacterial inocula- with or without PHA accumulation- were used to perform biofilm assays. Biofilm formation capability (BFC) was tested, after a week, by crystal violet (CV) microtiter plate assay. Planktonic cells were measured at 570nm and the BFC was determined as the ratio CV/OD_{570}. When the inocula had accumulated PHA, *P. extremaustralis* / pGEc4 and KA-08 presented lower BFC than *P. extremaustralis*. KA-08 showed the lowest BFC, with high planktonic growth. We also evaluated surfactant production by "drop collapse" assay, showing that surfactant production was more important in all strains when PHA was previously accumulated. These could indicate an association between both metabolisms, for example the existence of a surfactant precursor provide by PHA degradation. These results are interesting to understand the hydrocarbon bioremediation in resistant forms of growth as biofilm.

MI-P70.
INFLUENCE OF ANR ON POLYHYDROXYBUTYRATE SYNTHESIS IN *Pseudomonas extremaustralis*
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Polyhydroxyalkanoates (PHAs) are reserve polymers accumulated during unbalanced growth conditions. PHAs enhance survival and stress tolerance in bacteria and have biotechnologycal interest. We analyzed an Antarctic bacterium, *Pseudomonas extremaustralis*, that is able to accumulate large amounts of polyhydroxybutyrate (PHB). Genes of PHB synthesis, located in an adaptability island, shows an Anr-box in the promoter region. Anr is a global anaerobic regulator in Pseudomonas. We have previously characterized the microaerobic metabolism of *P. extremaustralis* and cloned anr gene. The regulation by Anr on PHB genes is still unknown. We constructed an anr mutant of *P.extremaustralis* by crossover PCR deletion technique. To study the role of Anr on the PHB synthesis we used controled bioreactor cultures under different aeration conditions. Wild type biomass was higher than mutant biomass in all conditions Mutant strain showed a pleiotropic phenotype. In microaerobiosis the content of PHB in the wild type was 51% (w/w) while in the anr mutant was only 21%. In aerobiosis the PHB content was low for both strains. Results showed a relationship between Anr and PHB accumulation. Further studies of PHB genes expression by Real Time PCR will corroborate the action of Anr. Microaerobic PHB production could help to reduce cost of the process resultant from aeration.

MI-P71.
SURFACTANT PRODUCTION IN *Pseudomonas* ISOLATED FROM NATURAL ENVIRONMENTS: ROLE IN BIOREMEDIATION
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A desirable characteristic for a microorganism suitable for hydrocarbon bioremediation is its aptitude to enhance the hydrocarbon bioavailability, thus the present work's aim is to characterize the biosurfactant production in bacteria isolated from natural samples.
Two strains: KA-08 and KB-08, isolated from activated slugde grown with diesel or kerosene as sole carbon source, were selected to be tested by their surfactant capability. Their rRNA 16S gene was sequenced and a BLAST analysis showed that both strains had high sequence similarity with species belonging to the genus *Pseudomonas*.
To analyze the presence of surfactant, both isolated strains and an Antarctic isolate characterized in our lab, *P. extremaustralis*/pGEc4, were assayed by the "drop collapse" assay. The putative surfactants were pre-purified by acid precipitation and organic extraction, and the chemical nature of the compounds was analyzed by TLC. The hydrocarbon solubilization ratio was assayed by a free cell supernatant hexane extraction, and analyzed with GC.
The three analyzed strains: *P. extremaustralis*/pGEc47, KA-08 and KB-08, showed high biosurfactant production. In *P. extremaustralis*, the surfactant seems to be a lipoprotein, while in KA-08 and KB-08 appear to be a glycolipid. On the other hand, the GC patterns shown that the solubilization/ degradation pattern differs from strain to strain.

MI-P72.
***IN VITRO* IMMUNOMODULATORY EFFECT OF LACTIC ACID BACTERIA: EFFECT OF LIPID RAFTS DISRUPTION**
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Probiotic modulation of host immunity is a beneficial effect attributed to several lactic acid bacteria (LAB), but the molecular mechanisms remain unclear. *In vitro* characterizing of immune responds to these bacteria may give an indication of how these events occur *in vivo*. We studied if disruption of lipid rafts could influence the effect of five LAB strains on cytokines production by peripheral blood mononuclear cells (PBMCs). Untreated (control) and disrupted-rafts PBMCs (dr-PBMCs) by MβCD teatment were co-incubated with bacterial strains at 37ºC for 4 hours. TNF-α and IL-10 were detected in culture supernatants by ELISA. The results show that *L. acidophilus* CRL1014, *L. rhamnosus* CRL1505 and *L. reuteri* CRL1101 have a similar stimulating effect on TNF-α release by control cells (30 times base line value) and this effect is decreased in dr-PBMCs by 42% of the control cells value. *L. casei* CRL75 and *S. thermophilus* CRL1190 has a lower TNF-α stimulating activity and it was reduced in dr-PBMCs by 30% and 50% respectively. *L. reuteri* strain stimulates 20 times baseline value of IL-10 secretion by control cells, also reduced by dr-PBMCs treatment (12%). Other strains show a similar pattern of stimulation of IL-10 (3 to 5 times baseline value) with small differences between control and dr-PBMCs. Studies are currently in progress to further define the rafts role.

MI-P73.
EFFECTS OF GENOMIC CONTEXT ON *Escherichia coli* DNA MUTATION RATE
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In *Escherichia coli* the transitory hemimethylated state of adenines in GATC sequences provide the strand discrimination signal to direct the DNA mismatch repair system (MRS). Strains deficient in any of the principal components of the MRS (MutS, -L, -H, and Dam proteins) are mutators. It has been reported using plasmid heteroduplexes that a decrease in the number of GATC sequences within these vectors lowered the efficiency of mismatch repair *in vitro* and *in vivo*. We analyzed the effect of genomic GATC density on mutation rate in *E. coli*. We introduced a mutated copy of a gene able to confer antibiotic resistance within a genome region with a high or low density of GATC sequences and analyzed the reversion rate of the mutation. Our results show, unexpectedly, that the reversion rate was lower in strains containing the mutated gene in a genomic region with a low density of GATC sequences, than those located in a high density context. Moreover, the reversion rate of the mutated gene located in the low GATC density region of a MRS deficient strain was lower than the reversion rate of the mutated gene located in the GATC high density region of a wild type strain. Two possibilities are discussed: 1- Other(s) *cis* or *trans* acting factor(s) influence the mutation rate; 2- The high and low GATC density genomic regions differently affect the transcription rate of the reporter gene.

MI-P74.
ENDONUCLEASE ACTIVITY OF *Pseudomonas aeruginosa* MutL PROTEIN
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Replication errors can be corrected by the mismatch repair system found in most organisms. In *Escherichia coli*, and a reduced number of bacteria, MutH protein nicks the non-methylated DNA at a hemi-methylated GATC site, providing the signal to distinguish the parental strand from the newly synthesized strand. Most genomes do not contain a MutH homologue. Therefore, most organisms must use a different nicking endonuclease. It was recently described that some MutL proteins have an endonuclease activity. The endonuclease active site probably involve a DQHA(X)2E(X)4E and a C(P/N)HGRP motif. We started the analysis of the first conserved motif of *P. aeruginosa* MutL protein by mutating its D and first E amino acids. Both mutant proteins are inactive *in vivo*. We also analyzed the endonuclease activity of the C-terminal region (250 aa) of the wild type and mutant proteins. *In vitro* analysis, using supercoiled plasmid DNA as substrate, showed that the wild type and mutant proteins display an endonuclease activity. These results suggest that although the D and the first E amino acids of the conserved region are essential for *in vivo* mismatch repair, they are not essential for the MutL endonuclease activity, suggesting that structural modifications of the mutant proteins affecting some other function of MutL may be responsible for their *in vivo* lack of function.

MI-P75.
INTERACTION OF THE C-TERMINAL REGION OF *P. aeruginosa* MutS WITH THE DnaK CHAPERONE
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MutS recognizes misspaired bases and it is required to activate downstream events in the postreplicative DNA Mismatch Repair System. In a previous work, we determined that the C-terminal region of *P. aeruginosa* MutS is involved in the interaction with other factor necessary for the correct function of the MRS. To analyze if the C-terminal region of MutS (Cter) interacts with other protein, the DNA encoding this domain was fused in frame to the maltose binding protein (MBP) gene, and expressed in *P. aeruginosa*. The purified MBP-Cter protein consistently copurified with the chaperone DnaK, which was absent in MBP purified preparations. The *P. aeruginosa* cells expressing the fusion MBP-Cter acquired a phenotype similar to that previously described for *dnaK* mutants in other bacteria: diminished viability, cell filamentation and high temperature sensitivity. Similar phenotypes were detected by overexpressing the full length MutS protein. On the contrary, overexpression of a C-terminal deletion MutS mutant did not produce phenotypic changes compared to the wild type strain. We propose that DnaK interacts with the C-terminal region of MutS and the overexpression of the repair protein produces a complete sequestration of DnaK. We are currently analyzing the interaction MutS-DnaK in vitro and generating *P. aeruginosa dnaK* mutant strains to characterize its phenotype.

MI-P76.
ROLE OF PolIV, ImuB AND DnaE2 IN *Pseudomonas aeruginosa* STRESS-INDUCED MUTAGENESIS
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Here, we studied the role of PolIV, ImuB and DnaE2 in *Pseudomonas aeruginosa* (PA) stress-induced mutagenesis. We constructed PAO1 *dinB*, *imuB* and *dnaE2* mutants and analyzed their role in UV-induced mutagenesis. After UV irradiation, PAO1 showed a 2.6 fold increase (*P= 0.038*) in the mutation frequency confirming that PA displays a subtle UV-induced mutator phenotype. PolIV deficiency resulted in levels of UV-induced mutagenesis similar to the wild type strain (ratio 3.6; *P= 0.019*). In contrast, *imuB* (ratio 0.83, *P= 0.09*) and *dnaE2* (ratio 0.72, *P= 0.2*) showed no increase in the mutation frequencies after induction, indicating that UV-induced mutagenesis in PA might depend on ImuB and DnaE2 but not on PolIV. We further investigated the role of these DNA polymerases in stationary-phase mutagenesis using a plasmid-based test system that allows the detection of -1 deletions and substitutions. These plasmids contain the reporter gene *pheA* harboring loss-of-function mutations that produce phenol monooxygenase inactivation, but whose reversion allows bacteria to grow using phenol as the sole carbon source. Results obtained from the incubation of PAO1 carrying the plasmids on phenol selective plates showed that PA displays stationary-phase mutagenesis. We are currently analyizing *dinB*, *imuB* and *dnaE2* strains in order to study the role of these DNA polymerases in stationary-phase mutagenesis.

MI-P77.
STUDY OF DE ROLE OF PolIV IN *Pseudomonas aeruginosa LasR* MUTAGENESIS
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High prevalence of *lasR*-deficient *Pseudomonas aeruginosa* occurring naturally in Cystic Fibrosis chronic lung infections has been shown. However, mutational mechanisms acting on LasR inactivation remain poorly investigated. We previously linked the Mismatch Repair System-deficiency with the emergence of *lasR* mutants, showing that *lasR* variants reproducibly emerge at a high frequency from a hypermutator *mutS* strain. Here, we explored the involvement of transient hypermutability in *lasR* mutagenesis by studying the role of the error-prone DNA polymerase PolIV (*dinB*). A *dinB* null mutant derived from a *mutS* strain was constructed and the emergence of *lasR* variants was analyzed. Notably, *dinB* disruption in a hypermutator background (*mutSdinB*) resulted in 3 fold increase ($P=0.04$) in the *lasR* emergence frequency respect to the *mutS* strain. Furthermore, over-expression of PolIV in the *mutSdinB* strain resulted in 16 fold decrease ($P=0.04$) in *lasR* emergence. Analysis of the *lasR* mutational spectra in *lasR* variants derived from the *mutS* strain, displayed that *lasR* inactivation was entirely due to base substitutions (80% C-T transitions). In contrast, 20% of *lasR* variants emerged from the *mutSdinB* strain contained -1 frameshift and 80% base substitutions with only 38% being C-T transitions. These results suggest that Pol IV could play an antimutator role in *lasR* mutagenesis.

MI-P78.
SIMPLE SEQUENCE REPEATS AND MUCOID CONVERSION IN MISMATCH REPAIR-DEFICIENT *Pseudomonas aeruginosa*
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In *Pseudomonas aeruginosa* (PA), the main pathway for mucoid conversion is mutagenesis of the *mucA* gene, frequently due to -1 bp deletions in a simple sequence repeat (SSR) of 5 Gs (G^5-SSR_{426}). We have recently observed that this *mucA* mutation is particularly accentuated in Mismatch Repair System (MRS)-deficient cells grown *in vitro*. Here, we evaluated the role of G^5-SSR_{426} in conversion to mucoidy under a MRS-deficient background. Thus, *mucA* alleles were engineered with different contents of G:C SSRs, and tested for their effect on the mucoid conversion frequency and *mucA* mutational spectra in a *mutS*-deficient PA. Deletion of G^5-SSR_{426} severely reduced the emergence frequency of mucoid variants, with no preferential site of mutagenesis within *mucA*. Moreover, although mutagenesis in *mucA* was not totally removed, this was no longer the main pathway for mucoid conversion, suggesting that G^5-SSR_{426} biased mutations towards *mucA*. Mutagenesis in *mucA* was restored by the addition of a new SSR (C^6-SSR_{431}), and even synergistically increased when G^5-SSR_{426} and C^6-SSR_{431} were present simultaneously, with mutations being restricted to -1 bp deletions within any of both G:C SSRs. These results confirm a critical role for G^5-SSR_{426} in *mucA* mutagenesis of MRS-deficient cells, and shed light on the contribution of SSR-dependent hypermutability in PA mucoid conversion.

MI-P79.
GENETIC DETERMINANTS INVOLVED IN Cr(VI) RESISTANCE IN *Streptomyces* sp. MC1
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Biological transformation of Cr(VI) to Cr(III) provide environmentally friendly approach to remediation. *Streptomyces* sp. MC1 showed ability to reduce Cr(VI). Our aim was the evaluation of genetic determinants involved in this mechanism. *Streptomyces* sp. MC1 was screened for of plasmids by alkaline lysis and pulsed field gel electrophoresis. Chromate reductase gene was investigated, a pair of primers was designed using sequences of database, and used in PCR reactions. The amplicon was sequenced and compared with the databases. Phylogenetic trees were plotted.
The techniques used did not allow plasmid isolation, however an amplicon of 222 bp was obtained. It showed similarity to proteins of aldehyde-dehydrogenase NAD-dependent family. Also, it showed similarity with actinomycetes proteins: putative aldehyde-dehydrogenases, ferredoxin reductase and proteins involved in heavy metals resistance.
The amplicon translated sequence showed a 23.7% of similarity with the chromate reductase of *T. scotoductus* LTD-01. It is remarkable that the published chromate reductase protean sequences show a average similarity of only 27.3%.
This putative chromate reductase gene was localized at chromosomal level. A fragment amplified from *Streptomyces* sp. MC1 was related to FMN oxidoreductases, suggesting that Cr(VI) resistance and reduction by *Streptomyces* sp. MC1 could be related to this protein family.

MI-P80.
MOLECULAR BASES OF METAL/OPERATOR SELECTIVITY IN MerR MONOVALENT METAL ION SENSORS
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Metal ions are essential in biology and play key roles in the structure-function of a large number of proteins. They can also be toxic, especially at high concentrations. Intracellular metal ion concentrations must therefore be tightly controlled to maintain normal metabolism. In *Salmonella*, two related transcriptional regulators, CueR and GolS, ensure resistance against Cu and Au ions. CueR is essential to maintain copper homeostasis, while GolS is dedicated to protect the cell from the toxic effects of gold. We have identified amino acid residues at the GolS metal-binding loop that direct Au(I) recognition. In addition, we determined the operator sequences recognized by these regulators, and established the nucleotide bases responsible for the regulator-operator selectivity among GolS and CueR regulated promoters. Here, by using *in silico* modeling, site- and loop-directed mutagenesis, as well as reporter expression assays, we identify the regions in both transcriptional regulators responsible for the recognition/selection of their innate operators. In addition, we establish the role of specific amino acids residues that govern both, monovalent metal ion sensing and divalent metal ions exclusion from the metal binding loop. Our results highlight the mechanisms employed by these regulators to modulate the expression of their target genes without affecting global metal homeostasis.

MI-P81.
COPPER-TOLERANCE IN *E. coli* MEDIATED BY POLYPHOSPHATE LEVEL AND Pit METAL/PHOSPHATE TRANSPORTER

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We have previously shown that *E. coli* cells grown in media containing >37 mM phosphate maintained in stationary phase a high polyphosphate (polyP) level and enhanced-cellular fitness. Cellular polyP level plays an important role in basic metabolism and in stress responses. The main enzymes associated to polyP metabolism are polyphosphate kinase (PPK) and exopolyphosphatase (PPX). Metals tolerance has been correlated with polyP level in several microorganisms. This tolerance may be due to the metals sequestration by the polymer and to the activation by metals of PPX, which degrades polyP leading to the formation of metal-phosphate complexes to be exported by the inorganic phosphate transporter (Pit) system. However, these events have not been described yet in *E. coli* for copper. Here, we analyzed the copper-tolerance of cells grown in media with different phosphate concentrations and their relationship with the polyP level. A high copper-tolerance was shown in stationary-cells grown in high phosphate medium. On the other hand, the intracellular polyP level was decreased when cells were incubated with $CuSO_4$. In stationary phase, the *pitA* mutant strain grown in high phosphate medium was more sensitive to $CuSO_4$ than the wild type strain. The copper-tolerance in stationary phase could be due to the polyP degradation and the later transport of metal-phosphate complexes through Pit.

MI-P82.
S-LAYER IN *Bacillus thuringiensis* AND HEAVY METAL RESISTANCE

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In several bacteria, the S-layer constitutes the most external envelope which allows multiple interactions with the medium. A single protein is responsible from such structure, but certain species would replace or vary this protein according to the external conditions. One interesting property is the possibility to absorb metals, allowing the bacteria to pursue its growth. In a strain of *B. thuringiensis var isreliensis* (BTI) one S-layer has been sequenced (*slp*). In this work the presence and characterization of S-layers from BTI strains was performed in view of investigating the possibility of metal biosorption. The S-layer pattern and abundance was analyzed in different cultural conditions: Variation of temperature, medium composition and presence of heavy metals.
Null mutation of the *cry* gene from the 4Q2 strain unable to synthesize the bioinsecticide crystal was found to have an increased resistance to metals compare to wild-type and this was related to the S-layer production and might influence biosorption capacity.

MI-P83.
INTRACELLULAR ZINC ACCUMULATION INCREASES MCCJ25 RESISTANCE IN *Escherichia coli*

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We have observed an intrinsic resistance to the antibiotic MccJ25 in several *E. coli* strains. In order to identify genes responsible for this phenotype, a genomic library in MudII4042 was constructed from MC4100 Mucts Δ*yojI* (pEG109) and used to infect the sensitive strain AB1133. Muductants were selected in the presence of MccJ25. Plasmid DNA was extracted from a MccJ25-resistant clone, digested with HindIII and cloned into pACYC177. This plasmid, named pAC521H, conferred resistance to MccJ25 when transformed into a sensitive strain. The fragment carried by pAC521H was sequenced and located on the 41-min region of the *E. coli* chromosome. Additional subclones of this plasmid in the vector pACYC184 were constructed. One of them, containing a 4 kb fragment encompassing the genes *znuA, znuB* and *znuC*, which encode for the zinc importer ZnuABC, was able to confer resistance to MccJ25. Several experiments showed that ZnuABC neither acts as a MccJ25 pump nor activates one. Interestingly, the individual expression of these genes did not affect the susceptibility to MccJ25, suggesting that the complex ZnuABC is required to confer resistance to the antibiotic. Moreover, the absence of the native zinc exporter, ZntA, leads to an increased resistance to MccJ25. Taken together, the results suggest that the intracellular accumulation of zinc increases the resistance to MccJ25 in *E coli*.

MI-P84.
CDM DELAYS GLYCOPROTEIN TRANSPORT HINDERING VIRUS REPLICATION WITHOUT AFFECTING CELLULAR PROTEINS

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The 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM) displays antiviral and immunomodulating properties. CDM exerts its antiviral action by affecting the traffic of viral glycoproteins, and provokes a delay in the transport of the transferrin receptor (TfR). Here, to extend our understanding about the effect of CDM on viral and cellular glycoproteins we studied: the binding ability of TfR by the transferrin-rhodamine (Tfn-rho) uptake assay; the TNF-alpha transport by immunofluorescence staining; and the subcellular localization of viral proteins in cells infected with a triple fluorescent-tagged HSV-1 (YK608) by confocal microscopy. CDM did not affect the Tfn binding ability of TfR since it co-localized with Tfn-rho in recycling endosomes of control and CDM-treated cells. Besides, in macrophages stimulated with LPS, CDM did not impede the transport to the plasma membrane of active TNF since higher levels of TNF were detected within the Golgi complex compared with LPS alone, and later, its fluorescence was widespread on the cell surface. Nevertheless, confocal microscope images evidenced that intracellular localization of VP26, gB and VP22 viral proteins were markedly affected by CDM.
Then, the delay on glycoprotein transport caused by CDM would account for the strong inhibition on virus multiplication without interfering with the bioactivity of cellular glycoproteins.

MI-P85.
ROLE OF L-SIGN AND DC-SIGN IN JUNIN ARENAVIRUS ENTRY INTO HOST CELLS

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Cell tropism of viruses is regulated by interactions between viral and cellular factors during the replication cycle. The entry and dissemination of viruses in several families can be mediated by C-type lectins such as hDC-SIGN and hL-SIGN. DC-SIGN is expressed in dendritic cells and has been identified as a receptor for different enveloped viruses. In this report we studied JUNV ability to be internalized by hDC- or hL-SIGN. Immunofluorescence assays showed that infection of Vero cells was enhanced by the over expression of hDC-SIGN or hL-sign, whereas infection of non-permissive murine cell line, 3T3, can be rescued by the expression of hDC-or hL-SIGN. Treatment with mannan reduced infection, in immunofluorescence and PFU assays, of this lectin expressing cell lines, whereas anti-hDC/hL-SIGN antibodies blocked infection as seen by PFU assay. Therefore these lectins can act as a novel attachment factors that mediate entry of JUNV. In addition, we demonstrate that the human C type lectins hL-SIGN and hDC-SIGN enhances transduction by JUNV pseudovirus particles in cells devoid of the JUNV receptor, hTfR1, indicating that C-type lectins can mediate virus entry in the absence of the virus cellular receptor. Altogether, this study characterizes the endocytic pathway utilized by the arenavirus JUNV.

MI-P86.
MAPPING OF THE Z-BINDING AND OLIGOMERIZATION REGIONS WITHIN THE TACARIBE VIRUS NUCLEOPROTEIN

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Arenaviruses, such as Tacaribe virus (TacV) and its closely related pathogenic Junin virus (JunV), are enveloped viruses with a bipartite negative-sense RNA genome which encodes the nucleocapsid protein (N), the precursor of the envelope glycoprotein complex (GP), the polymerase (L) and a RING finger protein (Z), that is the driving force of arenavirus budding. We have established a plasmid-based system which allowed the successful packaging of TacV-like nucleocapsids along with Z and GP of JunV into infectious virus-like particles (VLPs). Using this system, we have demonstrated that Z-N interactions are required for assembly of the nucleocapsids into arenavirus particles. To identify the region(s) on the N molecule involved in the interaction with Z, N mutants carrying either C-terminal, N-terminal or site-directed mutations were analyzed for their ability to form a complex with Z, as detected by coimmunoprecipitation. The assembly of mutant N proteins into the VLPs was also analized by Western blot. We found that the 210-residues C-terminal region of N, which comprises a putative Zinc finger motif, is required for both N-Z interactions and the incorporation of N into VLPs. Our findings also indicated that the N-terminus of N (residues 1 to 332), which is dispensable for the incorporation of N into VLPs, is involved in N-N interactions.

MI-P87.
TACARIBE VIRUS Z PROTEIN RESIDUES INVOLVED IN Z-Z INTERACTIONS AND VIRAL RNA SYNTHESIS INHIBITION

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The arenavirus Tacaribe (TacV) comprises a single phylogenetic lineage together with the four South American pathogenic producers of severe hemorrhagic disease. TacV genome encodes four proteins: the nucleoprotein (N), the glycoprotein precursor, the polymerase (L), and a RING finger protein (Z). TacV-Z is a multifunctional protein that has a key role on virus morphogenesis. In addition, Z is able to self-associate, and exhibits an inhibitory effect on viral RNA replication and transcription through its interaction with the L polymerase (J.Virol. 77:10383-93, 2003). We have previously shown that the region comprised between the residues G36 and R85 of Z is sufficient to maintain Z-L interactions and Z inhibitory functions. To learn more about the roles of individual amino acids in the different interactions of Z, a panel of point mutants was created by in vitro mutagenesis. The interaction between Z mutants and L protein was analyzed by a coimmunoprecipitation assay and a minireplicon system was used to examine the effect of mutations on viral RNA synthesis. The capacity of Z mutants to self-associate was also evaluated by coinmunoprecipitation. Our results show that single amino acid changes selectively interfere with Z-Z or Z-L interactions.

MI-P88.
PHOSPHATIDYLINOSITOL-3-KINASE/Akt CELLULAR PATHWAY IN JUNÍN VIRUS PROTEIN TRANSLATION

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In this work we studied the participation of the phosphatidylinositol 3-kinase/Protein kinase B (PI3K/Akt) cellular pathway in the translation mechanism of Junin virus (JUNV). Since this pathway plays a central role in the regulation of the cellular cap-dependent translation and JUNV mRNAs are capped, we evaluated if activation of this pathway was necessary for an efficient translation of viral proteins. When we analized JUNV replication in Vero cells treated with 20 µM Ly294002 (PI3K inhibitor), at different times p.i., we observed an inhibitory effect only when the inhibitor was added at early times. Then we evaluated if the uptake of JUNV was responsible for this activation using chlorpromazine, an inhibitor of JUNV entry. We did not observe Akt activation in the presence of this compound indicating that internalization of JUNV into cells was responsible for this activation. Finally, we tested the participation of eIF4E in the translation of JUNV proteins using rapamycin and a negative dominant 4E-BP plasmid. In both cases we did not observe an inhibitory effect on viral proteins expression and virus yield. These results suggest that, although JUNV mRNAs are capped and the virus can activate the pathway implicated in the regulation of cap-dependent translation, this pathway, or at less eIF4E translation factor, is not implicated in the translation of JUNV proteins.

MI-P89.
CHARACTERIZATION AND EXPRESSION OF RECOMBINANT HUMAN ANTIBODIES AGAINST *Trypanosoma cruzi*

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Patients chronically infected with *Trypanosoma cruzi* (*T. cruzi*) develop Chagas heart Disease (cChHD). Their antibody response is suspected to be involved in cardiac pathogenesis. Due to the clinical relevance of the anti-*T. cruzi* antibodies (Abs), more knowledge about their repertoire, epitope recognition and fine specificity is required. For this purpose, recombinant human anti-*T. cruzi* single chain variable fragments (scFvs) Abs were isolated from a cChHD patients-derived libraries using *phage display* technology. The libraries were subsequently panned against *T. cruzi* total extract and the selected monoclonal anti-*T. cruzi* antibodies bearing (his)6 tag at C-terminus, were produced in *E. coli* and purified from periplasmic extracts. We have characterized two human monoclonal anti-*T. cruzi* scFv, scFv 6B(6) belongs to the VH 3-30*18 family recognizes a 175 kDa protein and scFv A2R1, that belongs to the VH 3-73*01 family recognizes two proteins of about 47.5 kDa in *T. cruzi* Western blot. Flow cytometry and microscopic analysis revealed that both scFv recognize only permeabilized parasites indicating cytoplasmatic protein targeting. Also the selected scFv clones showed cross-reactivity with other kinetoplastids as *T. brucei* and *C. fasciculata*. These results show that phage display technology is an effective method for selection of human recombinant Abs against parasite antigens.

MI-P90.
CLONING AND CHARACTERIZATION OF THE BSL-LIKE GENE IN *Toxoplasma gondii*

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BSL protein Ser/Thr phosphatases have been shown to modulate the strength of the signal delivered to the transcriptional effectors of the steroid-hormone pathway in plants. BSL phosphatases belong to a poorly characterized group of PPP phosphatases found in several organisms, except metazoans and fungi. Diagnostic features of BSL phosphatases are two recognisable domains and a set of conserved positions in the catalytic domain. These phosphatases are only found in land plants, green algae and certain groups of protists, thus spanning a broad range of both unicellular and pluricellular organisms. In order to understand how signalling pathways present in unicellular organisms were co-opted in the transition to multicellularity, we set out to characterize the homologous gene in Toxoplasma gondii. Toxoplasma was chosen as a tractable model for unicellular organisms, loosely related to photosynthetic organisms through an ancestral event of secondary endosymbiosis. The TgBSL gene was only predicted from the annotated genomic sequence. We first isolated the full-length coding region of TgBSL from tachizoites' RNA, and found several alternatively spliced forms. We also report the initial characterization of this single-gene encoded protein phosphatase from Toxoplasma gondii

MI-P91.
ALTERATIONS IN REDOX BALANCE AND ERGOSTEROL SYNTHESIS IN TcCPR OVEREXPRESSING *T. cruzi* EPIMASTIGOTES

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We are interested in the physiological significance of the NADPH dependent P450 reductases expressed in *T.cruzi*. Increased resistance to classical trypanocidal compounds was previously demonstrated in TcCPR-B overexpressing *T. cruzi* epimastigotes. Overexpression of TcCPR-A resulted to be lethal, showing abnormal cell shapes and altered DNA content. Taking together subcellular localization at ER and the biochemical behavior of recombinant TcCPR-C as Cyt P450 partner in reconstituted systems, we investigated its participation in lanosterol demethylation, a well described pathway catalized by CYP51, a cytochrome P450 family member in *T. cruzi*. Transgenic parasites as well as control epimastigotes were incubated with [3H]mevalonolactone, a precursor in ergosterol, the characteristic sterol in trypanosomatids and yeast cells.. An increase in ergosterol content could be observed in overexpressing TcCPR-C epimastigotes. Redox balance (NADP+/NADPH and NAD+/NADH ratios) was also investigated with or without induced oxidative stress by H2O2 treatment. Interestingly, TcCPR-C parasites present the most affected redox metabolism with a strong increase in NADP+/NADPH ratio, no longer modified by peroxides as it does occur in control parasites. NAD+/NADH ratio significatively decreased in transgenic lines compared to control.

MI-P92.
SUBCELLULAR LOCALIZATION OF NADPH DEPENDENT CYTOCHROME P450 REDUCTASES FROM *Trypanosoma cruzi*

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The presence of TcCPR-A and TcCPR-B in cytosolic fractions and TcCPR-C in microsomal fractions from *T. cruzi* epimastigote cells was previously demonstrated by Western blot using polyclonal mouse antisera raised against the recombinant proteins. To study specific subcellular localization of TcCPRs by indirect immunofluorescence we designed different constructs using the vectors pTREX and pTEX-GFP. Therefore we analyzed their co-localization with cytosolic GFP in GFP-expressing parasites and the endoplasmic reticulum and glycosome markers calreticulin/BiP and GAPDH, respectively. TcCPR-A was detected in the cytosol coincident with GFP localization. TcCPR-C showed a strong co-localization with the ER markers around the nucleus and kinetoplast. A different approach consisting in GFP fusions at the C-terminal end of each expressed CPR supported the subcellular localization observed for TcCPR-A and TcCPR-C using the respective antisera. We could also analyze TcCPR-B concluding a cytosolic pattern of localization similar to the one observed for TcCPR-A. Subcellular localization of TcCPR-A and TcCPR-C was assessed using immunogold electron microscopy. TcCPR-A signal was not homogeneous in the cytosol, confirming previous results obtained by immunofluorescence. TcCPR-C was located in some areas of the endoplasmic reticulum, closely associated with the mitochondrial and nuclear membrane

MI-P93.
A Wee1 HOMOLOGUE FROM *Trypanosome brucei*
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Cyclin B-Cdc2 kinase activity is required for triggering entry into mitosis in yeast and mammalian cells. Wee1 is a cdc2-inhibitory kinase that prevents premature activation of the mitotic program phosphorylating the Tyr-15 residue in the ATP domain of cdc2. The proteins that regulate cyclinB-Cdc2 complex at the G2/M transition of the *Trypanosome brucei* cell cycle are not known. We have recently identified a wee1 orthologue in the *T. brucei* genome (TbWee). We evaluated if TbWee is able to rescue the *Schizosaccharomyces pombe* mutant. *S. pombe* wee1-50 strain was transformed with either the pREP3x vector alone, pREP3x-*S. pombe* wee1 or the pREP3xTbWee. Cells transformed with pREP3x grew normally whereas overexpression of TbWee caused the cells to increase in size indicating partial rescue of the mutant. To further characterize TbWee, we purified TbWee1-His protein from Sf9 insect cells and used the recombinant protein in kinase assays with histone H1 as substrate. Histone phosphorylation wasn´t detected but we observed autophosphorylation of TbWee on tyrosine residues. Using RNAi, we determined the essential role of TbWee for cell cycle progression. Hence TbWee exhibits functional properties that are characteristic of wee kinases.

MI-P94.
BIOCHEMICAL CHARACTERIZATION OF A METALLOCARBOXYPEPTIDASE OF *Trypanosoma brucei*
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Metallocarboxypeptidases (MCP) of the M32 family of peptidases have been identified in a number of prokaryotic organisms. Members of this family are absent from eucaryotic genomes with the remarkable exception of those of trypanosomatids. The genome of T. brucei, the causative agent of Sleeping sickness, encodes one such MCP, which displays 72% identity to the characterized TcMCP-1. As its orthologue, the T. brucei enzyme is cytosolic and acts best on Furyl-Acryloyl-AK at pH 7.3 with a Km of 260 µM. Divalent cations such as Ni^{2+} and Zn^{2+} had little effect on TbMCP-1 activity., but increasing amounts of Co^{2+} inhibited the enzyme. Despite having similar tertiary structure, both MCPs display different substrate specificity with respect to P1 position. Whereas TcMCP-1 cleaves Abz-FVK-(Dnp)-OH (where Abz: o-aminobenzoic acid and Dnp: 2,4-dinitrophenyl) TbMCP-1 had no activity on this substrate. Comparative homology models and sequence alignments using TcMCP-1 as template, led us to map several residues that could explain this difference. To examine the role that these residues could play in P1 preference, site-directed mutagenesis was undertaken replacing the TbMCP-1 specific residues by those present in TcMCP-1. We found that the substitution of Ala414 by Met414 led TbMCP-1 to gain activity on Abz-FVK-(Dnp)-OH, thus showing that this residue is involved in specificity determination.

PL-P01.
EFFECT OF NITROGEN FERTILIZATION ON THE ACTION OF ANTIFUNGAL COMPOUNDS

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Phenols have an important role in several plant-pathogen interactions; the concentration of these varies depending on the cultivar, photoperiod and the availability of Nitrogen (N). Our aim was to define the involvement of phenols in reaction to late blight under field conditions and different levels of N. Experimental design was a sub-split plot where the main plots consisted of two potato cultivars, Pampeana INTA (resistant to late blight) and Frital INTA (susceptible), the sub-plots were two frequencies of application of fungicides and the subsubplots were two levels of N, optimal and excessive. There was effect of cultivar on the concentration of phenols and the severity of disease at 82 days after planting (DAP) and 102 DAP. The phenols presented significant differences between levels of N at 102 DAP and the amount of phenols was higher in plots with optimal N in the first and second sampling. We found no differences in the concentration of phenols because of the application of fungicides but there were increases in severity in the plots without fungicides in the second and third assessment. In conclusion, changes in the concentration of phenols due to the application of N and cultivar but not related to the application of fungicides were observed. Therefore, resistance is the product not only of the level of phenols but also of the combination of several factors.

PL-P02.
MRCV P7-2 ENHANCES RNA SILENCING AND INHIBITS THE ACTION OF VIRAL SILENCING SUPPRESSOR PROTEINS

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RNA silencing is a highly specific gene expression regulatory mechanism involved in important biological processes such as genome stability, development and adaptive responses to biotic and abiotic stresses. In addition, it represents one of the main defense pathways against viral infections. As a counter defense response, most viral pathogens evolved to encode proteins known as RNA silencing suppressors with the capacity to inhibit the pathway at different steps. Using agroinfiltration assays on *Nicotiana benthamiana* 16C we show that Mal de Río Cuarto virus (MRCV; Fijivirus, Reoviridae) encoded P7-2 protein has the capacity to enhance local gene silencing. We also found that MRCV P7-2 inhibits the action of Tomato bushy stunt virus P19, Tomato etch virus HcPro and Turnip crinkle virus P38, three well known viral suppressors of gene silencing that target different steps of the silencing pathway. Interestingly, a P7-2 counterpart is absent in *Nilaparvata lugens* reovirus genome, the only insect Fijivirus which is unable to replicate in plants. This work shows that MRCV P7-2 is the first reported viral encoded protein with the capacity to enhance local gene silencing and to inhibit the action of viral suppressors. The presence of a viral protein with these effects on gene silencing might unravel a new mechanism by which viruses could regulate gene expression during disease development.

PL-P03.
FUNCTIONAL ANALYSIS OF POTATO *SNAKIN-1* GENE PROMOTER IN TRANSGENIC PLANTS

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SNAKIN-1 (*SN1*) gene encodes for a peptide isolated from potato tubers with *in-vitro* and *in-vivo* antimicrobial properties. We had previously reported the cloning, sequencing and analysis of a 1421bp promoter region placed upstream of *Solanum tuberosum spp. tuberosum cv* Kennebec *SN1* gene and its tissue-specific regulation in the model plant *Arabidopsis thaliana*. The aim of this work was to study the specific expression and regulation of SN1 activity in potato upon the analysis of transgenic lines harboring *SN1* promoter region fused to betaglucuronidase (GUS) reporter gene. GUS activity was tissue- and time-specific being highest in storage and reproductive organs during the first weeks of potato plant development. To gain more insight into the factors that regulate potato SN1 activity, the influence of different growth regulators, biotic and abiotic conditions on *SN1* promoter activity was also analyzed. *SN1* promoter was strongly induced in response to shock high or low temperatures or after gibberellic acid treatments. Nevertheless, the promoter activity remained unchanged after wounding, light treatment, salt stress or fungal pathogen infections. In conclusion, these results are similar to the ones reported for heterologous expression and suggest that *SN1* promoter is a candidate sequence potentially useful for plant genetic engineering approaches.

PL-P04.
ANALYSIS OF A *Xanthomonas axonopodis pv. citri* HAIRPIN IN HOST AND NON-HOST PLANTS

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Plant-pathogenic bacteria colonize their hosts through the secretion of virulence effector proteins by the type III protein secretion system. This system is codified by the hrp (for hypersensitive response (HR) and pathogenicity) cluster and it is indispensable for pathogenicity in the host plant and induction of HR since it mediates the translocation of effector proteins across the bacterial membrane and the walls and plasma membranes of plant cells. HR is a rapid, local, programmed cell death that is induced upon recognition of the pathogen and inhibits pathogen growth within the infected plants. *Xanthomonas axonopodis pv. citri* (Xac) hrp cluster has a *HPA1* gene that encodes a harpin. Harpins are heat-stable, glycine-rich proteins that associate stably with synthetic membranes and form ion-conducting pores. We constructed a Xac *hpa1* deletion mutant and analyzed the role of HPA1 in citrus canker as well as its capacity as an HR inducing peptide in non-host tobacco plants. Although we observed that HPA1 could induce HR in non-host plants, elicitation of HR in citrus leaves by this harpin depends also on the presence of other bacterial determinants. These joined actions enhance basal immune responses and resembles pathogen-associated molecular pattern (PAMP)-triggered immunity, suggesting a role for HPA1 as a PAMP.

PL-P05.
A PUTATIVE NOVEL REGULATOR OF PATHOGEN-INDUCED PLANT DEFENSES
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In plants and animals, the incorporation of a methyl group in the carbon 5 of cytosine (5mC) generates a stable epigenetic mark that regulates gene expression. The 5mC levels can be modified by environmental conditions and thus affect the responses to stress. We have recently demonstrated that the genome of *Arabidopsis thaliana* severely reduces the content of 5mC upon the attack of *Pseudomonas syringae pv. tomato* (Pst). Infected plants also display decondensation of centromeric chromatin. Pst-induced DNA hypomethylation occurs in the absence of DNA replication suggesting it may result from the action of helix-hairpin-helix DNA glycosylases, which mediate active DNA demethylation in Arabidopsis. Here we evaluate the involvement of a novel putative Arabidopsis DNA glycosylase, ACDI (Abnormal Chromatin Decondensation in Infection), in the activation of defences against Pst. We constructed Arabidopsis T-DNA and siRNA mutant plants impaired in ACDI expression, as well as ACDI over-expressing lines. Abnormal features of chromatin condensation and susceptibility to Pst were found in these plants. Our results suggest that this putative glycosylase may participate in the phenomena of Pst-induced chromatin decondensation and the regulation of biotic defense responses.

PL-P06.
METABOLIC PROFILING ANALYSIS OF POTATO WITH ALTERED EXPRESSION OF THE ANTIMICROBIAL GENE *SNAKIN-1*
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Snakin-1 (StSN1) is a broad-spectrum antimicrobial cysteine-rich peptide isolated from *Solanum tuberosum* whicht was shown to be active against pathogens both in vitro and in vivo. We have previously obtained and characterized potato transgenic lines overexpressing or silencing *StSN1* gene. Based on the enhanced resistance of the overexpressing lines to different pathogens, we decided to explore the metabolic changes undergoing in lines with altered expression of *StSN1*. To this end, we carried out metabolic profiling on potato leaves using a gas chromatography–mass spectrometry approach. For this purpose, we chose three transgenic lines with higher expression levels of *StSN1* than wild type genotype, and other five with repressed levels. Using data-mining tools, the metabolic profiles from these experiments were analyzed to determine possible clusters. Integration of these data with previous results from transcriptional profiling analysis and phenotypic characterization of these lines, will allow us to deep into the knowledge of the in-vivo role of this gene

PL-P07.
THE ROLE OF CHLOROPLAST-GENERATED REACTIVE OXYGEN SPECIES IN THE RESPONSE TO A VIRULENT PATHOGEN
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Plants growing in their natural environment are exposed to a wide variety of pathogens. Upon infection they activate defence mechanisms including the induction of defence-associated genes and a localised cell death (LCD), conjointly known as the hypersensitive response (HR). There is a concomitant production of reactive oxygen species (ROS) in diverse cellular compartments reported to play a decisive role in the deployment and modulation of the HR. Transgenic tobacco plants expressing a cyanobacterial flavodoxin (Fld) in chloroplasts, an electron shuttle of prokaryotes and algae, display enhanced tolerance to several abiotic stress conditions by specifically preventing ROS formation in plastids. Fld antioxidant protection in chloroplasts also prevents the onset of LCD upon exposure to a non-host bacterium leaving unaffected other aspects of the HR. We use here Fld as a tool to probe the role of chloroplast generated ROS in the plant response to a virulent pathogen, namely *P. syringae pv tabaci*. Under normal irradiation conditions, Fld expression delays the appearance of LCD lesions and oxidative stress symptoms in infected leaves of transgenic plants in comparison to wild-type siblings, while failing to provide protection when plants are incubated in the darkness. These observations are discussed in the context of the role played by plastid-generated ROS in this type of interaction

PL-P08.
REVEALING ROLES OF AUXIN IN THE PLANT DEFENSE RESPONSE AGAINST BIOTIC STRESS
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The phytohormone auxin plays a central role in many aspects of plant growth and development. However, recent studies have associated novel auxin functions with plant defense mechanisms. In our laboratory a Solanum tuberosum *IAR3* gene (*StIAR*3) was isolated from a potato cDNA library. *StIAR3* shows 78% homology with an *A. thaliana* IAA-amido-hydrolase (*IAR3*), an enzyme involved in the release of active auxin through the hydrolysis of inactive aminocid-IAA conjugates. *StIAR3* is differentially induced by *Fusarium eumartii* infection in potato tubers. In addition, in silico expression analysis using microarray data shows that *StIAR3* is up-regulated by *Manduca sexta* infection and methyl jasmonic treatment in Solanaceae and by *Pseudomonas syringae* and *Phytophtora infestans* infections in *A. thaliana*. Thus, we hypothesized that auxin regulation by conjugation/hydrolysis takes part of the plant adaptative defense response against biotic stress. In order to study in deep the IAR3 functions, VIGS (Virus Induced Gene Silincing) strategy was performed. We analyzed the expression of *StIAR3* in silenced and non-silenced tobacco plants. Bioassays with fungal elicitors were carried out to evaluate the hypersensitive response. These results will allow us to evaluate the impact of IAA-amido conjugates and auxin balance in the plant defense response to fungal pathogens.
Supported by UNMdP and CONICET

PL-P09.
MULTIPLE TOSPOVIRUS RESISTANCE BY iRNA IN PLANTS

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Tospoviruses are among the ten most detrimental plant viruses in the world causing severe economic losses in a wide range of vegetables and ornamental crops. In Argentina, the three main species of this genus are TSWV, GRSV and TCSV, affecting mainly lettuce, tomato, potato, pepper and several ornamental plants.
Here we present a RNA interference resistance strategy based in the generation of double stranded RNA with sequence identity to a highly conserved region of the nucleocapsid gene of TSWV, GRSV and TCSV. In order to induce the host iRNA pathway to target this viral gene a 173bp region was obtained by multiple alignment of the species, amplified, cloned and inserted as an inverted repeat flanking an intron in a plant expression vector.
Nicotiana benthamiana plants were agroinoculated with this construction and challenged against each of the Tospovirus species in transient expression experiments. At 35 days post inoculation 100% of TSWV, 70% of GRSV and 60% of TCSV plants displayed a symptomless phenotype and no virus presence was detected by DAS-ELISA.
The approach presented here, based in a hairpin RNA construct targeting a short and highly conserved viral sequence, combines high specificity, a reduced off-target effect, does not relay in viral protein expression, and results in a high frequency broad range virus immunity to three tospoviruses.

PL-P10.
POSSIBLE ROLE IN LIPID SIGNALLING OF STRAWBERRY CLASS TAU GSTs EXPRESSED IN A BIOTIC INTERACTION

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In plants, Glutathione S-transferases (GSTs) belong to a family of proteins grouped into four main classes (tau, phi, theta and zeta), playing major roles in protection from biotic and abiotic stresses. We have isolated 17 GSTs class tau sharing high identity with other plant GSTs from strawberry leaves 48 hpi with the fungal strain M23. Proteins presented identities ranging from 29 to 99%, showing conservation in residues involved in GSH binding, and diversification in the H site at the C-terminal domain. Based in total sequence alignments and 3'UTR motifs, five different groups were found in FaGSTs. Comparative phylogenetic analyses among Arabidopsis and strawberry tau class GSTs indicated that the proteins expressed in the biotic stress in leaves are orthologues of the AtGSTs induced by salicylic acid (SA) and in response to cyclopentenones. These results agree with the increased values of malondialdehyde assessed as TBARS in leaves extracts 36 hpi, indicating that the generation of lipoperoxide precedes the induction of *FaGSTs*. We have also observed that total SA was increased in phloema exudates in challenged strawberry plants. The expression changes in *FaGSTs* transcripts were confirmed using real time PCR. Different approaches for functional studies of these paralogous genes in strawberry will help to clarify the role of class tau GST members in biotic interactions.

PL-P11.
EXTRACELLULAR SUBTILISIN-LIKE PROTEIN FROM *Colletotrichum spp.* INDUCES DEFENSE RESPONSES IN PLANTS

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We have earlier reported that the avirulent isolate M23 of *C. fragariae* protects the cv Pájaro of strawberry against anthracnose and that culture supernatant derived from that strain induced HR, autofluorescence, oxidative burst, accumulation of salicylic acid and callose deposition. Analysis of the active fraction indicated that the defense inducing agent consists in a 37 kDa protein. The aim of this work is to characterize the protein secreted by the isolate M23 and to study its activity in other strawberrry cultivars and in *Arabidopsis thaliana.* The purified protein was separated by SDS-PAGE and electroblotted in 10 mM CAPS (pH 11). The band excised was subjected to Edman gas-phase microsequencing. Each amino acid sequence obtained was compared to annotated sequences using psi-BLASTP. Partial sequence of the purified protein revealed that it belongs to the family of the subtilisin-like serin-proteases and the mature protein lacks the N-terminal protease inhibiting domains found in homologous proteins. Phytopathological experiments showed that defense eliciting protein exhibited the ability to confer resistance to other strawberry cultivars in different degrees. Experiments also showed that the purified protein could also induced the accumulation of hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$) and callose deposition in *Arabidopsis thaliana*.

PL-P12.
DIFFERENTIALLY EXPRESSED GENES IN POTATO SPROUTS AFTER POTASSIUM PHOSPHITE APPLICATION

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We are studying the role of phosphites in disease control management, yield and potato tuber quality. In brief, our previous results showed a number of promising properties associated with these compounds. When we applied potassium phosphites (KPhi) at 3 litre ha^{-1} to seed tubers immediately after cutting, this promoted early emergence, an increased in stem number and diameter, early tuber initiation and an increased in the number of tubers per plant. In addition to these physiological effects, phosphite treatment also resulted in greater resistance in seed tubers to *Phytophthora infestans*, *Fusarium solani* and *Rhizoctonia solani*. In order to understand the mechanisms which regulate these responses, we analyzed the changes in gene expression in tubers seeds at early stage of sprouting after KPhi treatment. Preliminary results of microarray analysis from potato sprouts treated or not with KPhi, showed that 26 genes were upregulated in the treated ones.
These genes were classified into 5 groups: plant defense, metabolism, abiotic stress, transcription factors and unknown genes. We performed semiquantitative RT-PCR assays of some of these genes to validate the results. Interestingly one of these genes was *CULLIN 1*, involved in jasmonic acid mediated signaling pathway. This result may support the hypothesis that phophites could be involved in triggering IR (induced resistant).

PL-P13.
ANALYSING THE EXPRESSION OF GENES ASSOCIATED WITH INDUCED RESISTANCE IN POTATO PLANTS TREATED WITH PHOSPHITES

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Phosphites (Phi) have the ability to protect plants against different pathogens, both through a direct effect in oomycete metabolism and by an indirect effect stimulating the plant´s natural defence responses. We have previously shown that KPhi foliar application to potato plants resulted in different protection levels against *Phytophthora infestans* depending on dose and plant age at application time.
In order to identify genes that are involved in induced resistant in plants treated with KPhi, we analyzed by RT PCR, the time course of transcript levels of two genes which encode predicted transcription factors involved in pathogen perception and defence gene expression. Preliminary results showed that *WRKY* and *NPR1* were differentially induced in plants both treated with Phi and infected with *Phytophthora infestans*, showing an earlier and highest induction than infected plants non treated with Phi. These results may allow us to hypothetize that Phi treatment might trigger a fast mechanism to protect potato plants to pathogen infections.

PL-P14.
PROTEIN WITH INHIBITORY ACTIVITY AGAINST MICROBIAL GLYCOSIDASES FROM LEMON

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Microorganisms have several glycosidases that degrade the polysaccharides of the plant cell wall and their involvement in pathogenesis has been demonstrated. In response, many plants produce proteins that specifically recognize and inhibit these enzymes. In a previous work we reported proteins that exhibit antimicrobial activity and inhibitory effects on microbial glycosidases. The aim of the work was to investigate the presence of proteins with inhibitory activity against microbial glycosidases in lemon peel (Citrus limon). The inhibitory protein (IP) was isolated from peel of mature lemon collected (Tucumán). The purification was carried out by chromatography techniques. The homogeneity was verified by SDS-PAGE. The IP in lemon peel was extracted with NaCl 1M, which indicates that the protein is bound to the cell wall. The IP was purified to apparent homogeneity by successive steps of salt precipitation, anionic exchange and gel filtration chromatography. IP was characterized in terms of their ability to inhibit microbial polygalacturonase, invertase and protease. The IP was able to inhibit 70 % of the *G. candidum* polygalacturonase activity (10 µg) and 65% of *S. commune* invertase activity. Protease activity from *Bacillus* was not affected. The data presented demonstrated that this protein can act on fungi glycosidades and may be considered as a defence-related cell wall protein.

PL-P15.
ROSMARINUS OFFICINALIS (ROSEMARY) REDUCE SUSCEPTIBILITY TO PLANT ARN VIRUSES

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Viruses cause many important plant diseases and are responsible for huge losses in many economically important crops in the world. Plant viruses can not be directly controlled by chemical application. Previously, we have shown that rosemary extracts reduce the growth of tobacco necrosis virus (TNV), decreasing significantly the number and size of virus lesions in *Nicotiana tabacum* plants. In this work we analyzed the biological role of two active principles of rosemary extract against several ARN viruses in *N. tabacum*. We have shown that the inhibition of virus infection is associated with the presence of micro-oxidative bursts and callose deposition. To investigate the roles of hormone-mediated signaling to TNV resistance by rosemary extracts, we analyzed the induction of genes involved in salicylic acid and jasmonic acid pathways. Our results indicate that both hormone-signaling pathways are involved in the activation of plant immune response to virus infection.

PL-P16.
SUPPRESION OF CALLOSE SYNTHASE GENE IN CITRUS CAUSES INCREASED SUSCEPTIBILITY TO *Xanthomonas*

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Citrus is an economically-important fruit crop which is severely afflicted by citrus canker, a disease caused by the bacterial phytopathogen, *Xanthomonas axonopodis pv. citri*. Identification of host genes with a role in resistance to canker would be an important step in development of new and sustainable strategies to manage the disease. However, the introduction of new genes for functional analysis through stable transgenesis continues to be a difficult process in citrus, because transformation efficiencies are generally low and competence for regeneration is very low or null. In this context, we have developed a post-transcriptional gene silencing system for *Citrus limon* to allow functional analysis of candidate genes. Double-stranded RNA expression vectors, encoding hairpin RNAs for the citrus *CALLOSE SYNTHASE* gene were delivered to lemon leaves by transient infiltration with transformed *Agrobacterium*. Phenotypic, histo-anatomic and molecular analysis showed that this vector was functional not only in lemon plants, but also in other species of the Rutaceas family. With this approach we demonstrate that plant cell wall-associated defence is the principal initial barrier against *Xanthomonas* infection in citrus plants.

PL-P17.

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF VOLATILE AND NON VOLATILE FRACTIONS OF ROSEMARY

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In this work the content and composition of polyphenols present in volatile and non volatile fractions isolated from two phenotypes of *Rosmarinus officinalis L*. grown under the same soil and climate conditions are compared. The relationship between the composition and the antioxidant and antimicrobial activities were studied. Patterns of bioactives were analyzed by GC/MS and HPLC, the antioxidant activity was studied by the DPPH assay and the antimicrobial activity by the broth dilution method.

Results showed that the chromatographic patterns of the non volatile polyhenols in both plants are qualitatively similar, although they differ significantly in the content of each main bioactive compound. The phenotype exhibiting the major content of non volatile diterpens resulted to posses the main antioxidant and antimicrobial activities. The volatile fraction of this plant also presented the highest antioxidant activity.

Essential oils and non-volatile polyhenol extracts are of interest due to their antioxidant and antimicrobial properties. In conclusion, both phenotypes of rosemary presented a typical pattern of bioactive molecules. associated with a specific antioxidant and microbicide action.

PL-P18.

APOPLASTIC HYDROPHOBIC PROTEINS INVOLVED IN TUBER DEFENSE RESPONSE TO *P. infestans*

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During infection, oomycetes secrete effectors into the plant apoplast where they interact with host resistance proteins. In response, large amounts of protease and protease inhibitors (PIs), are accumulated. We analyzed differentially expressed Apoplastic Hydrophobic Proteins (AHPs) in potato tubers from Innovator (resistant) and Spunta (susceptible) cvs, after wounding and *P. infestans* infection. Intercellular washing fluid was extracted from control, wounded or infected tubers at 0, 24 and 48 h and chromatographed into a PepRPCtmHR5/5 in FPLC. After elution with acetonitrile, fractions were analyzed by SDS-PAGE and proteins identified by MALDI-TOF-MS. Innovator cv. showed a higher basal AHP content and hydrophobicity than Spunta cv. In the latter, infection induced accumulation of patatins and PIs, whereas in Innovator cv. no changes in PIs accumulation were observed. In response to *P. infestans* infection, lypoxigenase, enolase, annexin p34 and glutarredoxin/cyclophilin were accumulated in both cvs. Hydrophobicity of AHPs was higher after 24 h of wounding and infection in both cultivars. These results suggest that an increase in AHPs concentration would be related with the protection against the oomycete and with the degree of resistance to pathogens. Finally, changes in hydrophobicity of Pis may induce changes in protease-inhibitor interaction affecting the defense response.

PL-P19.

A PLANT NATRIURETIC PEPTIDE FROM *Xanthomonas* MODIFIES HOST PLANT PHOTOSYNTHESIS

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Citrus canker, caused by *Xanthomonas axonopodis pv. citr*i (Xac), is one of the most serious diseases affecting citrus production worldwide. The pathogen enters host plant tissues through stomatal openings and wounds and then colonizes the apoplast causing the break of the epidermis due to cell hyperplasia. The infection is visualized as raised lesions on leaves, fruits and stems. Xac contains a gene encoding a plant natriuretic peptide (PNP), not present in any other bacteria. PNPs are a class of extracellular, systemically mobile molecules that elicit a number of plant responses important in homeostasis and growth. We expressed and purified this bacterial PNP (XacPNP) and demonstrated that this protein alters physiological responses including stomatal opening in plants. To better characterize the role of XacPNP in the interaction with the plant we measured chlorophyll fluorescence parameters and water potential of citrus leaves infiltrated with recombinant purified XacPNP and demonstrated that the peptide improves both the photosynthesis efficiency and the hydration condition of the tissue. Moreover, when we analyzed enzymes involved in the photosynthetic process we observed increased expression levels of them in the presence of XacPNP purified protein. Our findings suggest that the pathogen uses this plant-like peptidic hormone to modulate the host metabolism to its own advantage.

PL-P20.

PHOSPHORUS DEFICIENCY AND FLUORESCENT COMPOUNDS EXCRETED BY ROOTS AND SEEDS EXUDATES IN CROP PLANTS

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Confirming some previous results we show here that by inducing a stress condition (phosphorus shortage) some crops increase their excretion of fluorescent compounds in root exudates. In our experiments rapeseed plants entered into phosphorus shortage well before sunflower and soybean plants, which could be attributed to the seed size that limits the amount of phosphorus reserves in the seed. Chlorogenic acid is notoriously increased as well as scopoletin in rapeseed plant grown under phosphorus deficiency. Looking for a possible role for these secondary metabolites we tested the effect of root and seed exudates on the growth pattern induced on fungal growth. We observed the maximum induction of sclerotia formation with soybean seed exudates in *Macrophomina phaseolina*, although in our in vitro schemes the fungi finally overcame this condition and grew. It is as if both, an inducer of sclerotia and an inducer of myceliar growth were present in exudates, the effect of the latter somehow overriding the former. Distracting energy equivalents from a normal growing mycelium, inducing the formation of resistance structures like sclerotia, might be an opportunity to retard fungal invasion under a condition of extreme plant weakness.

PL-P21.

ELICITATION OF *Rubia tinctorium* CULTURES WITH METHYL JASMONATE TO INCREASE ANTHRAQUINONE PRODUCTION

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Plants produce a defense response against elicitation that involves the production of secondary metabolites, such as anthraquinones (Aqs), and the generation of reactive oxygen species (ROS). One of the agents widely used is methyl jasmonate (MeJ). AQs biosynthesis involves different metabolic routes: α–ketoglutarate and isochorismic acid (IC) are precursors of A and B rings. IC is produced from chorismic acid, the end-product of shikimate pathway (SP). The terpenoid pathway originates the C ring. The pentose phosphate pathway (PPP), which generates erithrose-4-phosphate (substrate of the SP), has been propose to be coupled to the proline cycle: the NADP+ generated by this cycle could act as cofactor of the first enzymes of the PPP. The aim of this work was to study the relationship between MeJ elicitation, ROS, proline cycle and AQs production in *R. tinctorum*. Cell cultures were treated with MeJ, diphenyl iodonium (DPI, an inhibitor of H_2O_2 generation) and with glucose/glucose oxidase (Glu/GOD, a H_2O_2 generating-system). MeJ increased AQs content (53%) after 48 hs. DPI addition reduced AQs accumulation in MeJ treated cells at 24 (67%) and 48 h of culture (49%). Glu/GOD induced AQs production (14 and 48%) after 24 and 48 h. MeJ treatment increased proline content at 48 h of culture (122%), while it was decreased in DPI/MeJ treatment (72%). Involvement of ROS is under study.

PL-P22.

SALICYLIC ACID MEDIATES CHANGES IN POLYAMINE METABOLISM OF TOBACCO PLANTS INFECTED BY *S.sclerotiorum*

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Polyamines (PAs) are natural aliphatic polycations with multiple functions. PA metabolism undergoes significant changes during plant responses to various stresses. Previously, our group found that PA levels vary both local and systemically in tobacco plants infected by *S. sclerotiorum*. The aim of this work was to gain insight into the role of salicylic acid (SA) in the modulation of PA metabolism during local and systemic responses of tobacco to fungal infection. Changes in PA metabolism of wild type (WT) plants induced by *S. sclerotiorum* infection were compared with those of transgenic (NahG) plants unable to accumulate SA. Locally, the levels of some apoplastic PAs increased after lesion development both in WT and NahG plants. When systemic levels of PAs were analyzed, putrescine levels of WT plants were found to be higher in infected plants than in controls, but in NahG plants the levels of this diamine and other PAs were lower in infected plants than in controls. The activity of enzymes involved in PA catabolism was not affected by infection of WT plants, neither local nor systemically, whereas in the NahG line the activity of these enzymes was different between control and inoculated plants. These results show that SA and PA metabolism are interconnected during local and systemic responses of tobacco plants to infection by the necrotrophic fungus *S. Sclerotiorum.*

PL-P23.

MODULATION OF POLYAMINE METABOLISM BY SALICYLIC ACID IN *Arabidopsis thaliana* AND *Nicotiana tabacum*

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Polyamines (PAs) are natural aliphatic polycations with multiple functions. Although plant PA metabolism is affected by pathogen attack, the signalling events involved in the regulation of PA metabolism during plant infection are not well known. In this work, the role of salicylic acid (SA) in the regulation of PA levels and the expression of genes involved in PA metabolism was investigated in the model plants *Arabidopsis thaliana* and *Nicotiana tabacum*. PA levels were modified when Arabidopsis plants were supplied with SA. Putrescine levels were higher in treated plants than in controls, as opposed to spermidine and spermine, both of which decreased in response to SA. The levels of arginine decarboxylase 2 and spermine synthase transcripts were higher in SA-supplied plants than in controls. Moreover, some modifications were observed in transcript levels of genes coding for enzymes involved in PA catabolism. Thus, transcripts of polyamine oxidase 4, an enzyme that oxidizes spermidine and spermine to produce putrescine, were significantly increased by SA treatment. PA levels and arginine decarboxylase transcripts of tobacco plants were increased by SA treatment. A high dose (200 µM) of SA induced the accumulation of the PA oxidation product 1,3-diaminopropane. As a whole, these observations show that SA, a key regulator of plant responses to biotic stress, modulates PA metabolism.

PL-P24.

BEHAVIOR OF SOYBEAN HEME OXYGENASE SUBJECTED TO UV-B RADIATION TREATMENT

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We have previously shown that heme oxygenase-1 (HO-1) plays a protective role against oxidative stress in soybean leaves subjected to UV-B radiation. The present study demonstrates that nitric oxide (NO) enhances this protection mechanism modulating HO-1 behavior. Pretreatments with 0.8-1.2 mM sodium nitroprussiate (SNP), an NO donor, prevented chlorophyll loss, H_2O_2 and $O_2^{\cdot-}$ accumulation and ion leakage in UV-B-irradiated plants (30 kJ m^{-2}). Treatments with SNP enhanced *HO-1* transcript levels after UV-B irradiation. Moreover, *HO-1* mRNA was increased 2.1-fold in SNP-treated plants, whereas subsequent UV-B irradiation augmented this over-expression up to 3.5-fold with respect to controls. HO activity showed a positive correlation with *HO-1* gene expression. cPTIO, an specific NO scavenger, negated the protection conferred by SNP. Findings here reported indicate that HO-1 over-expression involves NO and suggest that a balance between NO and ROS is required to trigger the expression of this antioxidant enzyme under UV-B radiation.

PL-P25.
COMPARATIVE RESPONSE OF HEMO OXYGENASE OF SOYBEAN NODULES TO SALT AND DROUGHT STRESS
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Heme oxygenase-1 (HO-1) induction plays a protective role in plant cells against oxidative stress. Salt and drought stress are among the most serious challenges to crop production worldwide. The aim of this study was to evaluate whether there is a link between drought and salt stress with soybean plants nodules HO-1 behavior. After 30 days growth, plants were subjected to salt and drought for 10 days. Under drought stress, there is an augmentation in TBARS levels (45%), CAT activity decreased by 20% and HO-1 protein levels were enhanced by 40%, respect to controls. Treatment with 100 mM NaCl increased TBARS levels (29%), CAT activity decreased by 30% and protein overexpression occurred, respect to controls. It is interesting to note that under 100 mM NaCl and drought stress CAT activity was inhibited, while HO is markedly increased.
Salt and drought treatments on nitrogen assimilation, glutamine synthetase (GS) and NADH-glutamate synthase (GOGAT) protein levels were evaluated. Both enzymes increased in drought conditions. GS remained similar to control and GOGAT increased protein levels with salt. In addition, HO activity and protein expression were significantly increased under salt and drought treatments.
Our data demonstrated that the up-regulation of HO could be protecting the soybean nodule nitrogen assimilation under saline and drought stress conditions.

PL-P26.
GENOMICS OF THE CADMIUM ACCUMULATION IN SOYA AND SUNFLOWER: AN APPROACH TO PLANT METALLOMICS
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Plants produce different types of peptidic defences against heavy metals: phytochelatins –enzymatically-synthesized peptides-, metallothioneins (MTs) –proteins deeply studied in the animal kingdom- and metallohistins –a new kind of protein acting against metal stress in plants. All these peptides constitute the plant response in front of an abnormal uptake (type or dose) of metals, besides their involvement in physiological metal metabolism pathways. Nevertheless, data on these systems (polymorphism, regulation, structure and function of these genes and corresponding proteins) in plants are scarce, so the aim of this study is the global characterization of the molecular mechanisms involved in cadmium accumulation in plants of interest in agriculture, such as soya and sunflower.
In silico analysis of ESTs banks with known MT sequences or motifs has rendered 7 and 8 sequences of probable MT genes for sunflower and soya, respectively. In both species, the sequences codify for isoforms belonging to the 4 different plant MTs types described. At the moment, the identified peptides are under studies for the determination of their structure/function relationship, physiological functionality and expression pattern.
This diversity in soya and sunflower MT families, and the typical plant MT type expression pattern, suggest that the differences are not only structural but also functional.

PL-P27.
METABOLIC CHANGES OF *Portulaca oleracea* UNDER DROUGHT STRESS CONDITIONS
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Portulaca oleracea is a C4 plant; however, under severe drought stress conditions it can change its carbon fixation metabolism into a CAM-like one. This work was carried out to answer whether the levels of small molecule metabolites such as osmoprotectants, free amino acids, organic acids and sugars are affected by strict drought stress in *P. oleracea*. Metabolic profile of this species under watered conditions and after withholding water during 21 days was performed in leaves through gas chromatography coupled with mass spectrometry (GS-MS). Several metabolites were identified in compositional analyses, such as organic acids; sugars; aminoacids; alcohols; and urea. However, only four analytes, urea, phenylalanine, pinitol and xilitol showed a significant drought-induced difference between well-watered and water-restricted samples, and other metabolites, such as malate, glutamine, alanine, galactose, glucose, fructose showed a significant difference between well-watered and water-restricted samples only at different time during the day, in agreement with the change in the type of photosynthetic metabolism. This work provides evidence suggesting that the change in the levels of several metabolites is important to resist drought stress conditions and to maintain CAM-like metabolism in *P. Oleracea.*

PL-P28.
CATALASE GENE FAMILY EXPRESSION IN TOMATO FRUITS (CV. MICRO-TOM) AFTER POSTHARVEST CHILLING
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Cold storage of fruits has been a main strategy to increase the shelf life of horticultural products. However, many fruits are sensitive and suffer chilling injury. Oxidative stress has been associated with the appearance of this injury and antioxidants or free radical scavengers have been shown to reduce it. We have evidence indicating that catalase (CAT) could be responsible for the chilling tolerance observed in Micro-Tom fruits. In tomato three *CAT* genes has been identified (*CAT1, CAT2* and *ER60*).The aim of this study was to analyze the expression of different *CAT* genes during postharvest chilling stress in Micro-Tom fruits. Mature green fruits were harvested, stored at 4ºC and transferred to 25ºC. CAT activity increased after the fruits were transferred to room temperature reaching to seven times the third day, but not during the cold storage. By bioinformatics screening we found a new putative *CAT* gene (*CAT3* from *Solanum lycopersicum*). We analyzed the expression of *CAT3* and the three genes previously identified. *CAT1* was the most abundant *CAT* gene expressed in mature green fruits and its transcription level increased thirty times after the fruits were transferred to 25ºC while the other family members didn't show significant differences. Our results suggest that CAT1 plays a key role in chilling stress tolerance, scavenging H_2O_2 after the cold storage of Micro-Tom fruits.

PL-P29.
DEVELOPMENT OF DROUGHT AND SALINITY TOLERANCE BY INTRODUCTION OF A CYANOBACTERIAL FLAVODOXIN

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Drought and salinity rank first among the major factors affecting crop yield and plant reproduction. Perturbation in water and osmotic homeostasis results in build-up of reactive oxygen species that poses a serious threat to cellular viability. Oxidative stress leads to decreased levels of ferredoxin (Fd), which is essential for electron distribution to a plethora of regulatory, dissipative and central metabolic pathways. Under these conditions, *cyanobacteria* and some algae induce the synthesis of the flavoprotein flavodoxin (Fld), which can provide a functional substitution of Fd in many of its crucial functions in the chloroplasts. Transgenic tobacco plants expressing Anabaena Fld in chloroplasts display enhanced tolerance to oxidative stress conditions and iron deficiency. In this work we show that in contrast to wild-type (WT) specimens these plants are also able to grow under extreme drought conditions. Physiological and biochemical data reveals a protection of the normal photosynthetic and primary metabolisms. Leaf disks of WT plants floated in NaCl solutions undergo extensive bleaching while the flavodoxin expressing counterparts maintain high levels of chlorophyll and caretonoids showing enhanced tolerance to salt-induced damage. The Fld gene-based strategy could be a useful biotechnological tool to improve crop yields and to gain at least some wastelands for agriculture

PL-P30.
ROLE OF HISTONE ACETYLATION IN THE UV-B DNA DAMAGE REPAIR IN PLANTS

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UV-B radiation induces the formation of photoproducts in DNA (DPCs), modifying the structural and dynamic properties of chromatin. Previous studies showed that, in maize, the acetylation of histones H3 and H4 N-terminus is increased after 8h of UV-B irradiation. Thus, we studied the role of histone acetyltransferases (HATs) in the UV-B DNA damage repair. Curcumin is an inhibitor of the activity of these enzymes. Maize leaves of the B73 genotype were treated with curcumin prior to the UV-B treatment. Plants treated with ethanol were used as controls. After the UV-B treatment, we measured increased DPCs accumulation in plants treated with curcumin in comparison with control plants. The experiment was repeated using *Arabidopsis thaliana* plants, including WT plants and T-DNA knockout mutants in *CHC1* and *SDG26* genes, which encode two chromatin remodeling proteins regulated by UV-B. As for maize plants, Arabidopsis DNA was more damaged in plants pretreated with curcumin than in control plants after the UV-B treatment. Moreover, *sdg26* plants were more susceptible to DNA damage than WT plants; this suggests that this line has an increased UV-B sensitivity. We are now investigating the effects of UV-B radiation in Arabidopsis knockout and RNAi mutants in two HATs, *ham1* and *ham2* mutants, which contain a MYST domain. The role of these proteins in DNA repair after UV-B exposure is under study.

PL-P31.
A 5-METHYLCITOSINE GLYCOSYLASE MUTANT, *ros1* HAS INCREASED TOLERANCE TO UV-B DAMAGE IN ARABIDOPSIS

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Ultraviolet-B (UV-B; 280–315 nm) radiation is an integral part of the sunlight and induces a broad range of physiological responses. The absorption of UV-B causes cellular damage and the formation of photoproducts (CPDs) in DNA, which affect the structural and dynamic properties of chromatin. In maize, chromatin remodeling has been implicated in the response to UV-B. Many of the chromatin-associated factors that have been characterized in plants mediate posttranslational histone tail modifications or DNA methylation. To further investigate the role of chromatin remodeling in DNA repair, we analyzed an Arabidopsis mutant with affected DNA methylation status. REPRESSOR OF SILENCING 1 (ROS1) catalyzes the release of 5-methylcytosine from DNA by a glycosylase-lyase mechanism and is required for release of transcriptional silencing of a hypermethylated transgene. We found that *ROS1* gene is UV-B downregulated and *ros1* deficient mutants accumulate significatively less CPDs than WT plants. By qRT-PCR, we determined that after UV-B exposure these plants have increased levels of some DNA repair genes and transcription factors of the UV-B-induced photomorphogenic pathway. We also found a higher expression of *CHS* in *ROS1* compared to WT. Our data suggests that ROS1, in a still unknown way, is involved in the modulation of UV-B responses in Arabidopsis.

PL-P32.
UV-B REGULATED miRNAs IN MAIZE AND *A. thaliana*

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Posttranscriptional mechanisms regulated by microRNAs (miRs) play a key role in several developmental processes and responses to environmental changes. In maize, some miRs were shown to be UV-B regulated by microarray analysis, including, miR172 and miR396. Some of the mRNA targets of miR172, *GL15* and *IDS1*, and of miR396, GRF1, 3 and 8, show opposite UV-B regulation than the miRNAs. In *A. thaliana*, miR172 target a set of AP2-like transcription factors (*AP2, TOE1, 2, 3, SNZ* and *SMZ*), most of them act as floral repressors. MiR396, targets seven GRF transcription factors (*GRF1-4* and *GRF 7-9*), which regulate leaf growth and development.
To study the UV-B regulation of *Arabidopsis* miR172 and miR396, quantification of miRs by Northern- blot and target mRNAs by qRT-PCR are being done. Preliminary results show that, as occurs in maize, miR172 decreases 2-fold in UV-B treated plants, while *TOE2* and *SMZ* mRNAs increased 2-fold and *AP2, TOE1, TOE3* and *SNZ* mRNA levels are not changed by UV-B treatments. UV-B treated plants flowered around 6 days later than under control conditions, correlating with the decrease in miR172 levels, and increased levels of *TOE2* and *SMZ* mRNAs. Also, UV-B treated plants have a smaller size than control plants, which may be due to a decreased GRFs expression measured by qRT-PCR. These results suggest miRNAs could mediate some UV-B responses in maize and *Arabidopsis*.

PL-P33.
UV-B REGULATION OF MAIZE FLS1 AND FNS ENZYMES
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Flavonoids are a highly diverse class of secondary plant metabolites found in vascular plants. These compounds have a significant impact in plant biology and benefits for human health. One group of these, the flavones, are synthesized from flavanones by the enzyme flavone synthase (FNS). Other flavonoids, the flavonols, are synthesized from the dihydroflavonols by the action of flavonol synthase (FLS). Here, we investigated the regulation of *ZmFLS1* and a putative *ZmFNS* by UV-B radiation in five maize land races from high altitudes and in the low altitude lines B73 and W23. *ZmFLS*1 and *ZmFNS* are induced by UV-B in most lines, demonstrating that these genes are under UV-B control. To relate the induction of *ZmFLS1* and *ZmFNS* by UV-B with its regulation by the P1 or C1/PL+R/B flavonoid regulators, we investigated whether the expression of these transcription factors is also UV-B regulated. UV-B significantly increases *P1* transcripts in all lines, being almost undetectable under the control conditions. In the absence of UV-B, *B* and *PL1* are expressed at variable levels in the different lines. After UV-B exposure for 8h, *B* and *PL1* showed an increase in their expression in most lines. Thus, in response to UV-B, the up-regulation of *ZmFLS1* and *ZmFNS* is possibly mediated by P1 and PL1+B, which are also induced by UV-B.

PL-P34.
GLUCANASE PROTEINS ARE INVOLVED IN FREEZING STRESS TOLERANCE IN *ARABIDOPSIS*
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Transcription factors (TFs) are key regulatory proteins in many plant stress responses. We previously reported that a sunflower TF belonging to the HD-Zip subfamily I confers freezing tolerance to transgenic Arabidopsis plants. This survey reports the molecular mechanisms involved in such tolerance. A transcriptome analysis performed with these plants showed increased transcript levels of glucanases, chitinases, thaumatin-like and pathogenesis related proteins. Based on these and qRT-PCR results, we obtained novel transgenic plants overexpressing the *Arabidopsis GLUCANASE* (At4g16260) and *PR2* (At3g57260) encoding genes. Both genotypes exhibited a normal morphological phenotype in normal conditions, but were more tolerant than WT plants to freezing stress. The tolerance presented by plants expressing the *GLUCANASE* was higher than in those which express *PR2*.
Apoplastic proteins extracted from cold-acclimated plants expressing the sunflower TF were able to inhibit the recrystallization of ice. On the other hand, apoplastic proteins extracted from transgenic and WT plants induced 3 hours at -8°C show a differential pattern in SDS-PAGE and the total protein concentration were almost twice in transgenic plants than in WT ones.
We propose that this TF confers cold tolerance to Arabidopsis plants via the induction of glucanase proteins.

PL-P35.
EFFECT OF UV-C TREATMENT ON GENE EXPRESSION IN DIFFERENT STRAWBERRY FRUIT TISSUES
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UV-C radiation of fruit has been tested to control postharvest diseases and delay some ripening processes. Nevertheless, there is little information concerning the mechanism by which beneficial UV-C induced changes occur. This work tests the effect of UV-C radiation on gene expression in different fruit tissue levels. Ripe strawberry fruits (cv. Strawberry Festival) were irradiated with UV-C (4.1 kJ m^{-2}). Control fruit received identical treatment with a Plexiglass filter. After treated, fruit were stored at 24 °C under white light, and samples from control (C) and treated (T) were taken at different time points. The expression of an *EXPANSIN* gene (*FaEXP2*) was analyzed by Northern-blot. At 4 h the expression was found to be higher in control fruit, consequently this time point was chosen for further analysis. This difference was confirmed by qPCR, but no significant change could be detected for *PECTIN METHYLESTERASE* (*FaPE1*, AY324809) and β-*1,3-GLUCANASE 2-1* (*FaBG2-1*, AY170375). Consequently, fruit were irradiated, but after 4 h of storage in the dark, different tissues were dissected (cortex and pith) and gene expression assessed separately. In this case, differences could be detected, mainly in the cortical region of the fruit, being not evident or less marked in the pith. These results suggest a certain degree of localization of fruit response to UV-C radiation.

PL-P36.
OXIDATIVE STRESS AND GROWTH MODIFICATIONS IN WHEAT SEEDLING SUBJECTED TO HEAVY METALS STRESS
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Germination and post-germination stage are critical in the plant life. Any disturbance of the optimum environmental conditions can limit the growth or affect survival and/or productivity of plants. In this work, the effect of different metals on germination and growth of wheat (*Tritucum aestivum L.*) was study. Seeds were floated for 72 h on distillated water or adding with 10 and 50 µM $AlCl_3$, $CdCl_2$, $CoCl_2$, $CrCl_3$, $HgCl_2$, $NiCl_2$, $ZnCl_2$. While most of the metals modified the seedling growth, Cd^{2+} and Hg^{2+} showed a similar behavior inhibiting germination with 50 µM metal and reducing significantly seedling growth with 10 µM metal. Cadmium was selected to continue the study. Seeds were treated with 1 and 10 µM $CdCl_2$, 0,5 µM paraquat (PQ) or 1 mM H_2O_2 for 48 h. Treatments decreased root length 28 and 55% for 1 and 10 µM Cd^{2+}, 54% PQ and 10% H_2O_2. Longitudinal sections showed changes in growth and cell proliferation. Treatments increased oxidation of proteins and catalase activity. Although membrane permeability increased with Cd^{2+} and H_2O_2, no cell death was observed by Evans Blue staining methods. These observations suggested that the oxidative imbalance in the system could be responsible of the growth inhibition observed in the cadmium treatment. More results are necessary to understand the relationship between the decrease in seedlings growth and the oxidative damages produced by metals.

PL-P37.
FUNCTIONAL CHARACTERIZATION OF GENES FROM THE *Arabidopsis thaliana OXR (OXIDATION RESISTANCE)* FAMILY
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Plants respond to oxidative stress setting up several defense lines. The first one involves scavengers that remove toxic molecules from the cell. When this barrier is overcome, proteins that prevent or repair the effect of oxidative damage in cell structures come into play. Human OXR1 (oxidation resistance 1) is a mitochondrial protein member of a conserved family of eukaryotic proteins involved in the prevention of oxidative DNA damage. We characterized two *Arabidopsis thaliana* genes, *AtOXR1* (At2g05590) and *AtOXR2* (At4g39870), that encode proteins with similarity to human and yeast OXR1. We found that AtOXR1 suppresses the oxidative mutator phenotype when expressed in an *E. coli* strain that lacks enzymes for the DNA repair system. Plants that overexpress these proteins show increased root length in MS medium and after paraquat treatment. Additionally, these plants have reduced levels of reactive oxygen species (ROS) and of transcripts from the ROS inducible *AOX1a* gene, encoding the mitochondrial alternative oxidase, under normal growth conditions and under abiotic stress generated by salicylic acid, 3-aminotriazole and UV-B treatments. We conclude that the Arabidopsis OXR proteins play a role in defense mechanisms against oxidative stress, preventing or repairing the damage generated by ROS toxicity.

PL-P38.
EPIGENETIC ANALYSIS ON A WATER STRESS-ASSOCIATED GENE IN TOMATO (*Solanum lycopersicon*)
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Our goal is to investigate the possible connection between epigenetics and water stress in plants, by analyzing the tomato *Asr1* gene, associated with water stress, as a model example. To test occurrence of cytosine methylation covering regulatory or coding regions, we used two restriction enzymes recognizing the site 5'CCGG3': HpaII, sensitive to CG methylation, and MspI, sensitive to CNG methylation.
Plant growth: plants were grown in pots with soil for 90 days, when watering was stopped. From that time on, samples were collected after 1, 2, 4, 7, 9 and 11 days.
DNA restriction: 100 ng DNA from each treatment was digested overnight by Hpa II or Msp I.
Monitoring cutting efficiency: by means of Real-time PCR, we analyzed a CCGG site 1700 bp 5' to the transcription start point and another one in exon 1, using either treated or untreated DNA as template. As a control, we used a primer pair whose amplicon does not contain such a site. For comparisons, Ct values were previously normalized to those from this non-relevant amplicon.
Conclusion: while the site in the regulatory region was constitutively methylated at both cytosines, remaining unchanged without irrigation, the cytosines studied in the coding region behave differentially: no methylation occurs in a CNG context but it does occur in a CG context, where the internal cytosine is demethylated as a consequence of water stress.

PL-P39.
FLAVONOIDS ARE SYSTEMICALLY INDUCED BY UV-B IN *Zea mays*
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Flavonoid concentration is increased by UV-B irradiation, but it is unknown if this is a local or systemic response. Nitric oxide (NO) is a diffusible molecule involved in the UV-B response. NO regulates the expression of chalcone synthase (CHS), a key enzyme in the synthesis of flavonoids. The aim of this work was to determine if maize flavonoids are local or systemically induced by UV-B, and what is the participation of NO in this response. We have used maize seedlings where the second leaf was sprayed with H2O or cPTIO (a NO scavenger), and then completely covered (C), partially covered (P) or uncovered (U) before to be UV-B irradiated. The results show a 60% increase in the NO concentration of U, 42% in P and 35% in C respectively. Flavonoid concentration increased 90% in C, 70% in P and 40% in U. Flavonoid concentration was reduced when leaves were pretreated with cPTIO before the UV-B irradiation. RT-PCR shows that CHS was up-regulated by UV-B in U, P and C, but downregulated with cPTIO. We have analyzed the subcellular localization of flavonoid and NO in UV-B irradiated plants. Flavonoid localization was coincident with the NO presence in the irradiated surface of the leaves and flavonoids were detected in vesicles. These results indicate that flavonoids are systemically induced by UV-B in a NO-related mechanism.
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PL-P40.
APOPLASTIC PEROXIDASE ACTIVITY IN WHEAT LEAVES IS INVOLVED IN THE LOW TEMPERATURE RESPONSE
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Previous reports have shown that winter wheat cultivars growing at low temperature (LT) reduce leaf area but enlarge epidermal cells, when compared with plants cultivated at optimal conditions. This response is less marked in spring cultivars. It has been proposed that apoplastic peroxidases contribute to the wall hardening by crosslinking/polymerizing proteins and polyssacharides and as a consequence they reduce the rate of cell growth. To evaluate the role of these enzymes in the LT response in wheat we obtained extracellular fluids (EF) from the basal (expanding) and distal (fully expanded) halves of second leaves of a winter (Pro INTA Pincén) and spring cultivar (Buck Patacón) growing at either 5°C or 25°C by a standard infiltration-centrifugation method. Peroxidase activity, measured using the general substrate tetramethylbenzidine (TMB, 6 mg/ml) was lower in the basal half than in the distal part of the leaves in all cases, in agreement with increased growth rates in the proximity of the meristems. Interestingly, the lowest peroxidase activity was that of the basal part of the winter cultivar at 5°C suggesting a connection between low peroxidase activity and the observed enlargement of cells in this cultivar at LT.

PL-P41.
DROUGHT-RELATED CHANGES IN SOLUBLE AND CELL WALL BOUND PEROXIDASE ACTIVITY FROM SUNFLOWER LEAVES

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Extracellular peroxidases are enzymes that catalyze the reduction of H_2O_2 with electrons transferred from a variety of molecules, classified as cell wall-bonded and soluble isoforms. They contribute to both the loosening and the stiffening of the cell wall, leading to opposite effects on growth. However, the precise role of apoplastic peroxidases in cell expansion has not been established. Furthermore, it is known that water stress reduces leaf growth and increases peroxidase activity. To assess the contribution of both isoforms to the observed inhibition of sunflower leaves growth in response to water deficit we isolated cell wall and extracellular fraction before the incubation with ferulic acid and tetrametilbencidine respectively in line HAR2 grown in chamber in well and stressed water conditions. Leaf area was measured daily to cover different developmental stages. Soluble peroxidases activity increased after the end of leaf expansion both in control and water stressed plants. In contrast, cell wall-bound activity increased along with leaf growth, reaching a peak before the end of leaf expansion. In addition, water stress decreased soluble peroxidases compared to non-stressed plants while increased the activity of the cell wall-bound isoforms. These differential responses suggest a different and probably regulatory role for soluble and cell wall bound peroxidases in leaf expansion.

PL-P42.
COMPARATIVE ANALYSIS OF HEAT-TREATED ORANGE FRUITS DURING DIFFERENT POST-HARVEST STORAGE CONDITIONS

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Heat treatment or curing is a common procedure prior to long time storage of citrus fruits. The aim of this study was to characterize changes at the molecular level induced by heat treatment on the proteome of orange fruit (*Citrus sinensis* L. Osbeck). Following two-dimensional PAGE, more than 50 differential protein spots were detected in juice vesicle tissue among all comparisons made. Heat treatment significantly affected the abundances of 16 proteins, while 25 differential proteins were found along the storage period. Identification of these citrus proteins by mass spectrometry and annotation according to the NCBI and *Viridiplantae* ESTs data bases revealed that 27% were stress-related proteins involved in cell rescue, defense and virulence, 24% were involved in metabolism, 20% were found to be storage proteins, and 15% belonged to the biogenesis of cellular components; being the rest related to minor categories. In order to analyse the changes produced by curing in the flavedo, different antioxidant enzyme activities were performed on this tissue. SOD and POD activities increased, APX activity decreased, whereas CAT and GR showed no significant differences. Furthermore, a marked putrescine induction was observed in cured flavedo. Overall, the present study provides the first analysis at the molecular level of citrus fruit responses to heat treatment.

PL-P43.
MOLECULAR CHARACTERIZATION OF StPPI, A *Solanum tuberosum* PROTON PUMP INTERACTOR

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Plasma membrane proton pumps (PM H^+-ATPases) are P-type ATPases that generate an electrochemical proton gradient across the plasma membrane, which drives the transport of solutes and regulate cytoplasmic and apoplastic pH. PM H^+-ATPases participate in various physiological processes such as phloem loading, stomata opening, mineral nutrition, salt and osmotolerance. The major regulators of the PM H^+-ATPases are 14-3-3 proteins, which interact with the ATPase C-terminus auto-inhibitory domain, thereby relieving its effect and stimulating proton pumping. A novel interactor of the PM H^+-ATPase, PPI1 (proton pump interactor, isoform 1), was identified in *Arabidopsis thaliana*. This protein binds to the PM H^+-ATPase and stimulates its activity *in vitro*.
In this work, we have identified and characterized a *Solanum tuberosum* homologue of the Arabidopsis *PPI1*, named *StPPI*. The gene structure was studied, establishing the position and size of introns and exons by a PCR approach. Southern blot analysis revealed that *StPPI* is a single-copy gene. Expression profiles of *StPPI* under different environmental conditions were determined by Northern blot, providing evidence of the participation of StPPI in abiotic stress responses. The full length *StPPI* was expressed as a fusion with His tag (His-StPPI) in *E. Col*i and the recombinant protein increased PM H+-ATPase activity *in vitro.*

PL-P44.
A STRATEGY TO REDUCE miRNAs ACTIVITY IN PLANTS BASED ON ARTIFICIAL miRNAs

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MicroRNAs (miRNAs) are 19-24-nucleotide ssRNA molecules that regulate gene expression and play a key role in plant development and morphogenesis. They are usually multigenic families whose members are expressed in a temporally and spatially regulated pattern with partially overlapping functions. miR164 family (a, b, c) regulate a group of NAC-domain containing transcription factors required for plant development. MiRNAs genetics knock out are useful but labourious and time-consuming gen-by-gen approaches. In this work, we present a novel tool to reduce the activity of the entire miR164 family. An artificial miRNA designed to recognize mature miR164 was cloned under the 35S promoter and transgenic *Arabidopsis thaliana* plants were generated. Transgenic expression of the artificial miRNA was confirmed by qRT-PCR in several lines. Phenotypic analysis of T1 lines showed morphological alterations that resemble those observed in miR164abc loss-of-function triple mutants. However, the severity of the phenotypes was attenuated gradually in further self-crossed generations. Northern blot analysis revealed changes in the expression level of endogenous miR164 in some transgenic lines. This approach could be a valuable tool, to study miRNAs functions as well as to use it biotechnologically. Although epigenetic regulation of the artificial miRNA might be playing a role limiting its usefulness.

PL-P45.
NODULE ORGANOGENESIS AND ISOPRENOID BIOSYNTHESIS IN ALFALFA-*Sinorhizobium* SYMBIOTIC INTERACTION

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We are interested in alfalfa (*Medicago sativa L.*) - *Sinorhizobium* interaction, specifically those aspects concerning recognition and nodule organogenesis. Early recognition in this symbiotic process depends of root exudates containing flavonoids and isoprenoids. The first step of isoprenoid biosynthesis is catalyzed by a thiolase II (EC 2.3.19). We have identified and cloned three alfalfa thiolase II genes and showed their root-specific expression. In addition, thiolase activity of two of them was experimentally demonstrated by using bacteria as heterologous expression system. We understand that this is the first report of root-specific thiolase II genes as well as of a functional validation of a plant thiolase II activity. Other aspect of the symbiotic interaction is the plant response leading to nodule formation. *Medicago truncatula* DMI3 gene codifies for a Ca^{2+}–calmodulin-dependent protein kinase required for the symbiotic process. We have identified and cloned its alfalfa ortholog gene (MsDMI3) that could be essential for the transduction of the Ca^{2+} signal induced by the perception of Sinorhizobium Nod factors. Real-time RT-PCR analyses indicated that MsDMI3 transcripts are expressed in roots and leaves. These two complementary research approaches are aimed to contribute to the understanding of nitrogen fixation in crop species.

PL-P46.
NOVEL ASPARTYL ENDOPEPTIDASES FROM FLOWERS OF BULL THISTLE

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Plant aspartic proteinases (APs)-EC 3.4.23- have been purified and characterized from a variety of tissues from several species, even though its functions are still unclear. In the flowers the APs are claimed to be involved in interaction pistil-pollen, senescence and stress response. In this work we characterize the APs of flowers of *Cirsium vulgare* (Savi) Ten. (Asteraceae), also known as bull thistle, as the first step in the study of the potential interaction of the zymogenic form with synthetic and natural membranes. Crude extract from mature flowers obtained at pH 3.0 presented milk clotting activity only inhibited by pepstatin, a specific aspartyl inhibitor. IEF and Zimogram showed an active band towards haemoglobin with a pI of 4. After a two-step-purification procedure, consisting on molecular exclusion (Sephadex G-25) and anionic exchange chromatography (Resource Q), only one significant active fraction was detected of 38 kDa determined by SDS-PAGE. The preproenzyme cDNA was obtained by RT of mRNA from immature flowers followed by PCR, primers used were designed from highly conserved regions among plant APs. At least two sets of clones were cloned in *E.coli* using a pGEM-Teasy vector and sequenced. Both sequences displayed a great identity with cyprosin precursor, one of the most studied members of the plant APs belonging to the peptidase family A1 (pepsin family).

PL-P47.
PLASMA MEMBRANE AQUAPORINS ARE DIFFERENTLY EXPRESSED IN HIGH AND LOW FIRMNESS STRAWBERRY CULTIVARS

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Fruit ripening studies are starting to consider aquaporin participation in fruit water status and its relevance in fruit physiology. In previous work, we reported the cloning of two strawberry fruit aquaporins showing differential behavior in terms of water permeability (Pf): high (FaPIP2,1) and low (FaPIP1;1). To understand its participation in ripening, we investigate their pattern expression from large green to 100% red stages of two cultivars with contrasting fruit firmness. Camarosa cultivar (firm fruit) showed high accumulation of *FaPIP1;1* and *FaPIP2;1* mRNAs. On the other hand, in the softer cultivar (Toyonoka), aquaporins expression was less marked.
To further characterize their functional properties, we confirm that the co/expression of these subtypes in Xenopus oocytes enhances water permeability, generating a response that surpasses the sum of their individual contribution. Moreover, this high water permeability is modulated by cytosolic pH, showing 98% reduction in its final value when pH_{int} is reduced to 6.0. In the same conditions, FaPIP2;1 expressed alone shows only partial inhibition (78%).
In conclusion, strawberry cultivars of different firmness present different expression levels of FaPIP2;1, an aquaporin that not only shows high water permeability properties but also modulates membrane Pf when coexpressed with FaPIP1;1.

PL-P48.
AN APPROACH TO THE STUDY OF THE MAIZE NADP-MALIC ENZYME FAMILY

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Genes encoding enzymes of the C4 cycle often belong to gene families having multiple copies. These findings led to the proposition that gene duplication, followed by functional innovation, was the genetic foundation for photosynthetic pathway transformation. In our studies on maize NADP-Malic Enzyme family we have isolated five transcripts encoding this enzyme The aim of this work was to study the role of each member of maize NADP-ME family. Predictive analyses and previous experiments clearly indicated that two proteins localize in the cytosol (ZmME3 and ZmME7) and two in the plastids (ZmME1 and ZmME2). On the other hand, ZmME4 amino terminal extension shows multiple transcription initiation sites and secondary structure that could reflect a complex transcription/translation regulation. Finally, as an approach to the functional annotation of each isoform, we performed a semi-quantitative analysis of each NADP-ME display in various tissues of maize, such as leaves, roots, grains, panicles, tassels, and embryos. The different expression indicating multiple functions of this gene family in maize. The recently available full genome sequence of sorghum, another C4 type grass, allowed us to discover that each maize isoform has a corresponding counterpart in sorghum, which may imply that certain NADP-ME fulfil particular roles in C4 metabolism.

PL-P49.

ANALYSIS OF AN *Arabidopsis thaliana* INVERTASE ENZYME INVOLVES IN THE PRODUCTION OF RADICAL BIOMASS

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Improving crop yield is a major goal in plant sciences. In higher plants, carbon is transported from source to sink organs mainly in the form of sucrose, where it is hydrolized by invertases and sucrose synthases. By QTL mapping has recently been shown that a vacuolar INV (vacINV) plays a key role in sink organs, particularly determining the root length in Arabidopsis. In this model, variation in vacINV activity was detected in a recombinant inbred lines population derived from a cross between two divergent accessions, LER and CVI. This analysis revealed that the CVI allele conferred both higher INV activity and longer roots. The main aim of this work is to understand the structural and functional differences of vacINV from both accessions. By DNA sequence analyses we found several polymorphisms between the CVI and Ler alleles at different coding positions leading changes in the protein sequence. Moreover, relative transcript abundance analyzed by qRT-PCR showed similar expression patterns evaluated in different organs and developmental stages of the plant. In parallel, a detailed phenotypic evaluation of the recombinant lines was performed and suggest differences in aerial biomass that could be related to differences in root growth. In order to assess the specific activity of the different vacINV alleles, recombinant proteins are being produced in an heterologous system.

PL-P50.

COMPREHENSIVE GENE EXPRESSION ANALYSIS OF ADVENTITIOUS ROOT FORMATION USING *Petunia* MICROARRAYS

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In order to target specific genes involved in adventitious root formation (ARF) in *Petunia hybrida* cv. Mitchell, a normalized cDNA library consisting of 4700 ESTs was constructed and used together with some other published or unpublished data bases for the production of microarrays representing 24,816 non-redundant unique sequences. Microarray design was carried out by generating 2-3 optimized independent probes with an average length of 36 base pairs. RNA of five different developmental stages of ARF including 0, 2, 6, 72 and 192 h after cutting (hac) along with different tissues as control like fresh leaf, wounded leaf and a root system from seeds was hybridized to the microarrays. Rank Product (RP) analyses were performed to identify significantly up or down regulated genes. Approximately 11,000 out of the 24,817 sequences belonged to ARF differentially expressed genes; and 1345 genes were identified to be ARF specifically induced. The majority of these genes were up regulated in the determination phase up to 6 hac. Performing blast against NCBI and TAIR8 revealed that primary metabolism are the category with most ARF specifically induced genes followed by, signaling, membrane transporters and secondary metabolism. Putative functions of the annotated genes being differentially expressed during ARF in the frame of the working model will be discussed.

PL-P51.

STUDY OF THE TRANSCRIPTIONAL REGULATION OF *Arabidospis* ASF1 HISTONE CHAPERONES BY E2F FACTORS

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ASF1 (anti-silencing function 1) is a conserved H3-H4 histone-chaperone first identified in yeast, which assembles or disassembles chromatin during transcription, replication and repair. In the *Arabidopsis* genome two *ASF1* homologs, *ASF1A* and *ASF1B*, were identified, but their roles in plants have not been studied yet. Here, we show that these genes are targets of E2F transcription factors, which regulate the transcription of genes required for cell cycle progression and DNA replication. By bioinformatic tools, we identified the E2F DNA-binding sites in *ASF1A* and *ASF1B* promoters. Through electrophoretic mobility shift assays (EMSA), we found that *Arabidopsis* E2F proteins, can bind to these E2F sites in vitro. These results were confirmed by chromatin immunoprecipitation (ChIP) studies, demonstrating that E2F binds directly to the *ASF1A* and *ASF1B* promoters in vivo. Furthermore, transgenic *Arabidopsis* plants overexpressing different E2F proteins (such as E2Fa, E2Fb, E2Fd and E2Ff) showed increased *ASF1B* expression. In addition, plants overexpressing the repressor E2Fc or a dominant negative version of DP, the essential dimerization partner of the *Arabidopsis* E2Fa, E2Fb and E2Fc proteins, showed decreased *ASF1A* and *ASF1B* expression. These results clearly demonstrate that members of the E2F family can regulate *ASF1A* and *ASF1B* gene expression.

PL-P52.

FUNCTIONALITY OF THE UNCHARACTERIZED CARBOXYL-TERMINUS OF HD-ZIP I PROTEINS

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Transcription factors exert their function essentially via protein-DNA and protein-protein interactions. For this purpose, they present different domains and motives. The HD–Zip family of transcription factors is characterized by the presence of a homeodomain DNA binding domain (HD) associated with a dimerization domain, the leucine zipper (Zip).

The subfamily I of HD–Zip proteins has been extensively characterized from a physiological and biochemical point of view. Although the members analyzed bind preferentially the same DNA sequence; their overexpression in Arabidopsis under the 35SCaMV promoter resulted in transgenic plants with significantly differential phenotypes.

The alignment of the sequences of a sunflower HD–Zip I protein and its homologous in other species showed that there is a high degree of conservation in the uncharacterized C-terminus. Similar results were obtained when a more extensive analysis of this region was performed with HD–Zip I members from Arabidopsis, rice and other species.

Arabidopsis plants expressing this complete sunflower protein share a similar phenotype with plants expressing a chimerical fusion of the C-terminus of this protein to the HD-Zip domain of the HAHB4 HD–Zip I protein, specially when grown in sucrose or ACC.

These results strongly suggest that this poorly studied region has an important role in the function of HD–Zip I proteins.

PL-P53.
FUNCTIONAL CHARACTERIZATION OF THE *Medicago* HD-ZIP TRANSCRIPTION FACTOR MTHB1 IN THE AERIAL ORGANS
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MTHB1 is a *Medicago truncatula* gene encoding a HD-Zip I transcription factor (TF); up-regulated by salt stress and hormones in roots. It was previously reported that this TF acts as a negative regulator of MTLBD1, a LOB-Like TF involved in lateral roots promoting; however, no information is available about the function of this gene in the aerial organs of the plant.
The promoter region of *MTHB1* was isolated by PCR and cloned into the pKGWFS7 vector directing the expression of the *GUS* reporter gene. Transgenic *Medicago* roots were obtained with this construct and were subjected to salt stress and ABA. These experiments indicated that both treatments enhanced *MTHB1* expression, in agreement with its potential function. Using the same construct, Arabidopsis plants were transformed and the expression pattern of GUS analyzed by histochemistry. This gene turned out to be expressed in roots, and in certain regions of the SAM. *MTHB1* expression pattern is consistent with those of the homologous in Arabidopsis, *ATHB7* and *12*.
TILLING mutant homozygous *Medicago* plants, unable to express a functional *MTHB1*, exhibited a particular aerial phenotype with multiple branches in comparison with the WT genotype.
The aerial expression pattern, together with the *mthb1* plants phenotype, suggests that MTHB1 is somehow involved in branch formation controlling SAM cells fate

PL-P54.
***FaPAL* EXPRESSION IN FRUIT TISSUE OF STRAWBERRY CULTIVARS WITH CONTRASTING ANTHOCYANIN ACCUMULATION**
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One of the most important traits that make strawberry fruit attractive to the consumers is the accumulation of anthocyanin pigments. Fruit color development during ripening is associated to the phenylpropanoid pathway. PAL is a key enzyme in this pathway, and then it has a determining role in strawberry fruit quality. In this work, we studied the level of anthocyanins during fruit ripening in external and internal tissues of cultivars that differ in color development (Camarosa, intense red colour in all fruit tissue, and Toyonoka, light red colour only in external fruit tissue). Toyonoka showed a lower accumulation of anthocyanins that was limited to external fruit tissue compared with Camarosa (highest pigment accumulation in both fruit sections). In addition, we cloned a *FaPAL* full-length gene and analyzed its expression in different strawberry plant organs. We observed *FaPAL* expression only in fruit tissues. During fruit ripening, *FaPAL* expression was only detected in the stages that showed some pigment accumulation in external and internal tissue of both cultivars, being the mRNA levels higher in Camarosa. PAL activity was higher in internal fruit tissue, showing no correlation with *FaPAL* expression in both cultivars. Finally, the higher *FaPAL* expression and PAL activity detected in Camarosa could be associated to the enhanced anthocyanin accumulation found in this cultivar.

PL-P55.
EFFECT OF PLANT REGULATORS ON THE EXPRESSION OF *BoPAO* AND *BoLOX1* DURING SENESCENCE OF BROCCOLI
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Plant senescence involves several events that occur at the final stages of development, or are initiated in response to a stress. The characteristics symptom is the loss of green color caused by chlorophyll degradation. Broccoli (*Brassica oleracea*) is a vegetable belonging to the group of Coles, included in the family of *Cruciferae*. Broccoli is harvested when their inflorescences are still immature, which causes considerable stress and triggers the senescence. In this work, it was studied by RT-PCR the effect of different plant regulators on the expression of two genes normally associated to senescence: *PHEOPHORBIDE A OXYGENASE* (*PAO*), involved in chlorophyll degradation and *LIPOXYGENASE 1* (*LOX 1*), associated to lipid degradation and to the synthesis of jasmonic acid. Broccolis were treated with cytokine and ethylene, in order to delay or accelerate senescence, respectively. It was observed that, after three days, the expression of *PAO* decreases significantly in both treatments. After 5 days, samples treated with ethylene increased the expression of *PAO* over control while cytokine treated samples showed a decrement of almost 50% in *PAO* expression. In the case of *LOX1*, it was observed that the expression significantly decreased in both treatments after 3 days. However, treatment with ethylene increased the expression of *LOX1* while cytokine did not affect this expression after 5 days.

PL-P56.
EXPRESSION OF TWO CHLOROPHYLL DEGRADATION GENES IN BROCCOLI UNDER POSTHARVEST HORMONAL TREATMENTS
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Harvesting of broccoli (*Brassica oleracea*) induces an early onset of senescence, characterized by a rapid degradation of chlorophylls. CHLOROPHYLLASE catalyzes phytol cleavage from chlorophyll and has traditionally been considered to be the first step in the chlorophyll degradation pathway. In recent publications, it has been stated that is PHEOPHYTINASE instead of CHLOROPHYLLASE the responsible for phytol cleavage during senescence. In the current work, we analyzed the expression by qPCR of known broccoli CHLOROPHYLLASEs (*BoCLH*) and a putative PHEOPHYTINASE from broccoli (*BoPPH*). Expression was analyzed using cytokine and ethylene treated broccoli and different sections of the florets were compared. Relative expression results from qPCR experiments showed no direct correlation between *BoCLH* expression and normal or hormone-regulated senescence. In the case of *BoPPH*, an increase in expression was found with the course of senescence followed by a gradual decrease. In cytokine treated samples, *BoPPH* expression was reduced and an increase was found in ethylene treated samples. These results suggest the possibility that phytol cleavage during chlorophyll degradation in postharvest broccoli is performed by BoPPH and not BoCLH, as was previously assumed, and thus reinforcing the recently arisen theory of PPH being the responsible for this important enzymatic step.

PL-P57.
MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF S-RNAses FROM A NATURAL POPULATION OF *Nicotiana alata*
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We report here the molecular and functional characterization of novel S-RNase candidates identified from a natural population of *Nicotiana alata*. Using degenerated oligonucleotides based on conserved domains of S-RNases, we identified by RT-PCR ten style sequences homologous to S-RNases, including three previously reported functional S-RNases and seven novel S-RNase candidates. Six of these candidates showed, by allele-specific RT-PCR, plant-specific expression in the natural population and style specific amplification, which increased gradually during bud maturation, consistent with the reported S-RNase expression. In contrast, the S63 sequence was present in all plants examined and was ubiquitously expressed in different organs and bud developmental stages. Genetic analysis confirmed the association with self-incompatible function in five S-RNase candidates while S5 and S63 appeared to be nonfunctional relic S-RNases. Phylogenetical analysis showed the *Nicotiana alata* S-RNases were included in eight transgeneric lines confirming that this species represents a broad sample of allelic variation at the S-locus of the *Solanaceae*.

PL-P58.
CLASS I TCP PROTEINS REGULATE ORGAN GROWTH AND DEVELOPMENT IN *Arabidopsis thaliana*
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TCP-domain proteins constitute a family of plant transcription factors generally involved in the regulation of cell growth and proliferation. *Arabidopsis thaliana* contains 25 different TCP proteins that can be divided into two classes. In order to analyze the role of class I TCP transcription factors, we transformed Arabidopsis plants with constructs that express *AtTCP11* and *AtTCP15* fused to the EAR repressor domain under the control of either the 35SCaMV or their own promoters. Transformed plants over-expressing *AtTCP11-EAR* exhibit lobed and asymmetric leaves, short pedicels and stems, and shortened siliques with a reduced number of seeds respective to wild-type plants. Expression of *AtTCP15-EAR* from the 35SCaMV promoter arrested seedling growth at early developmental stages. Supporting the idea that AtTCP15 is crucial for proper plant development, lines that express *AtTCP15-EAR* from its own promoter have severe phenotypic alterations showing asymmetric upward-curled leaves, reduced stem and pedicel elongation and, in the strongest cases, downward pointing flowers with organ alterations. We also generated plants that contain promoter fusions to the *GUS* gene to analyze the expression patterns conferred by both promoters. Taken together, the results indicate that the class I TCP proteins under study play an important role in the regulation of organ growth and development

PL-P59.
IMPORTANCE OF SITE II MOTIFS IN EJC CORE PROTEINS GENE EXPRESSION IN *Arabidopsis thaliana*
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The exon junction complex is involved in mRNA metabolism and is deposited on mRNA as a consequence of splicing. The Mago-Y14 heterodimer is a core component of this complex. We have observed that the promoters of the genes that encode both proteins are active mainly in meristematic regions in Arabidopsis. By means of a bioinformatic search, we detected the presence of site II motifs (TGGGCC/T) in the proximal regions of both promoters. These elements are involved in expression in meristematic cells in other genes. In order to confirm whether these elements are functional, we performed deletions of the respective promoters down to -500 bp (a fragment that included the site II motifs) and site-directed mutagenesis of the motifs. Transformed plants that express the *GUS* (â-glucuronidase) gene under the control of these promoter sequences were analyzed by histochemical and fluorometric assays. We found that the short versions produced the same expression patterns as those observed with larger fragments. In plants that contained promoters with mutations in the site II motifs, the expression disappeared. The importance of these motifs was confirmed through band shift assays using fragments from these promoters as probes and plant nuclear extracts. The results obtained suggest that site II motifs are essential for expression of Arabidopsis Mago and Y14 genes in meristematic tissues

PL-P60.
FINE-TUNING OF CHROMATIN IMMUNOPRECIPITATION (ChIP) FOR STUDYING DNA-PROTEIN INTERACTIONS IN TOMATO
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Our goal is to develop a Chromatin Immunoprecipitation ("ChIP") protocol suitable for tomato to search for DNA-protein interactions. For that purpose, we adjusted parameters to optimize the successive steps involved: Crosslinking (and its reversal afterwards): tissue was vacuum-infiltrated with different formaldehyde concentrations. The optimal one for achieving an efficient and reversible crosslinking turned out to be 1%, as determined by phenol extraction (since only non- crosslinked DNA is recovered in the aqueous phase). Chromatin extraction: nuclei were purified by means of filtration steps through nylon mesh, centrifugation in buffers of different density and incubation with Triton detergent to lyse chloroplasts. Recovered nuclei were stained with Sybrgreen and observed in a confocal microscope as 5-10 ìm fluorescent particles. DNA sonication: after testing various conditions, we ended up with 5 rounds of 10 seconds each at 15% amplitude, sufficient to obtain 200-1000 pb DNA fragments. Immunoprecipitation: we performed overnight incubation trials with ChIP-grade anti-H3 antibody and non-immune serum as positive and negative controls, respectively. Real-time PCR: primers specific for actin exon 2 resulted in 0.997 amplification efficiency. Samples precipitated by anti-H3 antibody contained 139.4 (SD = 95; n = 4) times as much actin DNA as the negative control (SD = 0.35; n = 2).

PL-P61.
BIOLOGICAL ROLE OFmiR398 IN TOBACCO
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MicroRNAs (miRNAs) are a class of non-coding small RNAs of roughly 21 nucleotides. They negatively regulate gene expression at the post-transcriptional level by perfect or nearly-perfect base pairing to complementary target mRNAs. Plant miRNAs were initially reported to be involved in growth and development patterning. Recently, miRNAs have also emerged as a class of gene expression regulators involved in different abiotic stresses. In Arabidopsis, the down-regulation of miR398 expression is important for the induction of the mRNA levels of two Cu/Zn superoxide dismutases (SODs) under high copper levels. SODs form the first line of defense against superoxide radicals. Surprisingly, results obtained in *Arabidopsis thaliana* indicate that the miR398 system is mainly implicated in copper homeostasis and has a minor role in the response against oxidative stress. In order to investigate the biological role of miR398 in plants from a broader perspective, we prepared transgenic tobacco plants with high levels of the miRNA. *Nicotiana tabacum*, cv Petit Havana expressing Arabidopsis *MIR398a* and *MIR398b* precursors from the 35S viral promoter were prepared. Overexpression of miR398 resulted in the nearly complete inactivation of the Cu/Zn SODs in tobacco, as judge by activity gels. The biological role of the miR398 system in tobacco plants will be discussed.

PL-P62.
TOMATO POLLEN TUBES AS A CELLULAR MODEL TO STUDY POLARIZED AND OSCILLATORY GROWTH
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Growing pollen tubes provide an excellent single cell system to study the mechanistic of polarized and oscillatory growth. Here, we show experimental basis for using tomato (*Solanum lycopersicum*) as a working model to study pollen tube growth regulation through tomato pollen receptor kinase LePRK2. Wild type (WT) pollen tubes show a non-continuous growth rate typical of pollen tubes. We found that Ca^{2+} oscillates with the same period as growth but out of phase as indicated by Fluo-4. To evaluate the participation of the actin cytoskeleton in pollen tube growth, we applied actin-cytoskeleton interfering agents. Promotion of actin depolymerization by 20 nM Latrunculin B resulted in an inhibition of pollen germination and a reduction in growth, abolishing the oscillatory growth behavior. Stabilization of microfilaments and actin-polymerization stimulation by 2 µM Jasplakinolide (Jas), caused depolarized growth in WT pollen tubes. Antisense-*LePRK2* pollen tubes show abnormal growth maybe by an impairment in transduction through Ca^{2+}-dependent pathways. In order to understand the role of LePRK2 in pollen tube, we will study its oscillatory tube growth compared to WT pollen. Oscillation and cytoskeleton studies in WT and these transgenic plants may constitute an appropriate tool to study the mechanisms that control pollen tube growth.

PL-P63.
***Arabidopsis thaliana* POLLEN TIPS INVOLVED IN POLLEN TUBE ELONGATION**
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Reproduction in flowering plants includes processes, like pollen germination and pollen tube elongation, where water and/ or solutes movements must be finely regulated. These regulations suggest the participation of aquaporins mediating water and solutes transport. *AtTIP5;1* and *AtTIP1;3* are the only aquaporins that are selectively and highly expressed in *Arabidopsis thaliana* mature pollen.
In a heterologous system (*Xenopus oocytes*), both TIPs showed urea and water transport but neither glycerol nor boric acid (Soto, FEBS Lett. 2008, 582). We found that *tip1;3* and *tip5;1* homozygous mutant plants showed significantly reduced pollen tube length under nitrogen starvation. This data suggests that AtTIP1;3 and AtTIP5;1 are involved in nitrogen metabolism during pollen tube elongation.
To understand the specific role of both TIPs, we generated transgenic plants that overexpress GFP-AtTIP5;1 and GFP-AtTIP1;3 fusion proteins studying their subcellular localization.

PL-P64.
DIFFERENTIAL REGULATION OF microARN miR398 TARGETS IN *ARABIDOPSIS*
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MicroRNAs (miRNAs) are a class of regulatory RNAs of ~21 nucleotides that posttranscriptionally regulate eukaryotic gene expression by directing mRNA cleavage or translational inhibition. They are involved in diverse physiological processes like development, differentiation and metabolism.
MiRNA miR398 targets two closely related Cu/Zn superoxide dismutases (cytosolic CSD1 and chloroplastic CSD2) that can detoxify superoxide radicals; and a subunit of Cytochrome C Oxidase, COX5b1. All these proteins bind Cu as a cofactor.
Copper deficiency in the growth media induces miR398, which causes the degradation of its targets, so that the available copper is re-directed to essential proteins. It has been observed that miR398 exhibits differential activity to its targets, being *CSD1* and *CSD2* more sensitive than *COX5b1* to the miRNA levels. Interestingly, genes regulated by miR398 contain target sites with specific sequences which have been conserved throughout evolution. To study this complex system we prepared transgenic plants that act as sensors of miR398 activity by overexpressing CSD2 and COX5b1 fused to GFP. Next, we introduced specific mutations in the miRNA target site of the sensors to study the mechanisms underlying miR398 activity. The action mechanism of miR398 will be discussed.

PL-P65.

EXPLORING THE ROLES OF TRANSCRIPTION FACTORS INVOLVED IN LEAF DEVELOPMENT BY ARTIFICIAL microRNAs

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In recent years, there have been several breakthroughs in post transcriptional gene silencing. Most of the novel technologies rely on the manipulation of small RNAs in order to reduce the expression of selected genes, allowing the study of a particular gene loss of function without the need of a mutant. Among these techniques, artificial microRNAs (amiRNAs) have proven to be particularly useful, due to their high specificity and efficiency to knock down target genes.
In this work we have designed and constructed a series of amiRNAs which specifically affect the expression of groups of genes involved in leaf development in *Arabidopsis thaliana*. The strategy consisted in the manipulation of the miR319 precursor in order to obtain a mature amiRNA of 21 nt with high complementarity against the genes of interest. The correct expression and processing of the amiRNAs was tested by small RNA blots, and their activity was evidenced by a decrease of the mRNA levels of the target genes. Plants overexpressing the different amiRNAs showed diverse morphological abnormalities such as uneven leaf curvature and a reduction of leaf lamina size. The obtained results allowed us to explore the roles of different transcription factors in leaf morphogenesis.

PL-P66.

CLONING OF *StCDPK1* AND *StCDPK3* PROMOTERS USING GENOME WALKER KIT AND FUSION TO A REPORTER GENE

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Transient changes in the cytosolic calcium concentration are sensed and decoded by calcium sensors, such as calcium-dependent protein kinases (CDPKs). Protein phosphorylation functions as a major mechanism involved in transducing external stimuli. Our group has cloned and characterized three CDPK isoforms, *StCDPK1*, *StCDPK2* and *StCDPK3*, which are differentially expressed during tuber development and sprouting. To perform functional studies, the promoter sequences of *StCDPK1* and *StCDPK3* were amplified using Genome Walker Kit. Genomic DNA libraries were generated using genomic DNA from potato. Gene specific primers were designed for *StCDPK1* and *StCDPK3*. Upstream sequences of both genes (*StCDPK1*, 2279 bp and *StCDPK3*,1841 bp) were amplified, cloned into pBI101 containing the reporter gene â-glucuronidase and transformed into *Agrobacterium tumefaciens* GV2206 strain. Two different potato subspecies (*Solanum tuberosum ssp andigena* and *tuberosum* var. Spunta) were transformed using leaf and tuber explants. In silico analysis using the PlantCARE was performed for both promoters. In the future, molecular characterization of the transgenic events and histochemical analysis will be performed and the pattern of tissue expression and distribution of *StCDPK1* and *StCDPK3* will be compared.

PL-P67.

A SMALL INTERGENIC REGION DRIVES EXCLUSIVE TISSUE-SPECIFIC EXPRESSION OF NEARBY GENES IN *ARABIDOPSIS*

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Transcription initiation by RNA polymerase II is unidirectional from most genes. Divergent genes, defined as nonoverlapping genes organized head-to-head, are highly represented in the *Arabidopsis* genome. Nevertheless, functional analyses of their intergenic regions are lacking. The At5g06290 and At5g06280 loci are head-to-head oriented and they encode a chloroplast located 2-Cys peroxyredoxin B (2CPB) and a protein of unknown function (PUF), respectively. In this study, we characterized the transcriptional activity of the small intergenic region in both orientations. This activity was analyzed by driving the GUS reporter gene during development and growth of *Arabidopsis* plants under physiological and stressful conditions. Results showed that this region drives expression either of *2CPB* or *PUF* in photosynthetic or vascular tissues, respectively. The promoter in both orientations showed similar regulation of GUS expression by temperature and other stress conditions such as mannitol, oxidative stress or fungal elicitor. These are the first evidences of an intergenic region that in opposite orientation directs GUS expression in different spatially localized tissues in a mutually exclusive manner. Additionally, this is the first demonstration of a small intergenic region that drives expression of a gene whose product is involved in the chloroplast antioxidant defence such as *2CPB*.

PL-P68.

FUNCTIONAL ANALYSIS OF THE *Solanum lycopersicum GDHB1* PROMOTER

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Glutamate dehydrogenase (GDH) catalyses the reversible amination of 2-oxoglutarate to form glutamate, using ammonium as a substrate. Although GDH is ubiquitous in plant tissues, its physiological role is a subject of controversy particularly in non photosynthetic tissues such as ripening fruits. In ripening tomato fruits a strong induction of NAD(H)- GDH paralleled the increase of glutamate concentration. A full-length gene encoding the â-subunit of *GDH* and its corresponding promoter were isolated in our lab. In silico analysis of this putative promoter revealed two groups of motif, one associated with physiological growing conditions such as ripening, nitrogen and carbon metabolisms, and another group associated with stress processes like drought and pathogen infection. We cloned 900pb of *GDH* promoter upstream of the â-glucuronidase (GUS) reporter gene in order to test the in vivo response of this promoter in Arabidopsis and tomato (cv. Micro-tom) plants. Transgenic plants were subjected to several treatments including ABA, SA, carbon and nitrogen sources. We found that the *GDH* promoter responded differently to the assayed conditions, corroborating some of the predictive *cis*-acting elements. In addition we found, for the first time, regulation of *GDH* by intermediates of the TCA cycle

PL-P69.
CHARACTERIZATION OF A FEMALE GAMETOPHYTIC MUTANT IN *Arabidopsis thaliana*
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Development and function of gametophytes are crucial for plant reproduction. However, relatively little is known about the molecular basis of embryo sac development. We characterized a Ds insertion line (MEE 42) with defects in female transmission efficiency. The phenotype of MEE 42 was studied by DIC microscopy of whole-mount preparations of ovules. Mutant ovules were found to be affected during post-pollination processes. Embryo development was arrested at one-cell zygotic stage. Endosperm development was also arrested. Some ovules appeared unfertilized, suggesting that the mutation is also affecting pollen tube (PT) guidance or fertilization. To further study the nature of the fertilization failure, we analyzed PT growth patterns by aniline blue staining. Although pollen tubes were found at the micropyle of both WT and mutant ovules, some mutant ovules showed abnormal PT growth patterns. To characterize the identity of the synergide cells -responsible for PT attraction- we tested the expression of a synergide marker. Similar GUS staining patterns were observed in WT and MEE42 mutant embryo sacs. The flanking sequence of the Ds element was confirmed for the 5' end of the DS element by using Ds and specific primers to be in At3g63080, encoding a glutathione peroxidase.

PL-P70.
EUKARYOTIC gamma CARBONIC ANHYDRASE HOMOTRIMERS BIND INORGANIC CARBON
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Gamma Carbonic Anhydrases (CAs) are widespread in Prokaryotes. In Eukaryotes,
homologous genes were found only in plant genomes coding for subunits of the mitochondrial Complex I. At present, only ãCA homotrimers of *Methanosarcina thermophila* (CAM) show reversible carbon dioxide hydration activity. However, homologous CcmM from cyanobacteria, was found to be inactive only conserving binding to bicarbonate. In the present work, it is shown that plant ãCA2 homotrimers bind $H^{14}CO_3$ but are unable to catalyse the reversible hydration of CO_2. These results suggest that plant ãCAs do not act as carbonic anhydrases but with a related activity possibly contributing to recycle CO_2 in the context of photorespiration.

PL-P71.
ATPase ACTIVITY AND IMMUNOSTIMULATORY PROPERTIES OF *Arabidopsis thaliana* HSP90 EXPRESSED IN *E. coli*
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The molecular chaperone Heat Shock Protein 90 (Hsp90) plays an important role in folding stabilization, activation of client proteins and also mammalian and pathogen Hsp90 showed immunostimulatory properties. For substrate activation, Hsp90 binds and hydrolyzes ATP. To gain insight on the biochemical and immunological properties of plant Hsp90, we expressed citosolic Hsp90 isoforms from *A. thaliana*. *AtHSP81.1*, *AtHSP81.2* and *AtHSP81.3* were directionally subcloned in *E. coli* Rosetta (D3) strain. In particular, recombinant AtHsp81.2 (rAtHsp81.2) over-expression was confirmed by SDS-PAGE and western blot using anti-His antibodies. rAtHsp81.2 was purified by Ni-NTA affinity chromatography and its molecular weight was determined by MALDI-TOF-MS. The purification procedure yielded 2mg/ml of 99% pure protein, suitable for further structure–function studies. rAtHsp81.2-ATPase activity was measured in vitro and the V_{max} and Km were determined. Finally, we evaluated the capacity of rAtHsp81.2 to induce in vitro proliferation of splenocytes from naive BALB/c mice. We observed that rAtHsp81.2 is able to stimulate proliferation of spleen cells as it was observed with Hsp83 of *Leishamania infantum*. LPS and ConA with or without polimixine B were used as controls.

PL-P72.
CHARACTERIZATION OF NADP DEPENDENT MALIC ENZYME FROM *Flaveria* SPECIES
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NADP-malic enzyme is a widely distributed protein involved in different metabolic pathways. In the most common C4 pathway for carbon fixation, the decarboxylation of malate is carried out by a NADP-ME. To understand the molecular events underlying the evolution of C4 photosynthesis, we have studied NADP-ME in *Flaveria* genus, which include C4, C3 and C3-4 intermediates species. Isoforms of plastidic NADP-ME are encoded by two genes in all species of *Flaveria*. Phylogenetic relationships placed these isoenzymes in two separates groups, called photosynthetic and no photosynthetic isoforms. We analyzed, by RT-PCR, the expression of these genes in developing leaves of the intermediate species *F. palmeri* and *F. sonorensis* finding out both genes are expressed in almost all the developmental stages tested. To compare the expression pattern we are setting up a quantitative protocol based on a Real time-PCR method. At the moment, the analysis showed a developmental and specie dependent expression of these genes. On the other hand, recombinant isoforms of NADP-ME from C4 *F. trinervia* and C3-C4 *F. palmeri* were purified and characterized. These enzymes showed different kinetics properties such as malate inhibition or substrates Kms, in spite of the high degree of sequence identity (more than 90%). Different expression levels and kinetic parameters suggest distinct function for two isoforms

PL-P73.
INFLUENCE OF EXPANSIN CARBOHYDRATE BINDING MODULE ON *IN VITRO* POLYGALACTURONASE ENZYME ACTIVITY

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Fruit softening is associated with cell wall disassembly mediated by the action of a set of enzymes and proteins. EXPANSINs (EXPs), a group of proteins with unknown enzymatic activity, and POLYGALACTURONASEs (PGs) are proposed to be involved in this process. It has been suggested that EXPs and PGs could cooperatively participate in ripening-associated cell wall degradation, though there is scarce experimental evidence that support such statement.
FaEXP2 encodes a putative strawberry expansin (FaEXP2) with a predicted carbohydrate binding module (CBM) in its C-terminal and an "expansin domain" in its N-terminal.
In order to evaluate the influence of expansin, we performed in vitro PG activity assays in the presence of full FaEXP2 or its CBM (CBM-FaEXP2). Both cDNAs were cloned, over-expressed in *E. coli*, and the recombinant polypeptides were purified. *In vitro* PG activity was measured using enzymatic extract from tomato, citrus pectin as substrate and the addition of FaEXP2 or full CBM-FaEXP2.
The presence of CBM/FaEXP2 in the reaction mixture decreased PG activity, and a slighter effect was found in the case of FaEXP2 also. These results suggest that CB-FaEXP2 could interact with pectins and then interfere with the activity of polygalacturonase enzyme

PL-P74.
PURIFICATION AND CHARACTERIZATION OF A JACALIN-RELATED PROTEIN FROM SUNFLOWER APOPLAST

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The proteomic analysis of apoplastic fluids (AFs) from sunflower seeds and seedlings let us the tentative identification of a 16 kDa band as a Jacalin-Related Protein (JRP). These plant lectins display a typical intracellular location and mannose or galactose affinity. The bioinformatic analysis of the matched sequence, a *Helianthus tuberosus* JRP, showed that it has a mannose binding motif and no signal peptide to enable its secretion. Purification of AFs on a mannose-agarose affinity matrix and MALDI/TOF-TOF MS confirmed the in silico assigned identity. Additionally the affinity purified fraction was able to agglutinate yeast and tomato cells. Polyclonal antibodies against the protein were obtained and used in immunoanalysis of sunflower seeds and seedlings. We obtained evidences that its content decreased through the germination being almost undetectable in 10 days-old seedlings. Furthermore, it remained unchanged in seeds incubated with 500 µM ABA or 100 µM Jasmonic Acid. We can conclude that the protein from sunflower AFs is a novel member of the mannose-binding JRP group with extracellular location.

PL-P75.
CARBON METABOLISM DURING PEACH FRUIT DEVELOPMENT

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Peach (*Prunus persica*) is a climateric fruit whose growth shows a double-sigmoid pattern. To gain knowledge about carbon metabolism during the fruit development, the metabolic pathways controlling the levels of organic acids and sugars were performed by activity measurements and qRT-PCR of transcripts encoding key enzymes. The results show that photosynthates translocated from vegetative organs, such as sucrose and sorbitol, are metabolized during rapid growth periods, mainly in the last stages of fruit development; however a great sorbitol decrease was observed after harvest. Key enzymes involved in sugar metabolism were analysed, which showed significant differences during fruit development. Interestingly, the different transcripts encoding Neutral Invertases displayed distinct pattern of expression. In the case of organic acids, a relatively higher metabolism during both rapid growth periods was observed. Regarding NADP-malic enzyme activity, it increased 8 times at end of growth, in agreement with a high respiratory rate. A significant decrease of total protein at the beginning of development was observed, which might work as an initial mechanism to obtain energy. Finally, the analysis of the metabolomic profile during peach development performed by GC-MS, indicates critical role of certain aminoacids metabolism, along with sugar and organic acid changes.

PL-P76.
CELL WALL METABOLISM IN "DIXILAND" PEACHES AFTER HARVEST AND HEAT TREATMENT

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Refrigeration of peaches during marketing and shipping affects fruit quality causing physiological disorders collectively named chilling injury (CI). To alleviate CI, different strategies have been applied before cold storage, among them heat treatment (HT) have been widely used. In this work, we analyze ethylene production, fruit firmness and the expression of enzymes and proteins involved in cell wall metabolism in peaches immediately after harvest (C), during ripening at 20ºC for 3, 5 and 7 d (C+3, C+5 and C+7), or after exposed to 39ºC for 3 d (TT) followed by 3 d at 20ºC (TT+3). For the group of fruit kept at 20ºC, a typical softening behaviour was observed, as well as an increase in ethylene production. In contrast, during the 3 d of HT, fruit maintained their firmness, which rapidly decreased after transfer to 20ºC. Relative expression of genes encoding proteins involved in cell wall metabolism was analyzed by qRT-PCR. Cell wall-related genes showed different expression patterns during ripening at 20ºC. However, TT decreased markedly the accumulation of all of them. Once TT fruits were transferred to 20ºC after HT, an increase in gene expression occurred to different extent depending on the transcript analysed. This study will help in the identification of the principal cell wall proteins involved in peach softening and their putative role in some CI symptoms alleviation.

PL-P77.
FUNCTIONAL ROLE OF GLU543 IN ALLOSTERIC FUMARATE REGULATION OF *A. Thaliana* NADP-MALIC ENZYME 2

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A. thaliana contains four NADP-malic enzymes (ME 1-4) that share a high degree of sequence similarity but differ in tissue and cellular compartment expression, kinetic properties and metabolic regulation. ME2 is the most regulated isoform and we found out that Arg115 is necessary for its activation by fumarate. However, this residue is conserved in others malic enzymes, activated or not by this compound. Recent studies with chimerical proteins determined that the region from aminoacid 303 to C-terminal end of ME2 is responsible for fumarate activation in the forward reaction. Analysis of this sequence revealed that a Glu residue at position 543 is always present in activated isoforms, but not in inhibited or not regulated isoenzymes. Therefore, mutated versions of ME2, named E543K and E543A, were generated by site-direct mutagenesis. The two mutants were expressed in *E. coli*, purified and analysed. The regulation by fumarate of both mutants was affected by the substitution, however the effect was more pronounced in ME2E543K in which Glu543 was replaced for Lys changing the charge of the site. In this way, our results allowed the characterization of a new residue involved in the activation by fumarate in ME2. This regulation would be important in vivo in Arabidopsis, which accumulate great amounts of fumarate, to levels comparable to those of starch and soluble sugars

PL-P78.
A MICROSATELLITE MOTIF WITHIN AN *INVERTASE* GENE IS ASSOCIATED WITH COLD SWEETENING IN POTATO

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Potato tubers stored at temperatures below 10°C for extended periods develop high levels of reducing sugars in a process known as cold sweetening. During cooking these reduced hexoses react with amino acids and the potato products (chips or fries) develop dark brown color and bitter taste. An association has been found between chip color and natural DNA variation at the *INVERTASE* locus invGE/GF on potato chromosome IX, that co-localized with a QTL for tuber sugar content. A microsatellite motif was found within the coding sequence of invGF gene and StI002 microsatellite was designed previously. Reducing sugar content and chip color were determined in genotypes of *Solanum tuberosum ssp. tuberosum* and *ssp. andígena* tubers at three times: at harvest and after storage for two months at 4°C or 10 °C. Also, StI002 variation was analyzed after PCR amplification of all genotypes. Of the nine *INVERTASE* alleles found, two were associated with chip quality. StI002_9 was associated with bad chip quality in the tuberosum genotypes. StI002_6 was associated with better chip quality in the *andigena* genotypes and was absent in *tuberosum* genotypes. This preliminary study points to StI002_6 as a good candidate for introgression into commercial *tuberosum* breeding programs. These results could be used for positive and negative assisted selection by molecular markers in potato breeding programs.

PL-P79.
NON CLASSICAL PROTEINS IN THE APOPLAST OF SUNFLOWER SEEDLINGS

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The apoplast or extracellular matrix of plants is a dynamic compartment involved in biological functions as diverse as cell growth and defense. In order to identify sunflower apoplastic proteins, a 1D-SDS PAGE followed by MALDI/TOF mass spectrometric analysis was performed.
Tentative identification was assigned using a sequence coverage of 20 %, a Mascott score of 62 and a peptide number of 4 as cut off. The analysis revealed novel proteins both in sunflower and the apoplastic fraction. Interestingly, a significant group of them was not expected to be extracellular since the sequences matched in the database lacked the typical signal peptide responsible for secretion. However, several controls allowed to discard a putative contamination of the apoplastic fraction with intracellular components.
The group devoid of signal peptide or non–classically secreted proteins was subdivided into unfolded proteins, thus Secretome P positive, and proteins negative for both signal P and Secretome P by in silico analysis. As unfolded proteins have been observed in the extracellular matrix of animal cells, the implication of these findings in the plant secretion mechanisms is discussed.

PL-P80.
TWO POTATO CDPK ISOFORMS DISPLAY DIFFERENT KINETIC PARAMETERS

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Changes in calcium cytosolic concentrations $[Ca^{2+}]i$ precede plant cells responses during growth, development and stress conditions. Distinctive calcium signatures are generated and the way Ca2+ signals are encrypted and decoded provides signaling specificity. Calcium-dependent protein kinases, CDPKs, are key calcium sensor in plants. In *Solanum tuberosum* five isoforms have been characterized. Isoforms StCDPK2 and StCDPK3 share 80% identity in amino acid sequence. Expression analysis revealed that both are expressed in leaves, stems, stolons and roots of potato plants but while *StCDPK2* mRNA is high in leaves and is induced in response to osmotic stress, *StCDPK3* expression is low in all tissues and is induced in early stolons. Recombinant StCDPK2::His6x and StCDPK3::His6x proteins were obtained and their kinetic parameters analyzed. Both isoforms have a similar Km for ATP (13-18 µM) and syntide-2 was the preferred substrate, but their Km and V_{max} for syntide differed: StCDPK2 (1,75 µM; 400 nmoles.min^{-1}.mg^{-1}), StCDPK3 (35µM; 9.5 nmoles.min^{-1}.mg^{-1}). In addition, StCDPK2 reached maximal activity at 2µM Ca^{2+} while StCDPK3 needed 50µM. Autophosphorylation of StCDPK2 was dependent on the presence of Ca^{2+} while StCDPK3 could autophosphorylate in the presence of 5 mM EGTA. Our results support the hypothesis that each isoform may have a distinct role in calcium signal transduction.

PL-P81.
A PLANT TRANSIT PEPTIDE DIRECTS PROTEINS TO THE HSP100 CHAPERONE SYSTEM

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Transit peptides (TP) are N-terminal extensions that route nuclear encoded proteins into plastids. Although Hsp100 chaperones have been implicated in precursor import into plastids, evidences for their interaction with TPs are lacking. Two plasmids were constructed for the expression of green fluorescent protein (GFP) and an amino terminal plant TP fusion to GFP, both with a six-histidine amino-terminal tag (His-GFP and His-TP-GFP). The constructs were transformed into *Escherichia coli* cells. The expression and purification conditions for both of them were assayed. After cellular lysis, the soluble fractions were purified using a Ni-NTA-Agarose resin. Products were analyzed by SDS-PAGE and Western blot using anti-His and anti-GFP antibodies. Under usual expression conditions (25 °C for 4 hs, 0.5 mM IPTG), His-GFP was purified satisfactorily but His-TP-GFP was not. Thus, the expression was optimized. Optimal conditions for expression were achieved at 0.5 mM IPTG, 18 °C for 16 hs and then, purifying the protein at 0 °C. Pull Down analyses were carried out using these two proteins as baits. Quantification of the inmunoblots showed that the His-TP-GFP retained about sixty times more ClpA (an *E. coli* Hsp100 chaperone) than His-GFP. These observations indicate the existence of an interaction between a plant TP and an Hsp100 protein from *E. Coli*.

PL-P82.
IDENTIFICATION OF TRANSCRIPTION FACTORS THAT INTERACT WITH THE ARABIDOPSIS *COX5B-1* PROMOTER

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The *Arabidopsis thaliana* nuclear gene *COX5b-1* (At3g15640) encodes an isoform of the zinc binding subunit 5b of mitochondrial cytochrome c oxidase. A promoter region located between nucleotides -333 and -259 of this gene is required for expression in vegetative tissues and contains elements with the core sequence ATCATT and CCACTTG which participate in induction of *COX5b-1* by sucrose and abscisic acid, respectively. Using an Arabidopsis transcription factor library, we performed a yeast one-hybrid screen and identified transcription factors that interact with this promoter region. Results obtained with HIS3 and lacZ reporter genes indicated that two transcription factors from the HD-Zip and MYB families are efficient transactivators in the yeast one-hybrid assay. Analysis of binding in vitro using recombinant proteins suggested that both proteins interact with the regions containing the ATCATT motifs, since binding was abolished upon mutation of these elements. These transcription factors are candidate regulators of the expression of the *COX5b-1* gene in vegetative tissues and its induction by sucrose

PL-P83.
PLANT SCO PROTEINS ARE INVOLVED IN COX BIOGENESIS, COPPER HOMEOSTASIS AND REDOX METABOLISM

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In *Arabidopsis thaliana*, *AtSCO1* and *AtSCO2* encode proteins with homology to the SCO proteins involved in cytochrome c oxidase (COX) biogenesis in other organisms. Phylogenetic analysis indicated that the two genes have diverged before the emergence of flowering plants. Heterozygote plants with a T-DNA insertion in the second exon of *AtSCO1* produce 25% abnormal seeds with defective embryos arrested at the heart or torpedo stage and no homozygote mutant plants can be detected. Embryos from abnormal seeds lack COX activity, suggesting that *AtSCO1* function is essential for COX assembly and for early stages of embryogenesis. Homozygote mutant plants obtained by complementation with the *AtSCO1* cDNA show a defect in pollen tube elongation, probably indicating a requirement of *AtSCO1* for pollen function. Plants that overexpress *AtSCO1* have altered responses to copper, including a different rate of root elongation, altered ratios of superoxide dismutase isoenzymes and increased activity of the miR398 promoter. Plants that lack *AtSCO2* expression develop normally but show retarded growth during the reproductive phase and alterations related with redox metabolism. The results suggest that *AtSCO1* is involved in COX biogenesis and that it also has a role in maintaining copper homeostasis in plants. AtSCO2, that lacks the copper binding motif, may participate in mitochondrial redox metabolism

PL-P84.
COPPER HOMEOSTASIS IN PLANTS: ROLE OF TWO *ARABIDOPSIS* METAL CHAPERONES INVOLVED IN COX BIOGENESIS

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In higher plants, copper (Cu) plays key roles in the photosynthetic and respiratory electron transport chains and is involved in crucial processes including ethylene perception, cell wall metabolism and oxidative stress protection. Cu deficiency induces plant chlorosis, mostly affecting young leaves and reproductive organs. We studied two *Arabidopis* proteins, AtCOX19 (At1g66590) and AtCOX17-1 (At1g53030), putative homologues of yeast metal chaperones involved in Cu delivery for cytochrome c oxidase (COX) biogenesis. *Arabidospsis* plants overexpressing *AtCOX19* and *AtCOX17-1* show a Cu deficiency phenotype, with severe disorders at the vegetative and reproductive stages, affecting normal pollen development and fertility. Although the Cu levels in 2-week-old seedlings measured by atomic absorption spectroscopy are comparable to those of wild-type, plants are Cu hipersensitive and exhibit decreased root length and reduced fresh weight, that is partially reverted by a copper-specific chelator. At the molecular level, overexpressing plants have higher expression levels of the stress responsive gene *AOX1a*, encoding the mitochondrial alternative oxidase, and altered expression levels of genes related with Cu metabolism. We conclude that the metal chaperones under study have a role in regulating cellular Cu homeostasis in addition to their known function in COX assembly.

PL-P85.
CHOROPLAST PROTEINS ARE DEGRADED BY CYSTEINE-PROTEASES IN SENESCENCE-ASSOCIATED VACUOLES

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The most obvious change during senescence of leaves is the degradation of chloroplasts, the photosynthetic organelles of plants. However, the mechanism underlying the breakdown of chloroplast proteins is poorly understood. "Senescence-associated vacuoles" (SAVs) with intense proteolytic activity develop in senescing leaves of Arabidopsis, soybean and tobacco. These vacuoles contain chloroplast proteins and high levels of cysteine protease activity. The main goal of this work was to identify the cys-proteases present in tobacco SAVs, and to determine their role in Rubisco degradation. Cys-proteases from isolated SAVs were irreversibly tagged with a biotynilated inhibitor; and inmunodetected. Western blots showed three main Cys-protease bands in SAVs at 64, 41 and 32 kDa, which are also the most important Cys-proteases in crude extracts from senescing leaves. None of these proteases could be detected in isolated chloroplasts. In in vitro autodigestion experiments with isolated SAVs, Rubisco degradation was completely abolished by incubation with E-64, a specific, irreversible inhibitor of Cys-proteases. Likewise, in vivo E-64 effectively reduced Rubisco degradation in leaf disks induced to senesce in darkness. These data strongly argue that Cys-proteases in SAVs are involved in the degradation of chloroplast proteins during senescence of leaves.

PL-P86.
SINK STRENGTH AFFECTS PHOTOSYNTHESIS AND RELATED ENZYMES CONTENT IN SENESCING MAIZE LEAVES

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We studied the evolution of photosynthesis and the content of chlorophyll, total soluble proteins, PEP carboxylase and RuBisCO in field grown maize leaves in response to changes in the source:sink ratio (the ability of plants to provide assimilates divided by the capacity of the grains to use them) during grain filling.
Treatments applied to the ear at 27 days from flowering (DFF): 1) heating (Q); 2) cooling (F); 3) removal (D); and 4) control (T).
Photosynthesis, chlorophyll content, total soluble proteins, PEPCase and RuBisCO enzymes were measured 3 times during grain filling on the third leaf above the ear.
F decreased and Q increased the rate of grain filling (about 10%) with respect to the control.
D, F and Q reduced photosynthesis compared with T at 65 DFF. At 100 DFF Q, F and T presented similar values of photosynthesis, whereas D showed no photosynthetic activity at all.
Total protein content was highest for T and lowest for D. F and Q did not significantly differ from T.
The amounts of PEPCase, RuBisCO and chlorophyll were lower in D than in T. F and Q also reduced the amount of these compounds in the leaves, but to a lesser extent.
Ear removal clearly reduced photosynthesis, chlorophyll, total protein and photosynthetic enzymes contents. Our results also indicate that variations in the rate of grain filling could also affect these indicators of leaf senescence.

Pl-P87.
A NEW APPRAISAL ON SUCROSE METABOLISM GENES IN MICROORGANISMS

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The increasing number of complete sequenced genomes of oxygenic photosynthetic organisms let us to continue our study on sucrose (Suc) metabolism proteins. Searches for homologs to *sps* and *spp, sus* and *inv* (genes encoding proteins of Suc synthesis, cleavage and hydrolysis) allowed to retrieve sequences of new putative genes. They were functionally characterized by expression in *E. coli* and by studies of the native proteins from cyanobacteria of the genera *Synechococcus, Prochlorococcus, Thermosynechococcus, Nostoc, Gloebacter and Microcystis* . The deduced aminoacid sequences of the characterized SPS, SPP and SuS were used to expand our phylogenetical analysis on Suc metabolism evolution. The presence of SuS proteins in *G. violaceus* and *M. aeruginosa* as the unique proteins responsible for Suc degradation allows to support the hypothesis of a distinct role for that enzyme in unicellular than in filamentous strains. By two different lines of evidence we conclude that the *sus* genes of those strains might have been acquired by lateral gene transfer. We conclude that whereas Suc seems to be essential in vascular plants, at least in some photosynthetic oxygenic microrganisms it is likely to be dispensable. Gene expression analyses suggest that its role in some microorganisms is related to environmental conditions.
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PL-P88.
CHLOROPLAST RETROGRADE SIGNALLING INVOLVED IN ALTERNATIVE SPLICING REGULATION

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With the aim of understanding the mechanisms that regulate alternative splicing in plants, we studied the *Arabidopsis thaliana* genes for RUBISCO ACTIVASE and the SR protein RSp31, whose alternative splicing patterns vary depending on light conditions. In both transcriptional units, light exposure causes an increase in the proportion of the shorter mRNA isoforms. Light intensity (not its quality) seems to be the factor that elicits changes in splicing patterns. Blue or red light produce similar effects as white light and different photoreceptor mutant plants behave as the WT in the alternative splicing response to light. The alternative splicing pattern modulation produced by light is affected by drugs acting on chloroplasts. The photosystem II inhibitor DCMU mimics the effect of darkness on alternative splicing. The involvement of a chloroplast signal was explored by studying the splicing pattern in leaves and roots. The light effect observed in leaves is also seen in roots but with a time delay. Even more, if leaves and roots are separated prior to light treatment the effect can only be seen in leaves. This suggests that a signal produced in chloroplasts could trigger changes in the alternative splicing patterns of different mRNAs acting at a systemic level. We are trying to get more information about the nature of this retrograde signal.

PL-P89.

NITRIC OXIDE AND POLYAMINES ARE INVOLVED IN THE REGULATION OF WHEAT NITRATE REDUCTASE

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Nitric oxide is a molecule which origin in plants is still discussed. Nitrate reductase is proposed to be involved in NO biosynthesis while polyamines (PAs), that share arginine as a common precursor with NO, are nitrogen compounds that can modulate NR. The aim of this work was to study a possible modulation of NR by NO and the effect of PAs on the NR. Wheat leaf segments were incubated with 10, 100 y 500 µM SNP; and with 0.1-1 mM Put, Spm and Spd during 6, 9, 16 and 21 h. At shorter times, 0.1 mM of the three PAs decreased significantly NR activity, while at 21 h, the activity increased. Only Put and Spm produced a similar effect when were used at 1 mM. To evaluate if NO participated in the initial inhibition caused by Put, we tested the recovery of the activity in the presence of c-PTIO and effectively, the effect produced by Put was mediated by NO. Sodium nitroprusside, produced a strong inhibition of the NR activity from the lowest concentration used. We confirmed that NO was involved in the inhibition using c-PTIO to reversed the effect. Although a clear inhibition of the activity was observed, no changes were observed in the NR protein expression. Then, we investigated the presence of the NO-tyr on the NR, with negative results. To summarize, NO and PAs participate in the regulation of NR, with NO as intermediate in the PAs effect and the mechanism involved still unknown.

PL-P90.

ALTERATIONS OF BRASSINOSTEROID HOMEOSTASIS IMPAIR ASCORBATE-GLUTATHIONE CYCLE IN TOMATO

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Ascorbate-glutathione (AA-GSH) system is a major antioxidant mechanism involved in plant adaptation to a changing environment, and brassinosteroids (BR), are plant steroidal hormones implicated in plant growth and development. The aim of this work is to study the AA-GSH system in tomato dwarf and over-growing plants resulting from deficiency (dx) or excess (35SD) of endogenous BR, respectively.
Under moderate irradiance, leaves of BR-deficient plants and BR-accumulating plants (35SD) displayed low AA accumulation with a more oxidised state than wt. However, GSH content remained unchanged. Plants with altered BR levels showed the activities of stromal ascorbate peroxidase and glutathione reductase (GR) lower than those observed in wt, while cytosolic ascorbate peroxidase (cAPX) and dehydroascorbate reductase activities were similar to those in wt. In addition, these plants showed decreased stomata conductance but only dx plants present an inhibition of photosynthesis. A sudden exposure of plants to a combined heat and high irradiance stress, did not affect AA levels but increased leaf GSH content and GR activity, and kept low activity of cAPX in both groups of plants with altered BR levels. Taken together, these results suggest that an altered BR homeostasis through either defect or excess of BR lead to similar disruptions of AA-GSH cycle

PL-P91.

AUXIN SIGNALING INTEGRATES GROWTH RESPONSES AND ADAPTATIVE MECHANISMS DURING SALT STRESS

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The plant hormone auxin is required for diverse aspects of plant growth and development. In addition, there is an increasing body of evidence on the participation of auxin signaling during the adaptative response to adverse conditions. We have previously reported that the *Arabidopsis thaliana* double mutant for auxin receptors, TIR1 and AFB2, modifies root growth and is rather tolerant to abiotic stress. The aim of this work was to study the mechanism involved in the adaptative response of *tir1/afb2* mutant under salt and oxidative stress. We demonstrated that *tir1/afb2* mutant presents lower levels of superoxide ion and hydrogen peroxide compared to wild-type. Furthermore, this double mutant displays increased gene expression and activity of antioxidant enzymes such as catalase, glutation-S-transferase and ascorbate peroxidase. Under salinity, total and reduced ascorbate content in *tir1/afb2* mutant was 3 folds higher than in wild-type plants. These data suggest that auxin could transduce and integrate developmental and environmental signals by modulating components of the antioxidant metabolism.
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PL-P92.

JASMONIC ACID, ROOT GROWTH AND ITS RELATION WITH AUXINS IN SUNFLOWER SEEDLINGS

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The jasmonates are naturally occurring in plants and have been accepted as a new kind of plant hormone. They act as signal molecules in plant defense and influence several aspects of plant growth and development, including root growth in certain species. The objective of this work was to investigate the effects of the jasmonic acid (JA) on the primary root length (PRL) in *Helianthus annuus* seedlings. Seedlings were treated hydroponically during four days with different combinations of JA, ibuprofen or salicylhydroxamic acid (IBU and SHAM, inhibitors of JA synthesis), NAA (an auxin) and NPA (an auxins transport inhibitor) and the effect on PRL was analyzed. The application of JA decreased the primary root growth in a dose-dependent way and the treatment with inhibitors of JA synthesis increased PRL, indicating that not only the exogenous but also the endogenous JA regulate the PRL. As auxins also inhibit the primary root growth, a possible interaction between JA and auxins was analyzed. Exogenous JA produced its phenotype even in the presence of reduced levels of auxins generated by treatment with NPA, and NAA produced its phenotype even in the presence of reduced levels of JA generated by treatment with IBU or SHAM. Therefore, these results indicate that the JA-induced primary root inhibition is independent of auxins in sunflower seedlings.

PL-P93.
HYDROGEN SULFIDE PARTICIPATES IN ABA-DEPENDENT STOMATAL CLOSURE
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Endogenous gases had emerged as a mainstream topic in signal transduction research both in animals and plants. Among them, hydrogen sulfide (H_2S) is an emerging signaling molecule with important functions in human physiology, it has been involved in sulfur-induced resistance in plant-pathogen interaction. In plants, it has been shown that L-cysteine desulfhydrase (L-CDES) releases H_2S during L-cysteine metabolism, while O-acetylserine(thiol)lyase (OAS-TL) consumes H_2S during L-cysteine synthesis. However, the mechanism of action and the biology of H_2S as a signaling molecule in plants it's still in its infancy.
In the present work we report that the H_2S donor NaHS induces stomatal closure in *Vicia faba* epidermal peels, in a dose dependent manner. In addition, when epidermal peels were treated with the inhibitor of L-CDES DL-Propargylglicine (PAG) ABA-dependent induction of stomatal closure was partially blocked. In order to correlate these effects at a whole plant level, we treated *Impatiens walleriana* plants with NaHS, and suspended the watering for four days Results show that H_2S treated plants were greener, and had a Real Water Content (RWC) 20% higher than control plants, after the imposed stress. Consequently it is postulated that H_2S is involved in the regulation of stomatal closure through its participation in some of the ABA evoked pathways.
Supported by UMNdP-CONICET and AMPCyT

PL-P94.
FIRST CHARACTERIZATION OF A NITRIC OXIDE SYNTHASE FROM THE PLANT KINGDOM
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Recently, it has been completed the genome sequence of the unicellular green algae *Ostreococcus tauri* (Ot), the smallest member of the plant Kingdom. In the genome of Ot it has been identified a gene sharing high similarity with those coding for nitric oxide synthase (NOS) of mammals. NOS enzymes generate nitric oxide (NO) from arginine using NADPH as electron donor. NO is involved in many physiological processes and associated with responses to (a)biotic stresses in organisms from all Kingdoms. To date, no gene sequence, cDNA or protein coding for a putative plant NOS have been found. In this study, we have conducted the cloning, expression and biochemical characterization of OtNOS. The OtNOS has been successfully expressed as a recombiant protein in E*scherichia coli*. OtNOS was showed as high-spin heme protein and shared spectral properties to those described for others isolated NOS. Activity of the recombinant OtNOS was determined by two methods (i) hemoglobin capture and (ii) NADPH oxidation. Bacteria expressing the recombinant OtNOS display high levels of NO after supplementation with the NOS substrate L-arginine and were more resistant than wt to the oxidant hydrogen peroxide. All together, these results describe for the first time the presence of NOS in plant Kingdom.
Financed by Conicet, ANPCyT and UNMDP.

PL-P95.
INTERACTING PARTNERS OF THE BSL PROTEIN PHOSPHATASES IN PLANTS
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The BSL protein phosphatases belong to a novel family of the PPP phosphatases found in several organisms. The only member of the family characterised to date is involved in the modulation of the brassinosteroid signalling pathway in *Arabidopsis thaliana*. BSL phosphatases are formed by a characteristic catalytic domain at the C-terminus, linked to a β-propeller domain at the N-terminus by a connecting region. Protein phosphatases are modular proteins, whose functional properties and/or subcellular localisation are defined by disparate interacting partners. To gain more insight on the processes these enzymes may act on in plants and to further precise their regulatory properties, we searched for interacting partners of one of the paralogs in Arabidopsis through yeast two-hybrid screens, using both domains separately. We found that the catalytic domain is able to homodimerise, which is an unusual feature among Ser/Thr phosphatases. We report the validation of this interaction and dissect its requirements, and show that it is common to all the members of the family in Arabidopsis, in a combinatorial way. Moreover, extending the assay to a broader phylogenetic range, including homologs in mosses and green algae, we show that this interaction appears to be a feature of this whole group of phosphatases. The functional consequences of this interaction are being explored

PL-P96.
BSL PROTEIN PHOSPHATASES IN *Arabidopsis thaliana*
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BSL phosphatases are members of a poorly characterised branch of the PPP family of Ser/Thr phosphatases. Found only in green algae, plants and alveolates, they stand apart among other PPP phosphatases by their structure: an N-terminal â-propeller domain linked by a connecting sequence to the C-terminal catalytic domain. To date, the only function ascribed to these proteins is the modulation of the brassinosteroid signal strength in plants. We set out to study their functions as a way to understand the evolution of signal transduction pathways in multicellular organisms. There are four members of the BSL family in *Arabidopsis thaliana*. We report the phenotypic and physiological characterisation of plants mutant for *BSL2* and *BSL3*. Both genes share high sequence similarity and their expression patterns extensively overlap. The BSL2 protein appears both in the cytoplasm and in the nucleus, and this pattern is observed even when each domain is expressed separately. Single mutants are normal, but when the function of both genes is disrupted, plants display a series of severe phenotypic defects ranging from reduced stature to complete sterility. Whereas the responses to the application of brassinosteroid are mostly unaltered, we detected an altered response to auxin. Finally, we analysed changes in global gene expression and found 829 significantly misregulated genes in the double mutants

PL-P97.

DIFFERENTIAL PROTEOME ANALYSIS CHARACTERIZATION OF ARABIDOPSIS HETEROTRIMERIC Gα SUBUNIT MUTANT

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In higher plants many classes of biotic and abiotic signals act through specific transduction mechanisms to coordinate plant development. Heterotrimeric G protein signaling is one of the most conserved mechanisms in eukaryotes. *Arabidopsis thaliana* possess only one gene encoding a unique α subunit protein: AtGPA1. The evidence revealed that GPA1 is a central molecule in transmitting hormone, light and other signals to different effectors molecules. GPA1 has been described as a positive modulator of cell proliferation and *gpa1* mutant leaves display a particular morphology. GPA1 mediated functions have been well described however only a few downstream effectors have been identified. Nowadays, proteomic is a powerful technique defining proteins that change in abundance, form, location and can be used to identify proteins involved in specific developmental or environmental changes. Here, we obtained the proteome profile of wild type and *gpa1* mutant leaves with the proposed to find differentially expressed proteins. Two dimensional gel electrophoresis revealed seven protein spots that showed statistically significant changes ($p < 0.05$) between the two phenotypes. Five proteins were more abundant in wild type plants and only two proteins were more abundant in *gpa1* mutants. Spot excision, trypsin digestion and peptide mass sequencing is being conducting to obtain protein identities.

ST-P01.
MOLECULAR CHARACTERIZATION OF PKA FROM *Yarrowia lipolytica*
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The dimorphic yeast *Yarrowia lipolytica* has gained interest in recent years due to its biotechnological and basic application. We have previously shown that the cAMP/PKA signal transduction pathway regulates different physiological processes in this fungus, including dimorphism, being cAMP a strong inhibitor of yeast to mycelium transition. In this work, several biochemical and structural characteristics of the PKA holoenzyme were studied. In this organism, both PKA catalytic (C) and regulatory (R) subunits are codified by single genes, named *TPK1* and *RKA1* respectively. Since in silico analysis of the N-terminal of R sequence showed low homology with the dimerization domain present in almost all eukaryotic R, we assessed the polymeric nature of the holoenzyme and of the R subunit by sucrose gradient analysis. Partially purified holoenzyme and R subunit were obtained from wild-type and tpk1 strains, and were characterised by phosphortransferase activity, cAMP-binding activity and western-blot recognition. The holoenzyme sedimented as a dimmer with an apparent sedimentation coefficient (S) of 5.6, while dissociated R and C subunits appeared as monomeric proteins with S of 3.6 and 2.5 respectively. Furthermore, cloning, expression and characterization of R subunits in *E. coli*, indicated that recombinant R appeared only as a monomeric protein.
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ST-P02.
TRANSCRIPTIONAL CROSS-REGULATION BETWEEN *Candida albicans* TPK1 AND BCY1 GENES
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In the present study, we performed a series of physiological and biochemical studies of a set of new generated *Candida albicans* PKA mutant strains, null for *TPK2* and carrying heterozygous *TPK1* and/or *BCY1 alleles*. The combination of deletions in strain tpk2 *TPK1/tpk1 BCY1/bcy1* rendered a mutant widely affected in its ability to react to changes in the environment, including dimorphism and biofilm formation. *In vitro* kinase assays along the growth curve showed that the defective new mutant, which exhibited an extremely low PKA specific activity at the stationary phase, vastly increased its specific activity at the logarithmic stage of growth which was not accompanied by a significant increase in neither mRNA transcription nor protein synthesis, suggesting a transient activation of enzyme activity. A *TPK1* reintegrated version of this mutant strain showed a phenotype similar to that of parental strain *tpk2/tpk2 BCY1/bcy1*. However, restoration of *TPK1* copy surprisingly led to an upregulation of BCY1 transcript. Increased cAMP binding levels consistent with the compensatory expression of the regulatory subunit were observed in this mutant. These results suggest an unexpected complex interdependence of the biosynthesis of the Tpk1 catalytic isoform and the Bcy1 regulatory subunit, whose molecular basis remains to be established.
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ST-P03.
THE EFFECT OF LEUCINE ON UGA4 GENE IS MEDIATED BY UGA3 AND UGA35 TRANSCRIPTION FACTORS
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The S. cerevisiae UGA4 gene encodes a permease capable of importing δ-aminolevulic acid and γ-aminobutyric acid into the cell. The expression of this gene depends on GABA induction and we have demonstrated that it is also regulated by the availability of extracellular amino acids through the amino acid sensor complex SPS (Ssy1, Ptr3, Ssy5). Two positive transcription factors, Uga3 and Uga35, act through the UASGABA element and are required for GABA induction. We have previously shown that the transcription factor Leu3 has an effect on UGA4 expression apparently through the UASGABA element. The aim of this work was to identify and study the molecules responsible for the effect of leucine on UGA4 gene and elucidate the mechanism by which this occurs. Promoter activity measurements indicate that the transcription factors Leu3, Stp1 and, in a lesser extent, Stp2 are involved in UGA4 regulation by leucine. Chromatin Immune Precipitation assays show that the transcription factors Uga3 and Uga35 bind to the regulatory region of UGA4, probably to the UASGABA element, in the presence of the inducer GABA but they lose part of this capacity when cells are pre-incubated with leucine. These results correlate with the low UGA4 induction achieved in the presence of this amino acid. ChIP assays also suggest that the transcription factor Leu3 is not bound to the UASGABA element in UGA4 promoter.

ST-P04.
R SUBUNITS ISOFORMS OF PKA FROM *Mucor circinelloides*: FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION
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In M. circinelloides PKA is a tetramer composed of two Regulatory subunits (R) and two Catalytic subunits (C).We demonstrated the existence of 4 genes for R subunit and 10 for C subunits.We disrupted pkaR1 and pkaR2 genes to study their role in regulation of morphological and cellular development. pkaR1Δ showed a reduction in growth and alterations in germination rates, cell volume, germ tube length, and asexual sporulation. In pkaR2Δ the only difference observed was that the germ tube emission was earlier than in the wild-type strain. The expression of all genes was detected by semiquantitative RT-PCR and they showed differential expression at different developmental stages.The expressions of the genes were also analyzed in pkaR1Δ which showed no variations in the expression of the other genes, and in pkaR2Δ which showed no expression of pkaR3 and an increased expression of pkaR4. Analysis of the nucleotide sequences of M. circinelloides pkaR genes reveled that they arose by gene duplication events, pkaR3 gene being less closely related to the other three.Our results suggest that these duplication events occurred very early in the zygomycete lineage. The results confirm that PKA is involved in M. circinelloides physiology and due to the existence of four R and ten C subunits there would be a multiplicity of possible PKA holoenzymes which could have different functional significance

ST-P05.
INTERACTING PROTEINS OF PKA REGULATORY SUBUNIT FROM *Saccharomyces cerevisiae*
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Common challenges to any cell are the processing of the extracellular stimuli it receives into intracellular signaling cascades that initiate a multitude of diverse biological functions. Subcellular targeting through the association with adaptor and scaffolding proteins has emerged as a key mechanism by which cells maintain signaling specificity. Compartamentalization of cAMP signaling is maintained by the clustering of cAMP signaling enzymes in discrete units by the scaffolding protein A-kinase anchoring proteins (AKAPs) through their interaction with Regulatory subunit. Our aim is to identify S. cerevisiae AKAPs to study PKA localization and to evaluate their participation in PKA activation. No anchoring proteins had been characterized up to now in yeast. We have identified anchoring proteins of Regulatory subunit from yeast PKA (BCY1) using TAP-affinity purification and MALDI-TOF-TOF identification. The interactions were verified by pull-downs and peptide arrays. The critical residues in the protein domain from the interacting protein were identified by Ala-scanning peptide array. We also demonstrate using BCY1 N-terminus mutants that the region 1-138 is necessary to the interaction with these proteins

ST-P06.
EXPRESSION REGULATION OF CATALYTIC SUBUNITS OF PKA FROM *Saccharomyces cerevisiae*
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Yeast cells respond to environmental changes by signal transduction cascades which are modulated according to the type of response. This modulation may involve gene activation or inactivation. We studied the expression regulation of two catalytic isoforms of PKA, Tpk1 and Tpk3, using reporter gene. We found that in wt strains, the transcriptional activity of Tpk1 promoter was increased during stationary phase. ß-galactosidase assays in wt strains grown to exponential phase in glucose or glycerol revealed that the expression was 10-fold increased using glycerol as a carbon source. Influence of PKA activity on these promoters was assessed. Strains with low PKA activity ($Tpk1^{w}$) showed a 4-5 fold increase of Tpk1 and Tpk3 promoters activity, while strains with high PKA activity (ΔBcy1) had a decreased activity for both promoters. We also studied the role of Msn2/4 on Tpk1 and Tpk3 expression. In strains lacking these stress responding factors, we found that Tpk1 promoter activity decreased and Tpk3 promoter activity remained invariable. In heat shock and osmotic stress conditions Tpk1 expression increased, while Tpk3 expression was not affected. The endogenous mRNAs levels from Tpks were also measured showing the same regulation. These results suggest differential regulation of Tpk1 and Tpk3 promoters depending on the carbon source, the stress condition and on the own cAMP-PKA pathway

ST-P07.
PKA CATALYTIC SUBUNITS LOCALIZE TO P-BODIES UNDER DIFFERENT GROWTH AND STRESS CONDITIONS
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PKA is a heterotetramer formed by two regulatory (R) and two catalytic (C) subunits. In S.cerevisiae, the R subunit is encoded by the BCY1 gene, and the C subunits are encoded by three genes functionally partly redundant: TPK1, TPK2 and TPK3. We have observed that during stationary phase, Tpk1 and Bcy1 showed cytoplasmatic localization, whereas Tpk2 and Tpk3 were accumulated in cytoplasmatic foci. Co-localization experiments in cells expressing Bcy1-Red1 and Tpk3-GFP during stationary phase showed that Tpk3-containing granules do not contain Bcy1. Co-localization experiments in cells expressing Tpk2-GFP or Tpk3-GFP and Dcp1-RFP or eIF4E-RFP during stationary phase revealed that Tpk2 and Tpk3 are associated with P-bodies and EGP-bodies. As part of our analysis, we assessed the localization of Tpk3 in response to glucose starvation, middle and high osmotic stress. Removal of glucose from cells in exponential growth or the addition of KCl triggered the accumulation of Tpk3-GFP containing bodies co-localizing with Dcp1-RFP. However, mild stress conditions (NaCl or ethanol), did not induce the Tpk3 accumulation. We observed that neither of the Tpks is required for P-body assembly. However the deletion of TPK3 accelerates P-body accumulation upon glucose deprivation, suggesting a role in the control of P-body formation.

ST-P08.
***IN VITRO* RECONSTITUTION OF THE CHIMERICAL TERMOSENSOR MS-DESK.**
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Membrane proteins play a major role in communicating with the environment by transmitting signals across membranes. While most sensors detect the presence of a chemical signal, our thermosensor DesK of Bacillus subtilis detects physical changes in the membrane in response to temperature shifts. In an attempt to understand the novel mechanism that triggers the activation of DesK, we worked with the purposely minimalized sensor, MS-DesK. The MS captures into one single chimerical transmembrane segment (TMS) the essence of the whole sensor having 5 TMS. Peculiarly, a group of hydrophilic aminoacids (Q9, K10, N12) are located in the N-terminal region, near to the interfase lipid-water. Site directed mutagenesis revealed that these aminoacids are needed for the detection of a temperature downshift. How could these aminoacids determine the kinase to phosphatase activity of DesK? We reconstituted the MS in unilamellar liposomes and studied the effect of temperature on MS activity. We found that a temperature downshift stimulates MS kinase activity. We also analyzed the role of unusual aminoacids comparing the activities of MS-mutants in vitro. This simplified system developed now opens up the way to unravel in molecular detail the mechanism of temperature sensing.

ST-P09.

Giardia lamblia: INVOLMENT OF PHOSPHATIDYLINOSITOL PATHWAY DURING ENCYSTMENT

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Giardia lamblia is a major cause of waterborne enteric disease worldwide. Disease transmission is dependent on the ability of the parasite to differentiate back and forth between two states: trophozoite and cyst. Trophozoites colonize the human small intrestine where they are exposed to high concentrations of conjugated bile acids. Although it is known that an increase in the bilis concentration triggers encystation in vitro, the molecular basis for its induction remains undefined. Previously, we demostrated the phosphatidylinositol (PI) pathway in *G. lamblia* trophozoites. Now, we analized the changes in the lipid kinase activities, in order to find a potential involvement of PI pathway on encystment, through the phosphorylation of endogenous substrates with with [γ^{32}-P] ATP. An increase of PI-K and PIP-K activities and a decrease of DAG-K activity were observed when trophozoites were induced to encyst. In addition, we analyzed these activities in trophozoites stimulated with bilis; the results showed a similar behavior than that observed in encysting trophozoites. We conclude, the trophozoites are able to respond to bilis through PI pathway and we suggest that this lipid signaling system participates in encystment process.

ST-P10.

FUNCTIONAL CHARACTERISATION OF *Trypanosoma brucei* UBIQUITIN CONJUGATING ENZYME CDC34

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The ubiquitin–proteasome system is a fundamental regulatory pathway for controlling protein stability in many cellular processes, such as signaling and cell cycle progression. In response to particular signals, specific regulatory proteins are tagged with a chain of ubiquitin molecules through the action of an enzymatic cascade composed of an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating enzyme, and an E3 ubiquitin ligase. E3s are responsible for binding substrates and for bringing substrates into the proximity of a ubiquitin-charged E2, which then transfers ubiquitin to the substrate. The importance of the UPS for cell cycle progression is exemplified by the central role of the ubiquitin-conjugating enzyme Cdc34, which co-operates with the SCF E3 ligase to regulate the levels of key cell cycle regulators. A major substrate of the Cdc34–SCF complex in *S. cerevisiae* is the CDK inhibitor Sic1, whose precise ubiquitin-mediated proteolysis is necessary for the G1–S-phase cell cycle transition. We investigate the functional role of CDC34 in *T. brucei* using RNAi and dominant negative overexpression. We show that RNAi of CDC34 in bloodstream form trypanosomes results in an *in vitro* growth defect with defects in cytokinesis furrow ingression. Cellular localization, RNAI interference induction *in vivo* and 2D-DIGE analysis are currently being investigated.

ST-P11.

LOCALIZATION OF THE PHOSPHODIESTERASE TCRPDEC2 AND ITS ROLE IN OSMOREGULATION IN *Trypanosoma cruzi*

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Trypanosoma cruzi, the causative agent of Chagas disease, possesses a complex life cycle during which it encounters extreme fluctuations in external osmolarity. It has been found that cyclic AMP levels increase when parasites are subjected to hyposmotic stress. Recently, we have described a cAMP-specific phosphodiesterase, TcrPDEC2, which possesses a FYVE domain, which is known to bind to membranes enriched in phosphatidylinositol 3-phosphate (PI 3-P). In addition, we have characterized TcVps34, the first phosphatidylinositol 3-kinase (PI3K) described in *T. Cruzi*.

In this work, we report a functional role for TcrPDEC2 in osmoregulation in *T. cruzi*. Treatment with TcrPDEC2 inhibitors affects their capability to respond to hyposmotic stress. Moreover, TcrPDEC2 showed to be localized in the contractile vacuole complex (CVC), a key organelle involved in the release of water. Furthermore, in TcVps34-overexpressing parasites this PDE is localized not only in the CVC but also in other intracellular structures, suggesting a possible role of TcVps34 in TcrPDEC2 localization. Finally, we demonstrated that transgenic parasites without the FYVE domain do not have phosphodiesterase activity.

Taken together, these results show that TcrPDEC2 plays a prominent role in vital processes such as osmoregulation and reveal the importance of the FYVE domain for the functionality of this enzyme.

ST-P12.

PROGESTERONE STIMULATES MAPK CASCADE IN *CAVEOLAE*-LIKE MEMBRANES OF *B. arenarum* OOCYTES

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Progesterone-induced amphibian oocyte maturation requires activation of MAPK pathway. We analyze the effect of tyrosine (tyr) kinase and Src inhibition on maturation and MAPK activation, and we evaluate the importance of *caveolae*-like membranes in signal transduction. Ceramide was compared to progesterone for its ability to induce tyr phosphorylation and methyl-ß-cyclodextrin (MßCD) was used for cholesterol modulation. Oocytes arrested in meiosis I were pretreated with either genistein (100-300µM) or daidzein or a Src-specific inhibitor and subjected to coincubation with either progesterone or ceramide for different periods of time. A detergent-free protocol was used to isolate *caveolae*-like membranes. Maturation was significantly inhibited when oocytes were treated with genistein or the Src inhibitor. Oocyte tyr phosphorylation and p42 MAPK expression decreased at genistein 300 µM while Src inhibition delayed the expression of the MAPK. Ceramide-induced maturation showed an increase in oocyte tyr phosphorylation and MAPK expression and both events were inhibited by genistein. Progesterone-induced oocyte tyr phosphorylation was inhibited by MßCD. Membrane fractions from mature oocytes registered tyr phosphorylation and MAPK activation. *Caveolae*-like membranes showed an association with MEK1 and MAPK suggesting that they can recruit signaling molecules involved in oocyte maturation.

ST-P13.
KLF6 FUNCTION INVOLVED IN C-JUN PHOSPHORYLATION BY JNK2 AND ITS EFFECTS IN APOPTOTIC RESISTANT CELLS

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KLF6 is a potential tumor suppressor gene product whose expression level responses to growth factors, tumor promoters and DNA-damage. We demonstrated that this transcription factor interacts with the c-Jun promoting its degradation by proteasome. JNKs kinases are the main regulators of c-Jun activity and stability. In the event of DNA damage signaling, JNKs also promote apoptosis involving sustained phosphorylation of c-Jun.
Interestingly, expression of KLF6 improves JNK2 activity leading to a transient increase of c-Jun phosphorylation in cells lacking JNK1 activity, correlating with KLF6 nuclear translocation. Under these conditions, apoptotic rate was induced after UV-radiation in *jnk1-/-* cells, which were initially relative resistant. How can apoptosis be triggered? It is well known that p53 can protect cells from death generating cell cycle arrest by p21 transactivation allowing DNA repair. However, if damage is extensive, p53 stimulates apoptosis through its pro-apoptotic target genes, *eg.* PUMA and NOXA. Abrogation of cell cycle arrest by phosphorylated c-Jun is essential to switch to the apoptotic program. We observed that over-expression of KLF6 after UV-radiation increases the p53mRNA level in *jnk1-/-* cells, though its target genes were not induced. In addition, KLF6 is also able to increase the p73 promoter activity whose product is essential for apoptosis in these cells.

ST-P14.
MKP-3 IS INDUCED BY HCG AND CYCLIC AMP IN MA-10 LEYDIG CELLS

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MKP-3 is a dual activity phosphatase specifically involved in the regulation of MAP kinases ERK1/2. In steroidogenic cells, trophic hormones activate steroid production and promote also cell proliferation. In the testis, lutheinizing hormone (LH) exerts these effects by a mechanism that involves PKA and ERK1/2 transient activation. Given that MKP-3 is induced by proliferative signals, we analyzed the effect of hCG (gonadotrophin hormone, an agonist of LH receptor) on MKP-3 expression in MA-10 Leydig cells. Semi-quantitative RT-PCR analysis showed that hCG (10 ng/ml) transiently increases MKP-3 mRNA levels. This effect reaches its maximum (2 fold) after 3 hs of stimulation and is mimicked by cAMP (0.75 mM 8Br-cAMP). The fact that a MEK inhibitor reduces the effect of hCG and of cAMP on MKP-3 mRNA levels, suggests that ERK1/2 participates in MKP-3 induction. In order to establish whether hCG/cAMP also induces MKP-3 by post translational modification, a Flag-MKP-3 chimera was over-expressed by transient transfection in MA-10 cells. Western blot analysis using an anti-Flag antibody, demonstrates that hCG and cAMP increase protein levels. Together, the present data indicates that in Leydig cells, MKP-3 is up-regulated by hCG/cAMP by a mechanism that includes actions at transcriptional and post translational levels.
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ST-P15.
STAR PROTEIN AND STEROIDOGENESIS ARE REGULATED BY HEME OXYGENASE ISOZYMES IN LEYDIG CELLS

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The aim of this study was to analyze the expression levels of both heme oxygenase (HO) isozymes and the influence of HO activity on steroid production in Leydig cells. We described the expression profile of HO-1 and HO-2 in MA-10 cells. hCG, db-cAMP, and hemin (HO inducer) treatment stimulated the expression of HO-1 and HO-2, and HO enzymatic activity. Hemin also inhibited hCG and db-cAMP- stimulated progesterone synthesis in MA-10 and testosterone in rat Leydig cells. We studied the effect of HO along the steroid synthesis, and found that StAR protein levels were decreased and the conversion of cholesterol to pregnenolone was inhibited by hemin, with no changes in the content of P450scc. We carried out a time course study of hemin action on hCG-stimulated StAR protein and mRNA. While StAR expression was markedly decreased, mRNA was significantly increased, suggesting that HO induction could regulate StAR by modifying its mRNA and protein levels. This results suggest that one of HO products (presumably CO) inhibits cholesterol transport to the inner mitochondrial membrane and steroidogenesis, possibly by binding to the heme group of the cytP450 enzymes; LH/hCG induces an enzymatic system with known antioxidant properties in LC contributing to a fine regulation of male reproductive function.CMM & BP must be considered with the same merit.
CONICET PIP5525, UBA X814 & PICT 05-38281 to OPP

ST-P16.
A DUAL ROLE FOR RAB3A IN THE ACROSOME REACTION OF HUMAN SPERM

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The sperm is endowed with a single secretory granule that undergoes a special type of regulated exocytosis (the acrosome reaction, AR). The AR relies on the same fusion machinery as typical exocytotic cells (i.e. SNAREs and Rabs). Rab3A is a GTPase that cycles between a GDP-bound (inactive) state that localizes at the cytosol and a GTP-bound (active) state that is attached to membranes. The role of Rab3A is controversial because in some models it stimulates exocytosis whereas in others behaves as an inhibitor. In human sperm, recombinant, prenylated Rab3A induces the AR in intact as well as SLO-permeabilized cells when is GTPγS-loaded. Different AR inducers like Ca^{2+} and 8-pCPT-2'-O-Me-cAMP (8pCPT, Epac-selective cAMP analogue) augment the levels of GTP-bound Rab3A. Here, we report that Rab3A distributed in the same proportion between cytosol and membranes in resting sperm. There was an enhanced binding of Rab3A to membranes after stimulating with A23187 or 8pCPT; but not with N6-Benzoyl-cAMP (a PKA-selective analogue), consistent with previous observations that Epac, but not PKA, exhibits a role in the AR. Interestingly, recombinant, prenylated and loaded with GTPγS, but not GTP, Rab3A exhibited an inhibitory role during the late stages of the AR. In summary, Rab3A seems to have a dual role depending on the stage it is added, this could explain the differences among models.

ST-P17.

ARF6 PROMOTES MEMBRANE FUSION BY GENERATING PIP_2 AND PA DURING ACROSOMAL EXOCYTOSIS

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During sperm acrosome reaction (AR) the plasma and the outer acrosomal membrane fuse at multiple points releasing hybrid vesicles and exposing the inner acrosomal membrane. AR is a special type of Ca^{2+}-regulated exocytosis. Our previous results suggested that AR needs phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidic acid (PA) after the opening of SOC channels. ADP-ribosylation factors (Arfs) are a family of monomeric GTP-binding proteins. In chromaffin cells, Arf6 activates both PI4P5Kinase and PLD1; both enzymes are thought to be coupled during signaling to support simultaneous increases in PIP_2 and PA. We analyzed if this small GTPase was involved in AR regulation. Western blot and indirect immunofluorescence demonstrated that Arf6 was present in human spermatozoa and localizes to the acrosomal region. Functional assays using the permeabilized sperm model demonstrated that myristoylated and GTPγS loaded-Arf6 triggered AR and that the GTPase is absolutely required for calcium-regulated membrane fusion. We defined that Arf6-induced membrane fusion requires PIP_2, PLD1 activity and consequently PA synthesis. By thin layer chromatography we demonstrated that Arf6-GTPγS increased PIP_2 concentration. We propose a model where Arf6 regulates PLD1 and activates a kinase to maintain a pool of PIP_2 in spermatozoa necessary for IP_3-dependent acrosomal calcium release.

SB-P01.
3-D STRUCTURE OF FRAGACEATOXIN C REVEALS THAT ACTINOPORINS FORM ALFA-HELICAL BARREL PORES

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Sea anemones are soft-bodied invertebrates that live attached to rocky substrates. Actinoporins are ~20 kDa soluble pore-forming proteins components of the anemones´s poison. After binding of monomers to the target membrane, and in a process that depends on the coexistence of lipidic phases, they undergo a conformational change that leads to oligomerization and insertion of the N-Terminal á-helices into the lipid bilayer to form an active pore. The accepted pore model is the so-called toroidal pore, which is composed of 4 protein subunits and the walls are formed by the N-Terminal á-helices and lipids. In this work, we present the 3-D crystallographic structure at 1.8 Å resolution of an actinoporin recently isolated from *Actinia fragacea*: fragaceatocin C (FraC). In the presence of detergent, FraC displays a nonameric crown-shaped structure where the N-terminal helices are still attached to the main body of the molecule, thus suggesting a putative pre-pore state. In contrast, the cryo-electron microscopy density map at ~30 Å resolution of FraC bound to PC-SM liposomes reveals a 8-fold radial symmetry pore in the membrane inserted state. Our results suggest that actinoporin pores are formed by a cluster of 8-9 α-helices without lipids lining the walls. The stability of this pore is sustained by the remainder of the proteins, which are tightly assembled at the membrane-water interface.

SB-P02.
LYSOZYME AMYLOIDOGENESIS *IN VITRO* IS DELAYED BY ITS NATURAL ACTIVITY INHIBITORS

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Several proteins can fold abnormally *in vivo* and have the capacity of forming fibrils, aggregates and deposits. The diseases linked to massive deposits of fibrillar aggregates are known as amyloidosis, and those formed by aggregation of misfolded LYZ share common characteristics with many neurodegenerative diseases as Parkinson, Alzheimer and Transmissible Spongiform Encephalopathies (TSEs). The mechanisms that determine how amyloidogenic proteins form oligomers and fibrils and why they are toxic are still elusive. Lysozyme (LYZ) is one of the most intensively used experimental models of amyloid formation. Two main approaches have been employed in order to delay or restrain their formation: fibril breaking or protein native state stabilization. Since the most cytotoxic species seem to be the oligomeric forms, we followed the latter strategy. We have previously shown that the stabilization of the native state of the LYZ by its natural activity inhibitors (N-acetyl glucosamine, N-acetyl chitobiose and N-acetyl chitotriose) hinder amyloid formation *in vitro* and that this fibril-growth inhibition correlates with ligand concentration and their affinities for LYZ. These results have been corroborated in the present study. Besides aggregates formed by Guanidinium Hydrochloride destabilization of LYZ in this work proved to have amyloid fibril characteristics by Atomic Force Microscopy (AFM)

SB-P03.
A THIOREDOXIN-LIKE [2FE-2S] FERREDOXIN FROM *Leptospira interrogans*

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Ferredoxins (Fds) are small soluble iron-sulfur proteins. Most of them are low-potential electron carriers that were long believed to consist of two phylogenetically distinct families: the [2Fe-2S] plant or [4Fe-4S] bacterial type Fds. The existence of a third family was initially suggested by the primary structure of a [2Fe-2S] protein from *Clostridium pasteurianum*, and was later confirmed by the crystal structure of a homologous protein from *Aquifex aeolicus*. The latter Fd displays a fold similar to that of thioredoxin. While homologous domains in large redox enzymes most likely function as electron carriers, the role of theses thioredoxin-like Fds is unknown. A different type of activity, perhaps a regulatory one, may be suggested by the very stable dimeric structure of these proteins, unusual among electron carriers.
We have identified and cloned a ferredoxin from *Leptospira interrogans* (LFd1), a parasitic bacterium of animals and humans. By co-expressing chaperons we succeeded in expressing and purifying the recombinant protein in *E. coli* with its Fe-S cluster properly bound. LFd1 displayed sequence and spectral similarities with thioredoxin-like Fds. By modeling *in silico* we predicted its probable 3D structure, which showed high similarity to *A. aeolicus* Fd4. We also determined that the protein is a dimer as was suggested for the *A. aeolicus* homologous protein.

SB-P04.
STRUCTURAL BIOLOGY ANALYSIS OF NOVEL FATTY ACID BINDING PROTEIN FROM *Ascaris suum*, AS-P18

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Ascaris lumbricoides causes the most common helminth infection of humans worldwide. A morphologically indistinguishable species, *Ascaris suum*, occurs in pigs and has become the best understood parasitic nematode in biochemical terms. Infective, second-stage larvae in Ascaris parasite may survive for up to 7 years, but little is known of the biochemical basis for such long term survival.
One of the most abundant components of the perivitelline fluid which surrounds the developing larva is the fatty acid binding protein, As-p18.
As-p18 is a developmentally regulated fatty acid binding protein and it is structurally related to the mammalian group of intracellular Fatty Acid Binding Proteins (iFABPs). However, while homology modeling shows that the sequence conforms well to a 10-stranded iFABP, there are indications of modifications to the binding site which could relate to ligand specificity. In addition, the unusual extra loops exposed on the surface of the protein might be involved in as yet unknown protein-protein interactions. Structural and functional studies will enhance our understanding of the unique features of As-p18 that may be related to the survival of the larva.
NMR resonance assignment is progressing and we expect to explore the protein's interactions with ligands.

SB-P05.
STRUCTURAL AND FUNCTIONAL STUDIES OF NA-FAR-1 A NOVEL FATTY ACID AND RETINOL BINDING PROTEIN

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FARs are a novel class of Fatty Acid and Retinol binding proteins with a structure that has no known counterpart. The majority of retinol binding proteins described to date are b-strand rich proteins while FARs are mainly a-helical. These proteins, which are confined to Nematodes, are secreted by the parasites into the surrounding tissues of the host, playing a central role in the establishment of the infection and making them candidate drug targets for antihelmintic control. Among the genes expressed by adult *N. americanus*, a FAR protein, Na-FAR-1, has been identified. *N.americanus* is a blood feeding nematode which causes anemia and growth stunt infecting aboriginal communities in the north of Argentina. We have optimized the purification conditions to obtain rNA-FAR-1 for NMR structural studies and functional ligand binding assays. Circular dichroism has confirmed a high content of a-helix. High quality NMR data have been obtained and full structure determination is in progress. Characterization of ligand-bound states have shown evidence of conformational changes. We have also analyzed the ligand binding capacity of Na-FAR-1 by fluorescence confirming that the protein binds retinol, oleic acid and DAUDA with high affinity. The results of the present work constitute a first step in the understanding of the biological function of the protein within the parasite.

SB-P06.
AN *IN SILICO* APPROACH TO STUDY EIIF4E INTERACTIONS

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eIF4E is a translation factor that interact with many postranscriptional gene expression regulators. The structure of eIF4E as well as some interacting complexes is known in humans and mouse. eIF4E is the only translation factor present in P-bodies, where it interacts with the helicase rck/p54 in human cells. In *Drosophila* the product of the gene me31b is the ortholog of rck/p54. In order to study the interactions and predict a model for the interaction eIF4E-me31b we used a suite of structural bioinformatics tools. Despite the lack of protein structural information in proteins database, homology models were build for these two proteins using information of ortholog proteins using the software MODELLER (http://salilab.org/modeller/). Several steps of energy minimization adjustment and loop refining were made. Finally, we built an interaction representation by means of refining models and, global and constrain docking with AUTODOCK, a suite of automated docking tools (http://autodock.scripps.edu/). Our data collection provided the basis for rational design of mutants aimed to experimentally define interactions within RNP complexes in P-bodies

SB-P07.
STRUCTURE AND STABILITY OF THE SNAIL NEUROTOXIN PV2

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PV2 is one of the major egg proteins from the perivitellin fluid of the eggs of the snail Pomacea canaliculata. It is an oligomer of ca.400 KDa with nutritive and neurotoxic properties. Here we characterize PV2 regarding oligomer structure, size and global shape, pH stability and resistance to proteolysis. Fluorescence and electrophoretic assays suggest PV2 is an octamer of 4 identical 98 KDa heterodimers composed of 67 and 31 KDa subunits, which are held together by disulfide bridges. Dimers are assembled into native PV2 by non-covalent forces. Proteinase K treatment suggests the 67 KDa subunit is more exposed. Analysis by small angle X-ray scattering (SAXS) showed that PV2 is an anisometric particle of 130 x 44Å, a size coincident with electron microscopy results. The 3D shape model presented is the first for an egg proteinaceous neurotoxin. Native PV2 isoelectric point is 6.2 and its stability against pH, studied by intrinsic Tryptophan fluorescence, showed that PV2 conformation is extremely stable under a wide pH range (2.0-12.0). PV2 was also resistant to trypsinolysis *in vitro* even under 0.1 % SDS, though Trypsin is able to digest the heat-denatured protein. Pv2 resistance to proteolysis by Trypsin and stability under a wide pH range suggests a novel mechanism to avoid digestion by predators that allow PV2 to maintain its neurotoxic properties when ingested.

SB-P08.
ORGANIZATION OF ACTIN FILAMENTS IN PERITUBULAR MYOID CELLS OF WKY AND SHR RATS

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Testicular peritubular myoid cells (PMCs), from seminiferous tubules (ST) of rodent form a monolayer between two basement membranes. These cells are contractile and express the cytoskeletal markers of true smooth muscle, such as α-isoactin, F-actin and myosin.
It is known that hypertension alters smooth muscle cells of blood vessel. The aim of this work was to analyze the organization of actin filaments (AF) of PMCs from testis of a hypertensive rat (SHR) and to compare with the organization of AF of PMCs from normotensive rat (WKY) using confocal microscopy. Segments of ST from VII-VIII stages were isolated, fixed with paraformaldehyde and incubated with antibody anti α- actin conjugated with FITC. The height of Z axis of PMCs-WKY was 3 ± 0.06 μm (x ± SEM) and PMCs-SHR was 4.82 ± 0.4 μm. The AF of PMCs from WKY and SHR rats were organized in two layers, one external (upper nucleus) with thick AF parallel to tubular axis and other internal (underneath nucleus toward to the seminiferous epithelium) with thin AF perpendicular to tubular axis. The external layer of AF in PMCs-WKY are distributed homogeneously in the whole cytoplasm and frequently are organized in bundles. However, in PMCs-SHR, the AF are restricted to the nuclear zone without formation of bundles. Results indicate that the cell morphology of PMCs, a kind of not vascular smooth cells, is affected by hipertension.

SB-P09.

CONSENSUS MODES, ROBUST DESCRIPTION OF COLLECTIVE MOTIONS FROM MULTIPLE-MINIMA NORMAL MODE ANALYSIS

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Understanding protein flexibility is essential for studying conformational changes, ligand binding and protein-protein interactions. Normal mode analysis is well suited for studying such motions, capturing the directions of lowest curvature on the potential energy surface. These directions have been shown to correspond to large-scale motions that are functionally significant. However, the described motions are those of a structure localized in a particular minimum of the energy surface. Here we describe a new theoretical framework for determining normal modes from an ensemble of closely related structures, which we call `consensus modes` (CM). The CM calculation assumes that the conformational potential energy surface can be better exploited when multiple-minima topological information is considered. CM calculated over an ensemble of structures issuing from a short molecular dynamics simulation provide a robust description of protein internal motions, and show high collectivity and symmetry properties. We adopted the apo form of the HIV-1 protease to demonstrate our approach. Low-frequency CM obtained from short molecular dynamics simulations describe collective motions that are "more collective", or global, than those obtained via standard techniques such as PCA. Such movements can be important for substrate or ligand binding, as we show in the case of the HIV-1 protease.

SB-P10.

CRYSTALLIZATION AND PRELIMINARY X RAY ANALYSIS OF NUCLEOSIDE DIPHOSPHATE KINASE 1 FROM *T. cruzi*

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Introduction: *Trypanosoma cruzi* is the etiologic agent of Chagas' disease. The Nucleoside diphosphate kinases (NDPKs) are enzymes involved in energy management and nucleoside balance in the cell. *T. cruzi* TcNDPK1, a canonical isoform. The objective of this work is obtaining protein's crystals, diffract and process the data for tridimensional structure resolution. Materials and Methods: TcNDPK1 was expressed in *E. coli* as a fusion protein with N-terminal His-tag. TcNDPK1 was overexpressed and purified by FPLC. Crystallization was assayed by sitting drop and hanging drop vapor diffusion method. Crystals was frozen and diffracted on synchotron x-ray radiation in Campinas (Brasil). The data set collected was reduced and merged using MOSFLM and SCALA programs. Results and Discussion: His-TcNDPK was overexpressed, purified and crystallized. The crystals are diffracted and collected the data to 3.5Å. The crystals belong to the trigonal space group P3, with unit cell parameters a=127.94, b=127.84, c=275.49. Structure determination is under way. These results will provide relevant information that could be the first step in rational drug design for treating Chagas' disease.

SB-P11.

EVOLUTION AND STRUCTURAL PLASTICITY OF METALLO-β-LACTAMASES

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Directed Molecular Evolution (DME) strategies are currently exploited to generate stable proteins, improve the catalytic efficiency, shape the substrate specificity of enzymes or design new activities. These studies also provide information on protein evolution.

We have shown that DME on the metallo-β-lactamase BcII from *B. cereus* gives rise to a variant with enhanced catalytic and resistance traits conferred by four mutations. Two of them, G262S and N70S, located under the active site floor, show a strong sign epistasis, which plays a crucial role in the evolutionary trajectory. The other two, V112A and L250S, are located far away from the active site, and their influence in resistance is not so obvious. Our purpose is to analyze how these mutations are able to enhance fitness and to identify the possible pathways that could lead to the acquisition of larger levels of resistance. We found that one third of the 24 possible evolutionary pathways are accessible. This restriction is mainly due to the fact that mutation G262S acts as a bottleneck in the evolutionary trajectory, providing a more evolvable genetic background than wt BcII.

We have also performed DME experiments on inactive mutants of BcII with active side substitutions, which proved reluctant to recover the native activity, suggesting that viable evolutionary pathways proceed by accumulating single base mutations.

SB-P12.

POLYMER-ASSISTED AGGREGATION OF 2-CYS PEROXIREDOXIN FROM RAPESEED (*Brassica napus*)

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Typical 2-Cys Peroxiredoxins (2-Cys Prx) are ubiquitous peroxidases that possess two conserved cysteines involved in the detoxification of reactive oxygen species and redox signaling. The composition of the milieu drives the partition of 2-Cys Prx into dimers, decamers and high molecular mass oligomers. Although a key to understanding the biological significance of the quaternary structure is the characterization of the assembly mechanism, factors that prompt 2-Cys Prx out of the solution are barely known. We found that ATP causes the reversible oligomerization of rapeseed 2-Cys Prx decamers (subunit molecular mass: 240 KDa) into species of large molecular masses (> 2 MDa). Searching for alternative modulators in these studies, we analyzed the aggregation of rapeseed 2-Cys Prx mediated by supramolecular structures. We found that critical concentrations of assemblies bearing positive charges cause the precipitation of the protein. Moreover, these results suggest that 2-Cys Prx decamers undergo conformational changes leading to a higher content of â-sheet, which causes a higher propensity to precipitate. The characterization of the driving forces behind the aggregate formation and the structural motifs necessary for the precipitation can help to develop efficient strategies directed to find ways of preventing and healing pathologies related to conformational diseases.

NS-P01.

LOCALIZATION AND NICOTINE MODULATION OF α7-ACETYLCHOLINE RECEPTOR IN HUMAN ENDOTHELIAL CELLS

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The α7-type neuronal nicotinic acetylcholine receptor (α7-AChR) is abundant in the central nervous system, though it also occurs in non-nervous tissues. Characterizing the role of α7-AChR in angiogenesis and in endothelial pathology is key for understanding the molecular and cellular basis of tobacco-related diseases. We studied the effect of nicotine on α7-AChR expression in the human umbilical vein cell line HUVEC by fluorescence microscopy. Cell-surface fluorescence of Alexa 488α-BTX-tagged α7-AChR varied in a time- and ligand-dependent manner. In order to determine whether α7-AChR occurred in ordered lipid domains ("rafts"), plasmalemma–enriched fractions were prepared by subcellular fractionation and resolved into detergent-soluble and detergent-insoluble fractions, and the distribution of α7-AChR analyzed by immunoblotting and binding studies. Trace amounts of α7-AChR were found in control HUVEC, increasing up to ~700% upon nicotine (50 μM) treatment. No differences were apparent between detergent-extracted fractions. Cholesterol depletion from endothelial cells was found to have little if any effect *per se* on the kinetics of wound repair mediated by cell migration. Nicotine alone enhanced the ability of cells to repair the mechanical wound. This "positive" effect of nicotine on would repair was drastically impaired in cells deprived of cholesterol.

NS-P02.

ORGANIZATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IS AFFECTED BY CYTOSKELETON-DISRUPTING DRUGS

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The cytoskeleton is assumed to participate in the protein clustering at cell surface, as the aggregation of AChR receptors at the neuromuscular junction. The effect of two cytoskeleton-disrupting drugs (cytochalasin D and jasplakinolide) on the organization of AChRs in CHO-K1/A5 cells was assessed by mathematical analysis of stimulated total emission depletion (STED) images. AChR clusters were found to be randomly distributed in control and treated samples at distances beyond 500 nm. For shorter distances (< than the prevailing diameter of clusters) no conclusion on the distribution could be reached owing to methodological restraints in the Ripley and Poisson analysis. However, drugs altered the arrangement of AChR nanoclusters, in a brightness-depending way. Drugs did not affect clusters brightness, but a significant increase in the proportion of the medium-size clusters and a diminution of the small-size ones was found in jasplakinolide-treated cells, suggesting a dispersive effect of AChR molecules within clusters. This dispersion was further corroborated by the diminution of the brightness/diameter ratio of clusters, and from Ripley´s analysis applied to patterns having simulated intra-clusters aggregation of AChR molecules. Taken together, our findings suggest that the cytoskeleton meshwork is involved in the anchoring and organization of the receptor at the cell surface.

NS-P03.

CIRCADIAN EXPRESSION OF RAR AND RXRβ IS MODIFIED IN THE HIPPOCAMPUS OF VITAMIN-A DEFICIENT RATS

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An endogenous time-keeping mechanism controls circadian biological rhythms in mammals. Previously, we showed clock and clock-controlled genes display a daily rhythmicity in the rat hippocampus. RAR and RXR nuclear receptors have been detected in the same area. Our objectives were to investigate whether RARα, RARβ and RXRβ exhibit a circadian variation in the rat hippocampus and whether vitamin A deficiency (VAD) modifies their daily expression. Twenty one-d old Holtzman rats were assigned to either a vitamin A-free diet (vitamin A-deficient group) or the same diet containing 4000 IU of vitamin A/Kg diet (control group) during 3 months. Half of the animals in each group were maintained under 12h-L:12h-D or 12h-D:12h-D during the last week. Total RNA was extracted using the Trizol reagent from hippocampus samples isolated every 4h. RARα, RARβ and RXRβ transcript levels were determined by RT-PCR and protein levels by Western blots. Regulatory regions of RARs and RXRβ genes were scanned for clock-responsive sites using MatInspector (www.genomatix.de). E-box and RORE sites were found on regulatory regions of retinoid receptors genes, which display an endogenously-controlled circadian expression in the rat hippocampus. VAD modified the daily rhythmicity of RARα and RXRβ probably, by modifying BMAL1 and PER1 oscillation.

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NS-P04.

CALPAIN MEDIATES ACTIVATION OF P19INK4D IN RESPONSE TO GENOTOXIC STRESS

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p19INK4d, a member of the INK4 family, stimulates DNA repair and reduces apoptosis in cells exposed to a variety of genotoxic agents. We have demonstrated that, in neuronal cells, the p19 gene is induced and the protein is phosphorylated in a CDK5-dependent manner in the presence of β-amyloid peptide.The β-amyloid peptide causes an increase in intracellular Ca^{2+} levels, activating the calpain protease, with in turn stimulates CDK5 activity. Therefore, Ca^{2+}-signaling could be involved in the induction and phosphorylation of p19. The aim of this work is to determine the mechanism that activates p19 and allows it to execute its protective role in neurons. The experiments were carried out in differentiated SH-SY5Y, Neuro-2A and HN9 cells and in cultures of hippocampal neurons. Treatment with β-amyloid peptide, a Ca^{2+} ionophore or neocarzinostatin caused an upregulation of p19 mRNA level and phosphorylation of the protein. However, not only p19 protein levels did not increase, but in some cases they were reduced following these treatments. Our hypothesis is that p19 would become cleaved and degraded by Ca^{2+}-activated calpain. Addition of calpeptin, a calpain-specific inhibitor, reduced p19 phosphorylation and prevented the increase in CDK5 activity in response to all genotoxic drugs. These results suggest that calpain could be involved in the activation of p19 following genotoxic stress

NS-P05.
ROLE OF LIPID RAFTS IN THE NEURONAL PLASTICITY MEDIATED BY M6A, A NEURONAL MEMBRANE GLYCOPROTEIN

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GPM6A is a stress/antidepressant-responsive gene in the hippocampus of different animal models. The gene expression is down regulated in both physically and socially stressed animals. M6a exerts regulatory actions on neurite/filopodium outgrowth in rat hippocampal neuronal culture through an unknown mechanism. The association of membrane proteins with specific microdomains such as lipid rafts (LP) in the plasma membrane is implicated in the regulation of extra and intracellular signaling pathways. The goal of this work was to test if M6a carries out its cell functions in such specialized membrane microdomains and if LP disruption could affect M6a plasticity. Using a discontinuous sucrose gradient followed by ultracentrifugation and SDS-PAGE-WB technique we identified the presence of M6a in the detergent resistant membrane fraction from both hippocampal tissue and cultured neurons. By microscopy we observed that M6a co-localizes with gangliosides, an LP marker. Futhermore, cultured neurons overexpressing M6a treated with MBCD (methyl-beta-cyclo-dextrin, an LP breaker) showed a drastic reduction in M6a ability to produce filopodia/spines. Taken together, our data indicate that M6a association to lipid raft membrane microdomains is involved in the neuronal filopodia/spines formation.

NS-P06.
THE GROWTH OF CHALCEDONY IN HUMAN BRAINS FROM ELDERLY PATIENTS

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The presence of autofluorescent chalcedony in autopsy-derived human brain tissue from older persons is documented by using a Leica laser scanning confocal microscope (LSCM). Three ion lasers were used: Ar 488; HeNe 543 and HeNe 633. Haematoxylin-eosin (H/E) stained sections from routine neurohistologic preparations were analyzed. These sections have been previously studied by a mineralogical microscope (Prado Figueroa *et al.*, 2008). The autofluorescent character of chalcedony allowed us to obtain three-dimensional images of the crystals. It is possible to visualize the degree of crystallinity (maturation) of chalcedony by using a LSCM. The occurance of mature prismatic quartz (chalcedony) was observed. Chalcedony is approximately 30 micron in size, distributed in patches or aggregates and it belongs to the rhombohedral (trigonal) crystal system. A less mature silica polymorph of about 1 or 2 micron in size, with different degree of crystallinity was also detected near of chalcedony. These autofluorescent crystallites were detected using an argon ion laser with the emission band at 457. Around of the chalcedony, the crystallites distribution were in spiral. Our images perhaps show the growth of chalcedony in the human brain, grain by grain, in an older person.

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NS-P07.
GENETIC BASIS OF HUMAN BRAIN EVOLUTION: TRANSGENIC ANALYSIS OF NPAS3 HUMAN ACCELERATED REGIONS

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Exceptional cognitive capacities set humans apart among primates. Comparative genomics revealed that phenotypic divergence between humans and close related primates is mainly due to changes in gene regulation rather than in protein-coding sequences. To search for genetic evidences of human evolution we analyzed 202 genomic regions evolving very slowly in vertebrates but significantly faster in the human lineage (termed human accelerated regions [HARs]) that have been recently identified. The largest cluster of HARs is located within 648 kb of the neuronal PAS domain protein 3 (NPAS3) gene. NPAS3 encodes a bHLH-PAS transcription factor that is broadly expressed in the developing mouse nervous system, and its dysfunction has been associated with schizophrenia in humans. In order to explore the participation of NPAS3 HARs on this gene regulation we generated transgenic mice carrying HAR mouse ortholog sequences upstream of Hsp68 minimal promoter and the reporter gene lacZ. Two Npas3 HARs were able to partially recapitulate the embryonic expression pattern of the endogenous gene suggesting their participation as Npas3 developmental enhancers. The identification of these HARs as functional NPAS3 enhancers will allow us to perform a comparative expression analysis of the human and mouse orthologs and to investigate their potential role in human brain evolution.

NS-P08.
AGE-ASSOCIATED CHANGES IN SYNAPTIC PI3K/AKT/GSK3β SIGNALING DURING IRON-INDUCED NEUROTOXICITY

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Phosphoinositide 3-kinase (PI3K)/Akt pathway and its downstream effector glycogen synthase kinase 3β (GSK3β) are key signaling pathways involved in synaptic plasticity and neuronal survival. In this work, the participation of synaptic PI3K/Akt/GSK3β signaling during iron-induced oxidative injury was characterized. Cerebral cortex synaptosomes obtained from adult and aged rats were exposed to Fe^{2+} (50 μM) for different periods of time and synaptosomal viability and the state of the PI3K/Akt/GSK3β pathway were evaluated. Mitochondrial function (MTT reduction), plasma membrane integrity (LDH leakage) and reactive oxygen species (DCF probe) were significantly affected in both age groups. However, aged animals showed a greater susceptibility to oxidative injury. In adults, Akt and GS3Kβ were activated only after 5 min, whereas in aged animals activation occurred after 5 and 30 min of incubation with the metal ion. Both Akt and GS3Kβ phosphorylation were dependent on PI3K activation. ERK1/2 activation was PI3K-dependent in adults, whereas in aged animals ERK1/2 activation was a PI3K-independent event and showed higher levels than that observed in adults. Results demonstrate that synaptic endings from aged animals undergo a greater susceptibility to iron-induced neurotoxicity with a differential profile in the activation of PI3K/Akt/GSK3β.

NS-P09.
NICOTINIC ACETYLCHOLINE RECEPTOR AND LIPID DOMAINS IN RECONSTITUTED MODEL MEMBRANES

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It has been proposed that the nicotinic acetylcholine receptor (AChR) is preferentially located in ordered lipid domains (rafts); however the reasons for this preference are not clear. Affinity purified AChR from *T. californica* and a synthetic transmembrane peptide (γM4, the AChR region in closer contact with lipids) were reconstituted into liposomes having a lipid composition resembling that of raft domains (PC:SM:Chol, 1:1:1). Their distribution in membrane domains was assessed by Forster resonance energy transfer between AChR intrinsic fluorescence and dehydroergosterol (fluorescent cholesterol probe). AChR exhibited no preference for any membrane domain. In contrast, γM4 peptide showed a distinct preference for raft-like domains. We characterized the lipid composition of detergent resistant and soluble fractions (DRMs and DSM, respectively) obtained after treatment of the liposomes, with or without protein, with 1% Triton X-100 at 4°C. DRMs and DSMs were enriched in Chol and POPC, respectively, and the presence of AChR or γM4 did not change this lipid distribution. However, AChR induced a significant increase in the amount of the DSM fraction, whereas γM4 caused an opposite effect. Thus, the AChR membrane location does not result exclusively from intrinsic physicochemical properties of the protein but is likely to depend on extrinsic factors.

NS-P10.
CELL CYCLE AND IT'S ROLE ON THE DERIVATION OF DOPAMINERGIC NEURONS FROM HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are derived from human pre-implantation embryos and can proliferate without apparent limit while retaining the ability to differentiate into multiple cell types. Efficient derivation of hESCs into neural cells is critical for future cell-based regenerative therapies. Continuous self-renewal of hESCs is accomplished by an abbreviated cell cycle due to reduction in the G1 phase when compared to adult somatic cells thus molecular mechanisms operative in undifferentiated hESCs hasten the cellular cycle to progress into the S phase. When the undifferentiated hESCs initiate the differentiation process, the time lapse in which they are held in G1 is amplified. The aim of this study is the optimization of a novel protocol to generate an efficient yield of dopaminergic neurons from hESC through the use of diverse morphogens such as sonic hedgehog and the characterization of the expression of cell cycle regulators during this process. By immunocitochemistry and qRTPCR we assessed the appearance of neuronal markers such as nestin, pax-6, Tuj1, tyrosine hydroxylase concomitant with the downregulation of stemness markers. Interestingly the induction and nuclear localization of cell cycle inhibitors, p19INK4d and p27Kip1 was observed. Understanding the expression outline of these cell cycle modulators will contribute to infer their role along neuronal development.

NS-P11.
AN ACYL-COA SYNTHETASE AND MITOCHONDRIAL ARACHIDONIC ACID AS KEY REGULATOR OF NEUROSTEROIDOGENESIS

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Stimulation of receptors and subsequent signal transduction results in the activation of arachidonic acid (AA) release and metabolism via the cycloxygenase, lipoxygenase or epoxygenase (citochrome P-450) pathways. How the cells drive AA to these pathways is not elucidated yet. Our laboratory showed a new mechanism that controls the level of free AA in adrenal, testis, heart and malignant cells. The hormone regulated mechanism involves the concerted action of acyl-CoA synthetase (Acsl4) and a mitochondrial acyl-CoA thioesterase (Acot2). Thus, generating AA, that serves as substrate of the lipoxygenase enzymes to regulate the steroidogenic acute regulatory (StAR) protein and cholesterol transport in the mitochondria, the rate-limiting step in steroid biosynthesis. It is known that the brain is also a steroidogenic tissue; however the transduction mechanism that controls the synthesis of neurosteroids is still unknown. To study this mechanism, we used as model primary culture of astrocytes from 2 days-old cerebellum which is known to express StAR protein under cAMP regulation. We demonstrate by RT-PCR, immunohistochemistry and Western blot that Acsl4 and Acot2 are expressed in astrocytes and that this expression is regulated by cAMP in parallel with the induction of StAR. In accordance with these findings, lipoxygenase inhibitors regulate the StAR protein induction.

D