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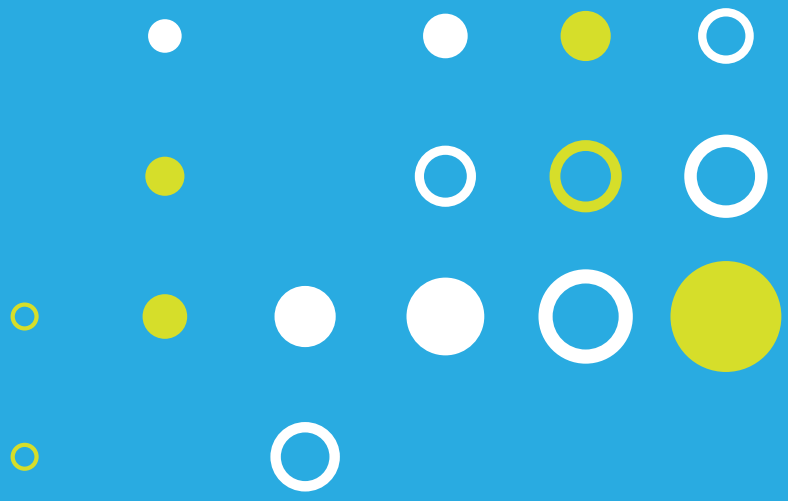

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Sociedad Argentina de
Investigaciones en Bioquímica
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- SAIB -

46th Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

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

November 30 - December 3, 2010

Puerto Madryn, Chubut
República Argentina

MEMBERS OF THE SAIB BOARD

DELEGATES OF SCIENTIFIC SECTIONS

-Cell Biology-
Dr. José Luis Daniotti
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Universidad Nacional de Rosario

-Microbiology-
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IBR-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas
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-Plant biochemistry and molecular biology-
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ACKNOWLEDGMENTS

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Consejo Nacional de Investigaciones Científicas y Técnicas
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European Molecular Biology Organization
(EMBO)

International Union of Biochemistry and Molecular Biology
(IUBMB)

ALUAR Aluminio Argentino S.A.I.C.

SAIB 2010 CONGRESS OVERVIEW

Tuesday, November 30th	Wednesday, December 1st	Thursday, December 2nd	Friday, December 3rd
	8:30 - 11:00 **Symposia** *Room A*: MicroRNAs and Small RNAs in Health and Disease (IUBMB)* *Room B:* Microbiology	8:30 - 11:00 **Symposia** *Room A:* Plant Biochem & Biol Mol* *Room B*: Cell Biology* *Room C (CENPAT-CONICET)*: A2B2C	8:30 – 11:00 **Symposia** *Room A*: MicroRNAs and Small RNAs in Health and Disease (IUBMB)* *Room B*: Lipids*
	11:00-11:30 Coffee break -*Exhibitors' room*	11:00-11:30 Coffee break-*Exhibitors' room*	11:00 - 11:30 Coffee break- *Exhibitors' room*
	11:30-13:00 **Oral Communications** *Room A:* CB (C01/06) and ST (C01/02) *Room B*: MI (C01/08)	11:30-13:00 **Oral Communications** *Room A:* CB (C07/12) and SB (C01/02) *Room B*: MI (C09/16) *Room C (CENPAT-CONICET):* LI (C01/06)	11:30-13:00 **Oral Communications** *Room A:* CB (C13/18) and NS (C01/02) *Room B:* MI (C17/24) *Room C (CENPAT-CONICET):* PL (C01/08)
	13:00-15:30 Lunch break	13:00-15:30 Lunch break	13:00-15:30 Lunch break
14:00-19:00 Registration	15:30-16:30 **Plenary Lecture** (*Room A*) Julio Collado-Vides	15:30-17:00 **Short Lectures (3)** (*Room A*) Junior Faculty's	15:30-16:30 **Plenary Lecture** (*Room A*) Hans-Peter Braun*
19:00-19:15 (*Room A*) **Opening Ceremony** Alberto R. Kornblihtt	16:30 – 18:30 **Posters** *CENPAT-CONICET* CB (P01/30) MI (P01/30) EN (P01/06) BT (P01/10) LI (P01/11) PL (P01/19) NS (P01/08) ST (P01/06)	17:00 – 19:00 **Posters** *CENPAT-CONICET* CB (P31/60) MI (P31/60) EN (P07/13) BT (P11/20) LI (P12/24) PL (P20/41) NS (P09/16) ST (P07/12)	16:30 – 18:30 **Posters** *CENPAT-CONICET* CB (P61/84) MI (P61/87) EN (P14/18) BT (P21/30) LI (P25/34) PL (P42/63) SB (P01/09) ST (P13/18)
19:15- 19:30 Tribute to Mariano Levin by Alejandro Mentaberry	18:30–19:00 Coffee break-*Exhibitors' room*	18:30 – 19:00 Coffee break-*Exhibitors' room*	18:30 – 19:00 Coffee break-*Exhibitors' room*
19:30-20:30 **IUBMB Lecture** Jennifer Doudna*	19:00-20:00 **Plenary Lecture** (*Room A*) Carlos F. Ibáñez	19:00-20:00 **Plenary Lecture** (*Room A*) John Cronan*	19:00 - 20:00 **EMBO Lecture** (*Room A*) Pascale Cossart*
20:30-21:30 **Sols Lecture** Miguel Ángel De la Rosa	20:00 – 21:30 **Film projection** *(Room A)* ***"Un fueguito"*** film by Ana Fraile on the life of César Milstein**	20:00 – 21:00 **Lecture** (*Room A*) Argentina´s Bicentennial Lecture Diego Hurtado De Mendoza	
21:30 Cocktail		21:30 SAIB Assembly (*Room A*)	21:30 Closing Dinner

*Activities in English; **Subtitled in Spanish and English

PROGRAM

TUESDAY, November 30th, 2010

14:00-19:00 **REGISTRATION**

19:00-19:15 ***OPENING CEREMONY (Room A)***

Alberto R. Kornblihtt
SAIB President
IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires

19:15-19:30 ***TRIBUTE TO MARIANO LEVIN***

Alejandro Mentaberry
Facultad de Ciencias Exactas y Naturales.
Universidad de Buenos Aires

19:30-20:30 ***IUBMB LECTURE***

Jennifer Doudna
Dept. of Molecular & Cell Biology, Dept. of Chemistry
Howard Hughes Medical Institute
University of California
"Regulatory RNA biogenesis for viral defense in bacteria"
Chairperson: Hugo Luján, Facultad de Medicina, Universidad Católica de Córdoba

20:30-21:30 ***"ALBERTO SOLS" LECTURE***

Miguel Ángel De la Rosa Acosta
Instituto de Bioquímica Vegetal y Fotosíntesis,
Centro de Investigaciones Científicas Isla de la Cartuja,
Universidad de Sevilla-CSIC, Spain
"Cytochrome c, a key protein for cell life and death"
Chairperson: Beatriz L. Caputto, CIQUIBIC-CONICET, Universidad Nacional de Córdoba

22:00 **COCKTAIL**

WEDNESDAY, December 1st, 2010

08:30-10:45 ***SYMPOSIA***

Room A

IUBMB SYMPOSIUM: "MicroRNAs and Small RNAs in Health and Disease I"

Chairpersons: Javier Palatnik, IBR-CONICET, Universidad Nacional de Rosario
Javier Martínez, IMBA, Austrian Academy of Sciences, Vienna, Austria

08:30-09:00 ***Javier F. Cáceres***
Institute of Genetics and Molecular Medicine, Edinburgh, United Kingdom
"Post-transcriptional regulation of microRNA biogenesis"

09:00-09:30 ***Sebastián Kadener***
The Alexander Silberman Institute of Life Sciences, Jerusalem, Israel
"miRNAs impart robustness to circadian gene expression and behavior"

09:30-10:00 ***Javier F. Palatnik***
IBR-CONICET, Universidad Nacional de Rosario
"Generation of microRNAs by different molecular pathways in plants"

10:00-10:30 ***Pablo Manavella***
Max Planck Institute for Developmental Biology, Tuebiguen, Germany
"Identification of miRNA biogenesis modulators by chemical genetics"

10:30-11:00 ***Ramiro Rodriguez Virasoro***
IBR-CONICET, Universidad Nacional de Rosario
"The miR396 regulatory network in plants: biological function and evolutionary divergence between species"

Room B

MICROBIOLOGY SYMPOSIUM

Chairpersons: Hebe Dionisi, CENPAT-CONICET; Diego Comerci, IIB-UNSAM - CONICET

08:30-09:00 ***Carlos Argaraña***
CIQUIBIC-CONICET, Universidad Nacional de Córdoba, Argentina
***"P. aeruginosa* and ciprofloxacin: bacterial resistance mechanism and mutagenic effect of the drug"**

09:00-09:30 ***Claudio Valverde***
Programa Interacciones Biológicas, Depto. Ciencia y Tecnología, Univ. Nac. Quilmes, Argentina
"The mighty tiny. Why to study prokaryote small regulatory RNAs?"

09:30-10:00 ***Francisco García-del Portillo***
Centro Nacional de Biotecnología-CSIC, Madrid, Spain
"On how *Salmonella* controls growth inside eukaryotic cells"

10:00-10:30 ***Miguel Angel Valvano***

Centre for Human Immunology and the Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada

"An intracellular opportunistic pathogen that alters Rho GTPases in macrophages"

10:30-11:00 ***COFFEE BREAK (Exhibitors' room)***

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

Room A

CELL BIOLOGY (C01/06) and SIGNAL TRANSDUCTION (ST-C01/02)

Chairpersons: Luis Quesada-Allué, Fundación Instituto Leloir-IBBA, CONICET, Universidad de Buenos Aires; Sandra Verstraeten, IQUIFB, Facultad de Farmacia y Bioquímica Universidad Nacional de Buenos Aires

11:00-11:15
CB-C01
CALCIUM-DEPENDENT ASSOCIATION OF ARGINYLATED CALRETICULIN WITH STRESS GRANULES
Carpio MA, Lopez Sambrooks C, Durand ES, Hallak ME

11:15-11:30
CB-C02
PROTEASOMAL DEGRADATION OF ARGINYLATED CALRETICULIN(RCRT) IN THE CYTOPLASM
Goitea VE, Hallak ME

11:30-11:45
CB-C03
ROLE OF VINCULIN IN THE PROCESS OF COLLECTIVE CELL MIGRATION OF THE NEPHROGENIC URETERIC BUD(UB)
Marquez MG, Guaytima E, Brandan YR, Favale N, Pescio L, Sterin Speziale NB

11:45-12:00
CB-C04
CONTRACTILE APPARATUS OF SEMINIFEROUS TUBULEMYOIDCELLS
Losinno AD, Morales A, Bertoldi MV, Amaya MC, Costantino VV, López LA

12:00-12:15
CB-C05
LOCALIZATION OF TRANSMEMBRANE-PROTEINS: ROLE OF CYTOPLASMIC MOTIFS AND TRANSMEMBRANE DOMAINS
Quiroga R, Maccioni HJF

12:15-12:30
CB-C06
MODELING FUSION/FISSION-DEPENDENT INTRACELLULAR TRANSPORT
Mayorga LS

12:30-12:45
ST-C01
EFFECTS OF LIPIDS OVER DESK, THE THERMOSENSOR *Bacillus subtilis*
Martin M, de Mendoza D

12:45-13:00
ST-C02
CHROMATIN RELAXATION TRIGGERS INDUCTION OF CDK INHIBITOR p19INK4d REGARDLESS OF DNA DAMAGE
Sirkin PF, Ogara MF, Giono LE, Cánepa ET

Room B

MICROBIOLOGY (C01/08)

Chairpersons: Paula Vincent, INSIBIO-CONICET, Universidad Nacional de Tucumán
Fabiana Bigi, Instituto de Biotecnología-INTA

11:00-11:15
MI-C01
CcpA REPRESSES THE DIVERGENT *cit* OPERON OF *Enterococcus faecalis* THROUGH MULTIPLE *cre* SITES
Blancazo V, Suárez C, Poncet S, Deutscher J, Magni C

11:15-11:30
MI-C02
A UNIQUE *cre* SITE CONTROLS TWO MALATE UTILIZATION OPERONS IN *Enterococcus feacalis*
Espariz M, Mortera P, Blancato V, Suarez C, Repizo G, Alarcón S, Magni C

11:30-11:45
MI-C03
THE Rcs SIGNAL TRANSDUCTION PATHWAY IS TRIGGERED BY ECA STRUCTURE ALTERATIONS IN *Serratia marcescens*
Castelli ME, García Véscovi E

11:45-12:00
MI-C04
REGULATION OF UNSATURATED FATTY ACIDS BIOSYNTHESIS BY GROWTH TEMPERATURE IN *Bacillus*
Chazarreta L, De Mendoza D, Altabe SG

12:00-12:15
MI-C05
CHARACTERIZATION OF THE PhoP/PhoQ SYSTEM IN *S. marcescens* AND ITS ROLE IN PATHOGENESIS
Barchiesi J, Castelli ME, García Véscovi E

12:15-12:30
MI-C06
A NOVEL PATHWAY FOR PROTEIN LIPOYLATION IN *Bacillus subtilis*
Martin N, Christensen QH, Cronan JE, De Mendoza D, Mansilla MC

12:30-12:45
MI-C07
ArgP AND Lrp REGULATE TRANSCRIPTION OF *lysP*, THE SPECIFIC LYSINE PERMEASE OF *E. coli*
Ruiz JA, Nikel PI, Jung K

12:45-13:00
CB-C08
ADAPTABILITY TO BIOFILMS IS HASTENED IN *P. aeruginosa* MUTATORS BY PHENOTYPIC DIVERSIFICATION
Luján AM, Molin S, Smania AM

13:30-15:30 ***LUNCH BREAK***

15:30-16:30 ***PLENARY LECTURE* (*Room A*)**

Julio Collado-Vides
Center for Genomic Sciences, UNAM, Mexico
"Regulon DB v 7.0: Transcriptional regulation of *Escherichia coli* K-12 integrated within genetic sensory-response units or GeSorgans"
Chairperson: Eleonora García Véscovi, IBR-CONICET, Universidad Nacional de Rosario

16:30-18:30 ***POSTERS (CENPAT-CONICET)***

Cell Biology (CB P01/28)	Microbiology (MI P01/30)
Enzymology (EN P01/06)	Biotechnology (BT 01/10)
Lipids (LI P01/11)	Plants Bioch. & Mol. Biol. (PL 01/19)
Neuroscience (NS P01/08)	Signal Transduction (ST 01/06)

18:30-19:30 ***PLENARY LECTURE* (*Room A*)**

Carlos Ibañez
Department of Neuroscience, Karolinska Institute, Stockholm, Sweden
"Signal decoding by a transmembrane receptor"
Chairperson: José Luis Daniotti, CIQUIBIC-CONICET. Universidad Nacional de Córdoba

20:00-21:30 **Film Projection (*Room A*)**

Sponsored by ALUAR Aluminio Argentino S.A.I.C.

*"**Un fueguito**", film by **Ana Fraile** on the life of César Milstein*
Presented by Alberto R. Kornblihtt

THURSDAY, December 2nd, 2010

08:30-10:45 ***SYMPOSIA***

Room A

PLANTS BIOCHEMISTRY & MOLECULAR BIOLOGY SYMPOSIUM

Chairpersons: Claudia Spampinatto and Paula Casati, CEFOBI-CONICET Universidad Nacional de Rosario

08:30-09:00 ***Fernando Carrari***
Instituto de Biotecnología-INTA and CONICET, Argentina
"Tackling the metabolism of tomato fruits by genetical-genomics approaches"

09:00-09:30 ***Diego Gómez-Casati***
CEFOBI-CONICET, Universidad Nacional de Rosario, Argentina
"Biogenesis of Fe-S- and heme- containing proteins in plants"

09:30-10:00 ***Eric Grotewold***
Plant Biotechnology Center and Dept. of Mol. Genetics The Ohio State University, Columbus
"Plant regulatory networks and development"

10:00-10:30 ***Marcelo J. Yanovsky***
IFEVA, Facultad de Agronomía, UBA-CONICET, Buenos Aires, Argentina
"A methyl transferase links the circadian clock to the regulation of alternative splicing"

Room B

CELL BIOLOGY SYMPOSIUM

Chairpersons: Marta Hallak, CIQUIBIC-CONICET, Universidad Nacional de Córdoba; Carlos Arregui, Universidad Nacional de San Martín - CONICET

08:30-09:00 ***Eduardo M. Castaño***
Fundación Instituto Leloir-IBBA (CONICET), Buenos Aires, Argentina
"Keeping the balance of amyloid β with proteases on both sides of the equation"

09:00-09:30 ***Gopal Thinakaran***
Department of Neurobiology, The University of Chicago, Chicago, U.S.A.
"Lipid raft targeting of Alzheimer's disease APP secretases"

09:30-10:00 ***Claudio O. Fernández***
IBR-CONICET, Rosario, Argentina; MPI for Biophysical Chemistry, Göttingen, Germany
"From structural to cellular biology of Parkinson disease: rational design of aggregation inhibitors"

10:00-10:30 ***Pablo Wappner***

Fundación Instituto Leloir-IBBA (CONICET), Buenos Aires, Argentina

"Genome-wide RNAi screen identifies novel genes required for the response to hypoxia"

Room C (CENPAT-CONICET)

SYMPOSIUM of the "Asociación Argentina de Bioinformática y Biología Computacional, A2B2C"

Chairperson: Alejandro Colman Lerner, IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

08:30-09:00 ***Ariel Chernomoretz***

Laboratorio de Bioinformática, FIL/IFIBA-CONICET, University of Buenos Aires. Argentina

"Looking for structure in gene expression profiles"

09:00-09:30 ***Diego U. Ferreiro***

Laboratorio de Fisiología de Proteínas. Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

"Localizing frustration in protein molecules"

09:30-10:00 ***Alejandro D. Nadra***

Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires

"Structure-based prediction of protein-DNA binding specificities"

10:30-11:00 ***COFFEE BREAK (Exhibitors' room)***

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

Room A

CELL BIOLOGY (CB C07/13) and STRUCTURAL BIOLOGY (SB C01/02)

Chairpersons: Cecilia D'Alessio, Fund. Instituto Leloir and IIBBA-CONICET, Univ. de Buenos Aires
María Carolina Touz, INIMEC-CONICET, Córdoba

11:00-11:15
CB-C07
SPECIFICITY OF TRANSMEMBRANE PROTEIN PALMITOYLATION
González Montoro A, Chumpen Ramirez S, Quiroga R, Valdez Taubas J

11:15-11:30
CB-C08
FATTY ACID SYNTHESIS IS REQUIRED FOR PROTEIN TRANSLOCATION DURING SPORULATION
Diez V, Schujman GE, Gueiros-Filho F, De Mendoza D

11:30-11:45
CB-C09
RT INTRODUCES SPECIFIC cDNA BACKGROUND SYNTHESIS IN RT-PCR ASSAYS: HOW TO WORK IT OUT?
Baez MV, Adrover MF, Muñoz M, Aguirre AI, Melendez M, Epstein AL, Kornblihtt AR, Jerusalinsky DA

11:45-12:00
CB-C10
QUANTITATIVE STUDY OF MOTHER-DAUGHTER ASYMMETRY IN Ace2 LOCALIZATION IN YEAST
Durrieu L, Munzer U, Bush A, Cedersund G, Colman-Lerner A

12:00-12:15
CB-C11
STUDYING THE LINK BETWEEN CHROMATIN AND SPLICING BY IMAGING APPROACHES
Schor IE, Llères D, Lamond AI, Kornblihtt AR

12:15-12:30
CB-C12
GENOME-WIDE SEARCH FOR ENDOGENOUS SMALL RNAs AFFECTING ALTERNATIVE SPLICING BY TGS
Allo M, Bertucci PY, Buggiano V, Gomez Acuña L, Kornblihtt AR

12:30-12:45
SB-C01
TWO MOLECULAR ENZYME FORMS AND REACTION MECHANISMS FOR THE GLYCOGENIN AUTOGLUCOPOLYMERIZATION
Issoglio FM, Carrizo ME, Romero JM, Curtino JA

12:45-13:00
SB-C02
TEMPERATURE-MEDIATED SELF-ASSOCIATION OF RAPESEED2-CYS PEROXIREDOXIN
Rimmaudo L, Aran M, Wolosiuk RA

Room B

MICROBIOLOGY (MI-C09/16)

Chairpersons: Gabriela Gago, IBR-CONICET, Universidad Nacional de Rosario; Juan Ugalde, IIB-UNSAM-CONICET

11:00-11:15
MI-C09
A NOVEL *B. suis* ADHESIN IDENTIFIED BY PHAGE DISPLAY MEDIATES BACTERIAL ADHESION TO EPITHELIAL CELLS
Posadas DM, Ruíz V, Bonomi HR, Martín FA, Zorreguieta A

11:15-11:30
MI-C10
Rhodobacter sphaeroides* CHEMORECEPTOR, McpH. EXPRESSION AND FUNCTIONAL ANALYSIS IN *E.coli
Herrera Seitz K, Studdert CA

11:30-11:45
MI-C11
***Brucella abortus* OXYGEN SENSING: THE PrrB/PrrA AND NtrY/NtrX TWO COMPONENT SYSTEMS**
Carrica MC, Paris G, Goldbaum FA

11.45-12:00
MI-C12
THE ROLE OF GumE IN POLYMERIZATION OF THE *Xanthomonas campestris* EXOPOLYSACCHARIDE XANTHAN
Galván EM, Kampel M, Ielpi L

12:00-12:15
MI-C13
INSIGHTS INTO THE CONFORMATION AND FUNCTION OF RapA2 A CALCIUM BINDING ADHESIN FROM *R. leguminosarum*
Abdian PL, Caramelo JJ, Russo DM, Vozza NF, Zorreguieta A

12:15-12:30
MI-C14
RHOMBOID PROTEASES DISPLAY UNIQUE DOMAIN COMBINATION AND NOVEL FUNCTIONS IN *Haloarchaea*
Gimenez MI, Madrid EA, De Castro RE

12:30-12:45
MI-C15
CHARACTERIZATION OF CELLULOLYTIC BACTERIAL STRAINS FROM NATIVE FOREST SOIL FROM MISIONES, ARGENTINA
Campos E, Talia P, Rorig M, Sabaris G, Gonzales S, Cataldi A, Bigi F, Grasso D

12:45-13:00
MI-C16
GENERATION OF THE FIRST AGRICULTURAL SOIL REFERENCE METAGENOME BY NEXT-GENERATION SEQUENCING
Rascovan N, Carbonetto B, Revale S, Mentaberry A, Alvarez R, Vazquez M

Room C (CENPAT-CONICET)

LIPIDS (LI C01/06)

Chairpersons: Antonio Uttaro, IBR-CONICET, Universidad Nacional de Rosario; María González Baró, INIBIOLP-CONICET, Universidad Nacional de La Plata

11:00-11:15
LI-C01
THIACETAZONE REVEALS SYNTHESIS OF NEW MYCOLICACIDS IN *Mycobacterium smegmatis*
Doprado M, Belardinelli JM, Morbidoni HR

11:15-11:30
LI-C02
c-Fos PHYSICALLY INTERACTS AND ACTIVATES SPECIFIC POLIPHOSPHOINOSITIDE SYNTHESIS ENZYMES
Cardozo Gizzi AM, Alfonso Pecchio A, Caputto BL

11:30-11:45
LI-C03
c-SRC AND TC45 REGULATE c-Fos TYROSINE PHOSPHORYLATION STATE AND PHOSPHOLIPID SYNTHESIS ACTIVATION
Ferrero GO, Caputto BL

11:45-12:00
LI-C04
Fe^{2+} INITIATED PEROXIDATION OF SONICATED LIPOSOMES MADE WITH RETINAL LIPIDS: EFFECT OF INDOLEAMINES
Fagali NS, Catala A

12:00-12:15
LI-C05
GLYCEROL-3PHOSPHATE ACYLTRANSFERASE 2 IS EXPRESSED IN SPERMATOGONIA AND UNDIFFERENTIATED CANCER CELLS
Pellon-Maison M, Cattaneo ER, Coleman RA, Gonzalez-Baro MR

12:15-12:30
LI-C06
ROLE OF C/EBP AND SP1 IN THE REGULATION OF CK AND CCT EXPRESSION DURING NEURONAL DIFFERENTIATION
Domizi P, Banchio C

13:00-15:00 ***LUNCH BREAK***

15:00-16:30 ***SYMPOSIUM* (*Room B*)**

SHORT LECTURES "JUNIOR FACULTY"

Chairperson: Sara Sánchez, INSIBIO-CONICET. Universidad Nacional de Tucumán

Sergio E. Álvarez
Instituto Multidisciplinario de Investigaciones Biológicas San Luis (IMIBIO-SL)
"Novel intracellular actions of sphingosine-1-phosphate to regulate NF-kB activation"

María Laura Fanani
CIQUIBIC-CONICET, Universidad Nacional de Córdoba
“Sphingomyelinase acting as a perturbator of membrane composition and structure”

Lucía F. Franchini
Instituto de Investigación en Ingeniería Genética y Biología Molecular (INGEBI-CONICET), Buenos Aires
"Human brain evolution: searching the genetic basis underlying our unique cognitive capacities"

16:30-19:00 ***POSTERS (CENPAT-CONICET)***

Cell Biology (CB P31/60)	Microbiology (MI P31/60)
Enzymology (EN P07/13)	Biotechnology (BT P11/20)
Lipids (LI P12/24)	Plants Bioch. & Mol. Biol. (PL (P20/41)
Neuroscience (NS P09/16)	Signal Transduction (ST P07/12)

19:00-20:00 ***PLENARY LECTURE* (*Room A*)**

John E. Cronan, Jr.
Department of Microbiology University of Illinois, USA
“Fatty Acid Synthesis is Not Only for Lipids”
Chairperson: Diego de Mendoza, IBR-CONICET. Universidad Nacional de Rosario

20:00-21:00 ***ARGENTINA´S BICENTENNIAL LECTURE (Room A)***

Sponsored by ALUAR Aluminio Argentino S.A.I.C.

Diego Hurtado de Mendoza
Escuela de Humanidades, Universidad Nacional de General San Martín, Argentina
"Ciencia y política en la Argentina: una interacción conflictiva (1930-2000)"
Chairperson: Eduardo Cánepa, Dpto. de Qca. Biológica, FCEN -Universidad de Buenos Aires

21:30 **SAIB GENERAL ASSEMBLY**

FRIDAY, December 3rd, 2010

08:30-10:45 ***SYMPOSIA***

Room A

IUBMB SYMPOSIUM: "MicroRNA and Small RNAs in Health and Disease II"

Chairpersons: Nora Calcaterra, IBR-CONICET, Universidad Nacional de Rosario;
María Elena Álvarez, CIQUIBIC-CONICET, Universidad Nacional de Córdoba

08:30-09:00 ***Sebastián Asurmendi***
Instituto de Biotecnología, CICVyA, INTA, Buenos Aires, Argentina
"Tobamovirus alter miRNAs systemically independently of virus presence"

09:00-09:30 ***Raúl Andino***
Department of Microbiology and Immunology, University of California, San Francisco - USA
"Antiviral immunity in *Drosophila*: systemic RNAi and viral suppressors"

09:30-10:00 ***María Carla Saleh***
Viruses and RNAi Lab, Institut Pasteur, France
"RNAi, the key player in antiviral response in insects"

10:00-10:30 ***Javier Martínez***
IMBA, Austrian Academy of Sciences, Vienna, Austria
"The elusive human tRNA ligase identified"

10:30-11:00 ***Alberto R. Kornblihtt***
IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires
"Small RNAs regulate alternative splicing through transcriptional gene silencing"

Room B

LIPIDS SYMPOSIUM

Chairpersons: Claudia Banchio, IBR-CONICET Universidad Nacional de Rosario
Ricardo Morbidoni, Facultad de Medicina, Universidad Nacional de Rosario

08:30-09:00 ***Mauricio G. Martin***
VIB Department of Developmental Molecular Genetics and K.U.
Leuven Department of Human Genetics, Leuven, Belgium
"Cholesterol loss during aging in hippocampal neurons. Causes and consequences"

09:00-09:30 ***Bruno Maggio***
Dpto. Química Biológica-CIQUIBIC, Fac. Cs. Químicas. UNC. Córdoba. Argentina.
"Manifold structural transduction by membrane lipids. What are lipolytic enzymes doing?"

09:30-10:00 ***David N. Bridley***
Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada
"Autotaxin an lysophosphatidate induce chemo-resistance and release breast cancer cells from Taxol-induced mitotic arrest and cell death"

10:00-10:30 ***Héctor Alvarez***

CRIDECIT-CONICET, Universidad Nacional de la Patagonia San Juan Bosco
Comodoro Rivadavia, Argentina

"Triacylglycerol metabolism in oleaginous bacteria"

10:30-11:00 ***COFFEE BREAK (Exhibitors' room)***

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

Room A

CELL BIOLOGY (CB-C13/18) and NEUROSCIENCE (NS-C01/02)

Chairpersons: María Teresa Damiani, IHEM-CONICET, Universidad Nacional de Cuyo.
Gladys Ciuffo, IMIBIO-SL, CONICET, Universidad Nacional de San Luis.

11:00-11:15
CB-C13
ANALYSIS OF ZEBRAFISH nolc1l: THE ORTHOLOGE OF THE HUMAN GENE INVOLVED IN TREACHER COLLINS SYNDROME
Weiner AMJ, Scampoli NL, Calcaterra NB

11:15-11:30
CB-C14
INSULIN DEGRADING ENZYME CORE PROMOTER IS REGULATED BY NRF-1 AND RELATED-ETS TRANSCRIPTION FACTORS
Leal MC, Castaño EM, Morelli L

11:30-11:45
CB-C15
THE RD1 CLUSTER FROM *Mycobacterium marinum* IS RESPONSIBLE FOR AUTOPHAGY ACTIVATION UPON INFECTION
Lerena MC, Colombo MI

11:45-12:00
CB-C16
AUTOPHAGIC ACTIVATION BY ALPHA-HEMOLYSIN FROM *Staphylococcus aureus*
Mestre MB, Colombo MI

12:00-12:15
CB-C17
LYSOSOMES PARTICIPATE IN EARLY STEPS OF TL(III)- MEDIATED APOPTOSIS OF PC12 CELLS
Hanzel CE, Verstraeten SV

12:15-12:30
CB-C18
COMPLEX I SYNDROME IN RABBIT HEART SUBMITTED TO ISCHEMIA-REPERFUSION. EFFECT OF ADENOSINE
Bombicino SS, Valdez LB, Zaobornyj T, Iglesias DE, Donato M, D´Annunzio V, Gelpi RJ, Boveris A

12:30-12:45
NS-C01
CELL CYCLE INHIBITOR, p19INK4d, AS A POTENTIAL NEURONAL SURVIVAL FACTOR
Ogara MF, Castillo DS, Berardino BG, Cánepa ET

12:45-13:00
NS-C02
MELANOPSIN X DISPLAYS A RADICAL TEMPOROSPATIAL SWITCH IN EXPRESSION DURING CHICK RETINAL DEVELOPMENT
Verra DM, Contín MA, Hicks D, Guido ME

Room B

MICROBIOLOGY (MI-C17/24)

Chairpersons: Alejandro Viale, IBR-CONICET. Universidad Nacional de Rosario; Rosana De Castro, IIB-CONICET, Universidad Nacional de Mar del Plata

11:00-11:15
MI-C17
ALKANE MONOOXYGENASE GENE DIVERSITY IN SUBANTARCTIC MARINE SEDIMENTS
Guibert LM, Loviso CL, Marcos MS, Dionisi HM, Lozada MLMA

11:15-11:30
MI-C18
LARGE-SCALE PROTEOMIC ANALYSIS OF SUMOYLATION TARGETS IN *Trypanosoma cruzi*
Bayona JL, Nakayasu ES, Alvarez VE, Laverriere M, Aguilar C, Sobreira TP, Choi H, Nesvizhskii AI, Almeida IC, Cazzulo JJ

11:30-11:45
MI-C19
STUDIES ON HEME TRANSPORT AND METABOLISM BY *Trypanosoma cruzi*
Cricco JA, Menendez Barvo S

11.45-12:00
MI-C20
***Trypanosoma cruzi* "HIGH MOBILITY GROUP B" PROTEIN IS A CHROMATIN ARCHITECTURAL FACTOR**
Cribb P, Perozzi M, Villanova GV, Serra EC

12:00-12:15
MI-C21
SELECTING A *Zygosaccharomyces rouxii* STRAIN WITH HIGH POTENTIAL FOR CONCENTRATE GRAPE JUICE SPOILAGE
Rojo MC, Sturm ME, Mercado L, Torres A, Combina M

12:15-12:30
MI-C22
DEVELOPMENT OF A MODEL-WINE MEDIA TO EVALUATE *Dekkera bruxellensis* GROWTH IN MIMIC WINE CONDITIONS
Sturm ME, Rojo MC, Ciklic I, Ramirez ML , Combina M

12:30-12:45
MI-C23
MOLECULAR CHARACTERIZATION OF A BENZOQUINONE REDUCTASE GENE IN THE FUNGUS *Beauveria bassiana*
Pedrini N, Juárez MP

12:45-13:00
MI-C24
FIRST COMPLETE SEQUENCE OF HEPATITIS A VIRUS IN ARGENTINA: RECOMBINATION BETWEEN SUBGENOTYPES IA/IB
Malirat V, Aguirre S, Scodeller EA, Mattion N

Room C (CENPAT-CONICET

PLANT BIOCHEMISTRY & MOLECULAR BIOLOGY (PL C01/08)

Chairpersons: Virginia Busi, CEFOBI-CONICET, Universidad Nacional de Rosario; María Inés Zanor, IBR-CONICET, Universidad Nacional de Rosario

11:00-11:15
PL-C01
Mutador* TRANSPOSON ACTIVATION INDUCED BY UV-B IN *Zea mays
Qüesta JI, Walbot V, Casati P

11:15-11:30
PL-C02
***Ostreococcus tauri* ADP-GLUCOSE PYROPHOSPHORYLASE: INSIGHTS INTO THE ACTIVATOR BINDING SITE**
Figueroa CM, Kuhn ML, Iglesias AA, Ballicora MA

11:30-11:45
PL-C03
ON THE REGULATION OF TRIOSE-PHOSPHATE METABOLISM BY PROTEIN PHOSPHORYLATION
Piattoni CV, Guerrero SA, Iglesias AA

11.45-12:00
PL-C04
PROTEOMIC AND METABOLOMIC ANALYSIS OF ORANGE FRUIT AFFECTED BY HEAT TREATMENT DURING POSTHARVEST
Perotti VE, Del Vecchio HA, Meier G, Bello F, Cocco M, Vázquez D, Podestá FE

12:00-12:15
PL-C05
STRUCTURE AND FUNCTION OF THE EXON JUNCTION COMPLEX IN *Arabidopsis thaliana*
Mufarrege EF, González DH, Curi GC

12:15-12:30
PL-C06
ROLE OF NITRIC OXIDE AND PHOSPHOLIPASE D IN STOMATAL CLOSURE AND DROUGHT STRESS
Distéfano AM, García-Mata C, Lamattina L, Laxalt AM

12:30-12:45
PL-C07
MECHANISTIC LINKS BETWEEN *KNOXI* AND *CUC* GENES DURING ARABIDOPSIS DEVELOPMENT
Spinelli SV, Martin AP, Viola IL, González DH, Palatnik JF

12:45-13:00
PL-C08
AN ESSENTIAL ROLE FOR NITRIC OXIDE IN AUXIN SIGNALING PATHWAY
Terrile MC, Calderon-Villalobos LI, Iglesias MJ, Paris R, Estelle M, Lamattina L, Casalongué C

13:00-15:30 ***LUNCH BREAK***

15:30-16:30 ***PLENARY LECTURE* (*Room A*)**

Hans-Peter Braun
Institute for Plant Genetics, Faculty of Natural Sciences, Universität Hannover, Hannover, Germany
"Special features of the mitochondrial NADH dehydrogenase complex in plants"
Chairperson: Eduardo Zabaleta, IIB-CONICET, Universidad Nacional de Mar del Plata

16:30-19:00 ***POSTERS (CENPAT-CONICET)***

Cell Biology (CB P61/84)	Microbiology (MI P61/87)
Enzymology (EN P14/18)	Biotechnology (BT P21/30)
Lipids (LI P25/34)	Plants Bioch. & Mol. Biol. (PL P42/63)
Structural Biology (SB P01/09)	Signal Transduction (ST P13/18)

19:00-20:00 ***"EMBO" PLENARY LECTURE* (*Room A*)**

Pascale Cossart
Department of Cell biology and Infection, Institut Pasteur, Paris, France
"The intracellular bacterial pathogen *Listeria monocytogenes*: a model system and a reference?"
Chairperson: Luis Mayorga, IHEM-CONICET, Universidad Nacional de Cuyo

21:00 ***CLOSING DINNER***

L01
IUBMB Lecture
REGULATORY RNA BIOGENESIS FOR VIRAL DEFENSE IN BACTERIA
Jennifer A. Doudna
Dept. of Molecular & Cell Biology, Dept. of Chemistry - Howards Hughes Medical Institute, University of California. E-mail: doudna@berkeley.edu

Many bacteria contain clustered regularly interspaced short palindromic repeats (CRISPRs) that confer resistance to invasive genetic elements. Central to this immune system is the production of CRISPR-derived RNAs (crRNAs) following transcription of the CRISPR locus. We identified the endoribonuclease (Csy4) responsible for pre-crRNA processing in *Pseudomonas aeruginosa*. The 1.8 Å crystal structure of Csy4 bound to its cognate RNA revealed that Csy4 makes sequence-specific interactions in the major groove of the CRISPR RNA repeat stem-loop. Together with electrostatic contacts to the phosphate backbone, these enable Csy4 to bind selectively and cleave pre-crRNAs using phylogenetically conserved serine and histidine residues in the active site. This RNA recognition mechanism explains sequence- and structure-specific processing by a large family of CRISPR-specific endoribonucleases. Csy4 assembles with crRNA into a multi-protein complex that targets complementary viral sequences for destruction. Structural insights into the assembly and function of this complex will be discussed.

L02
SOLS Lecture
CYTOCHROME *c*, A KEY PROTEIN FOR CELL LIFE AND DEATH
Miguel A. De la Rosa
Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja, Universidad de Sevilla-CSIC, Spain. E-mail: marosa@us.es

Cytochrome *c* plays a double physiological role and is thus essential for cell life and death. It acts as electron carrier within the mitochondrial respiratory electron transport chain and, it serves as a cytoplasmic apoptosis-triggering agent. Its functioning can indeed be modulated by Tyr nitration, which is one of the most common post-translational modifications.
We analyzed the nitration-induced changes in secondary structure, thermal stability, heme environment, alkaline transition and molecular dynamics of the five monotyrosine mutants of human cytochrome *c,* which have all their Tyr residues but one replaced by Phe. The resulting data, along with the functional analyses of the mutants, suggest that the specific nitration of Tyr46 and Tyr48 - which are both close to the heme propionate group - and the solvent-exposed Tyr74 impairs the electron transfer to (horse) cytochrome *c* oxidase, enhances the cytochrome *c* peroxidase activity, and blocks its ability to activate caspase-9.
In addition, a comparative proteomic analysis with human, algal, and plant cytochrome *c* allowed us to identify novel proteins that could act as physiological partners of cytochrome *c* under normal or programmed cell-death conditions. The finding of new protein partners of cytochrome *c* in differently evolved organisms will help us to understand the function of non-nitrated and nitrated cytochrome *c* in cell metabolism.

L03
EMBO Lecture
THE INTRACELLULAR BACTERIAL PATHOGEN *Listeria monocytogenes*: A MODEL SYSTEM AND A REFERENCE?
Pascale Cossart
Unité des Interactions Bactéries-Cellules, Institut Pasteur, Paris, France. E-mail: pascale.cossart@pasteur.fr

Our laboratory is analyzing host-pathogen interactions using the intracellular bacterial pathogen *Listeria monocytogenes* as a model system. This bacterium can induce several forms of a food borne disease called listeriosis, an infection which is mortal in 30% of the cases. Infection is mainly due to the capacity of the organism to live and replicate in both phagocytic and non phagocytic cells. It is also due to the property of the organism to be able to cross three host barriers during infection, the intestinal barrier, the blood brain barrier and in pregnant women the materno-fetal barrier. A series of approaches have highlighted that *Listeria* has evolved amazing mechanisms to switch from saprohytic life to virulence, to escape early innate host immune defense, to invade non phagocytic cells, to spread from cell to cell and to cross host barriers.
We will present an overview of the fascinating strategies used by this bacterium during infection, and then focus on recent data which have led us to discover new concepts in cell biology and new strategies used by pathogens to counteract the host cell during the early phases of infection.

L04
Plenary Lecture
RegulonDB v 7.0: TRANSCRIPTIONAL REGULATION OF *Escherichia coli* K-12 INTEGRATED WITHIN GENETIC SENSORY-RESPONSE UNITS OR GeSorgans
Julio Collado-Vides
Centro de Ciencias Genómicas, UNAM, México. E-mail: collado@ccg.unam.mx

The major challenge of genomic bioinformatics is to transform data into knowledge, by means of organizing and integrating information in ways that help to understand biology. We are facing the challenge of going beyond "stamp collection" and improving understanding of gene regulation. RegulonDB (http://regulondb.ccg.unam.mx/) is the primary reference database of the best known regulatory network of any free-living organism, that of *Escherichia coli* K12. We are expanding the description of mechanisms of regulation of initiation of transcription and operon organization, so that regulation is now part of a unit that initiates with the signal, continues with the signal-transduction to the core of regulation modifying expression of the affected target genes defining an adequate response. We call these genetic sensory-response units, or geSorgans. We initiated their high-level curation, with graphic maps and super-reactions with links to other databases. I will be placing into context this expansion of our work, and will describe in detail some gesorgans showing the difference between what we had previously and what we are planning to offer in the new version of RegulonDB. We believe that this is a step in the right direction to facilitate the understanding of gene regulation by placing it into a more integrated biological context, connecting mechanisms and physiology.

L05
Plenary Lecture
SIGNAL DECODING BY A TRANSMEMBRANE RECEPTOR
Carlos Ibañez
Department of Neuroscience, Karolinska Institute, Stockholm, Sweden.

Cell surface receptors translate ligand-encoded information into specific intracellular responses. A major unresolved issue is the mechanism by which receptors process information across the plasma membrane, including the molecular mechanisms of receptor activation and signal decoding, fidelity and propagation. This presentation will focus on the p75 neurotrophin receptor, a member of the TNF receptor superfamily, as a prototype for a wide variety of non-catalytic receptors that function via protein-protein interactions. Topics of discussion include the mechanism of receptor activation, how this triggers the activation of different intracellular networks, and how fidelity is maintained during processing of ligand-encoded information.

L06
Plenary Lecture
FATTY ACID SYNTHESIS IS NOT ONLY FOR LIPIDS
John E. Cronan
Departments of Microbiology and Biochemistry, University of Illinois, Urbana, USA
E-mail: j-cronan@life.illinois.edu

It is often overlooked that fatty acid synthetic pathway is involved in synthesis of essential coenzymes found in all three domains of life. The two coenzymes that will be discussed, lipoic acid and biotin, are covalently attached to the enzymes where they carry intermediates between active site (the classical "swinging arms" of Lynen). Lipoic acid is simply octanoic acid in which hydrogen atoms at C6 and C8 have been replaced with sulfur atoms . We report that this coenzyme is assembled on its cognate proteins in *E. coli*. A key intermediate in biotin synthesis is pimelic acid (heptanedioic acid), a seven carbon dicarboxylic acid. We report that this intermediate is assembled in *E. coli* by deceiving the fatty acid synthetic pathway using disguised substrates.

L07
Plenary Lecture
SPECIAL FEATURES OF THE MITOCHONDRIAL NADH DEHYDROGENASE COMPLEX IN PLANTS
Hans-Peter Braun
Institute for Plant Genetics, Leibniz University Hannover, Germany
E-mail: braun@genetik.uni-hannover.de

The mitochondrial NADH dehyrogenase complex (complex I) is the largest enzyme complex of the Oxidative Phosphorylation (OXPHOS) system and the main entrance site for electrons into the respiratory electron transfer chain. Complex I has several unique features in plants. Most notably, it includes 16 extra subunits, some of which introduce side activities into this respiratory enzyme. For example, subunits resembling an archaebacterial gamma-type carbonic anhydrase form an integral part of complex I in plants. These carbonic anhydrase subunits constitute a spherical extra domain which is attached to the membrane arm of complex I on its matrix exposed side. Novel data will be presented on the structure and function of the NADH dehydrogense complex in plants.

L08
JF-L01
NOVEL INTRACELLULAR ACTIONS OF SPHINGOSINE-1-PHOSPHATE TO REGULATE NF-κB ACTIVATION

Alvarez SE[1,2], Harikumar KB[1], Milstien S[1], Spiegel S[1]
[1]Virginia Commonwealth University, Richmond, VA USA and [2]IMIBIO-SL CONICET, San Luis, Argentina. E-mail: sealvarez98@gmail.com

Sphingosine 1-phosphate (S1P), a pro-survival lipid mediator, is produced inside the cell by the action of sphingosine kinases (SphK1 and SphK2). SphK1 binds TNF receptor-associated factor 2 (TRAF2), a key component in NF-κB signaling. Genetic data indicate that TRAF2 is necessary for polyubiquitination (Ub) of receptor interacting protein 1 (RIP1) that then serves as a platform for IKK complex recruitment and NF-κB activation. However, direct evidence that TRAF2 catalyzes the Ub of RIP1 is still missing. Here we show that S1P is required for TNF-induced Ub of RIP1 and phosphorylation of IKK and IκBβ, leading to NF-κB activation. These responses were mediated by intracellular S1P independently of its cell surface G protein-coupled receptors. Importantly, S1P specifically binds to TRAF2 at the N-terminal RING domain and stimulates its E3 ligase activity. S1P, but not related lipids, dramatically increased recombinant TRAF2-catalyzed Lys 63- but not Lys 48- linked Ub of RIP1 *in vitro.* Our data reveal that TRAF2 is a novel intracellular target of S1P, and that S1P is a missing co-factor for TRAF2 E3 ubiquitin ligase activity, suggesting a new paradigm for regulation of Lys 63-linked Ub. These results also highlight the key role of SphK1 and its product S1P in TNFα signaling and the canonical NF-κB activation pathway important in inflammatory, anti-apoptotic, and immune

L09
JF-L02
SPHINGOMYELINASE ACTING AS A PERTURBATOR OF MEMBRANE COMPOSITION AND STRUCTURE

Maria Laura Fanani and Bruno Maggio
Dpto. Qca. Biológica (CIQUIBIC), Fac. Ciencias Químicas CONICET, Univ. Nac. Córdoba. Argentina. E-mail: lfanani@gmail.com

Sphingomyelinase (SMase)-induced ceramide (Cer)-enriched domains in a lipid monolayer are shown to result from an out-of-equilibrium situation. This is induced by a change of composition caused by the enzymatic production of Cer in a sphingomyelin (SM) monolayer that leads to a fast SM/Cer demixing into a liquid-condensed (LC) Cer-enriched and a liquid-expanded (LE) SM-enriched phases. The morphological evolution of Cer-enriched domains shows domain shape annealing from branched to rounded shapes after quenching of SMase activity. The fast phase separation causes a transient enrichment of Cer into LC domains. As a consequence, higher intradomain repulsion leads to transient branched structures that relax to rounded shapes by decreasing the proportion of Cer in the domain. Sphingomyelinase action on membranes brings attention to the complex dynamics of lipid interfaces where several time-dependent events occur in a narrow range of space (nm-m) and time (s to min). The resultant membrane structure is consequence of the interrelation of events such as substrate degradation rate, product diffusion, lipid demixing, phase nucleation rate and slow diffusion of lipids in the LC phase. In this context, the fast action of SMase can be taken as a compositional perturbation that brings about important consequences for the structural dynamics.

L10
JF-L03
HUMAN BRAIN EVOLUTION: SEARCHING THE GENETIC BASIS UNDERLYING OUR UNIQUE COGNITIVE CAPACITIES

Francini LF
INGEBI-CONICET, Buenos Aires, Argentina. E-mail: franchini@dna.uba.ar

It has been hypothesized that the evolution of the unique human cognitive capacities is due to the acquisition of new temporal and spatial expression patterns of preexisting genes rather than changes in the protein-coding sequences. Using a combination of bioinformatics and functional studies including the generation of transgenic zebrafish and mice we are investigating differences in gene regulation which may have contributed to the evolution of the human brain. I am presenting here our results involving the functional characterization of regulatory elements of two key brain development genes that evolved faster in the human lineage. We have uncovered a novel enhancer of *DLL1,* a gene involved in the proliferation/differentiation switch of neuronal precursors that positively evolved in primates. We found that the primate-specific changes are crucial for this regulatory element function during brain development. In addition, we are functionally characterizing the largest cluster of the most rapidly evolving human elements yet identified (termed [HARs]) located within 648 kb of the *NPAS3* gene. We tested the ability of *NPAS3*-HARs to function as developmental enhancers. Our results indicated that the regulation of *NPAS3* and *DLL1* has been shaped during human evolution, suggesting that changes in its expression pattern could have been crucial for the evolution of the human brain.

IUBMB I-S01
POST-TRANSCRIPTIONAL REGULATION OF microRNA BIOGENESIS
Cáceres JF
MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, EH4 2XU, UK. E-mail: Javier.Caceres@hgu.mrc.ac.uk

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate the expression of complementary mRNAs and affect a great diversity of biological processes. Their biogenesis involves a nuclear phase catalyzed by the Microprocessor (Drosha/DGCR8) followed by a cytoplasmic step carried out by Dicer to produce the mature miRNA.
We have shown that hnRNP A1, a protein implicated in many aspects of RNA processing, promotes the Drosha-mediated processing of a miRNA precursor, pri-miR-18a, by binding to its conserved terminal loop (1, 2). By contrast, hnRNP A1 is a negative regulator of Let-7a in differentiated cells by antagonizing the positive role of the KH-type splicing regulatory protein KSRP (3). Altogether, these data suggest the existence of auxiliary factors for the processing of specific miRNAs that can have a positive or negative role in the production of individual miRNAs.
DGCR8 is a double-stranded RNA binding protein that recognizes the RNA substrate, whereas Drosha functions as the catalytic subunit.
We have used high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) to identify endogenous RNA targets of the microprocessor component, DGCR8 in mammalian cells. We will discuss the role of DCGR8 in the processing of a whole spectrum of cellular RNAs.

IUBMB I-S02
miRNAS IMPART ROBUSTNESS TO CIRCADIAN GENE EXPRESSION AND BEHAVIOR
Kadener S
The Alexander Silberman Inst. of Life Sciences, Jerusalem 91904, Israel. E-mail: skadener@gmail.com

Most organisms use circadian (24 hr) clocks to keep temporal order. In *Drosophila*, CLK and CYC activate the circadian system by promoting rhythmic transcription of several key clock genes. In the last year, we have found another layer of regulation operating in the *Drosophila* circadian clock: translational regulation by miRNAs. More precisely, we have found that the miRNA bantam regulate clk through three conserved binding sites. Importantly, we found that these sites are necessary for Drosophila circadian rhythms.
In the present work we have expanded our findings and further characterize the regulation of Clk by miRNAs. Interestingly we found that miRNA mediated regulation seems to serve a new type function. Using single cell live imaging on a cell culture system we demonstrated that miRNA-mediated regulation not only decreases the levels of Clk but also sharpens CLK-CYC mediated gene expression. This is achieved by diminishing the levels of leaking transcription to undetectable levels. By assessing in vivo circadian transcription we demonstrated that this regulation is key for the generation of coherent and high amplitude oscillations on CLK-CYC transcriptional activity. We are currently testing the effect of this regulation on intrinsic noise of the target gene.

IUBMB I-S03
GENERATION OF microRNAS BY DIFFERENT MOLECULAR PATHWAYS IN PLANTS
Mateos JL, Bologna NG, Palatnik JF
Instituto de Biología Molecular y Celular de Rosario, Suipacha 531, 2000 Rosario, Argentina. E-mail: palatnik@ibr.gov.ar

MicroRNAs are small RNAs around 21nt that regulate gene expression at the post-transcriptional level. They recognize target mRNAs by base complementarity and guide them to cleavage or translational arrest. In humans, microRNAs are considered to regulate 40% of the protein-coding genes and have been implicated in many diseases. In *Arabidopsis* and other plants, microRNAs regulate key biological aspects such as development and stress response.
MicroRNAs are defined by their unique biogenesis which involves the precise excision from the stem of a fold-back precursor. In *Arabidopsis*, they are processed by the type III ribonuclease DICER-LIKE1 (DCL1), which cleaves the precursor to release the stem segment containing the microRNA. While animal microRNA precursors have stereotypical shapes and sizes, the plant counterparts have a large variability.
Here, we present studies on the mechanisms underlying the biogenesis of plant microRNAs. In most cases the processing machinery recognizes structural features in the precursor below the microRNA to produce the cuts that release the small RNA. In a few others, however, the processing machinery recognizes the terminal part of the precursor and proceeds with the cleavages in a loop-to-base direction. The emerging picture highlights the plasticity of the small RNA pathways in plants.

IUBMB I-S04
IDENTIFICATION OF miRNA BIOGENESIS MODULATORS BY CHEMICAL GENETICS
Manavella P
Max Planck Institute for developmental biology, Tuebiguen, Germany. E-mail: pablo.manavella@tuebingen.mpg.de

MicroRNAs are post-transcriptional regulators that bind to complementary sequences of target mRNA transcripts, usually resulting in gene silencing. The production of miRNAs requires multiple and coordinated steps. Although a variety of proteins involved in their processing and function have been identified, their mutant phenotypes are not always the same. An alternative to traditional forward genetic screens is chemical genetics. This approach utilizes small molecules to perturb a signaling pathway, permitting the identification of relevant factors without a continuous perturbation in a gene product. Herein we report the development of a high-throughput assay for miRNA-mediated gene regulation and the identification of several miRNA inhibitors. The screening of a chemical library, compose by 10,000 structurally diverse small molecules, allowed us to identify a group of compounds with different levels and modes of inhibition. Measurement of endogenous miRNAs together with the expression level of their precursors and target genes confirmed the inhibitory effect. Additionally, the compounds have been tested for their ability to affect other small RNA pathways as well as non-plant pathways.

IUBMBI-S05

THE miR396 REGULATORY NETWORK IN PLANTS: BIOLOGICAL FUNCTION AND EVOLUTIONARY DIVERGENCE BETWEEN SPECIES

Rodriguez RE, Debernardi JM, Mecchia MA, Palatnik JF
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Leaf primordia are initiated by recruiting cells from the peripheral zone of the shoot apical meristem. Extensive mitosis proceeds until cells begin to enlarge. This process is regulated by the concerted action of various transcription factor networks, among them the GROWTH REGULATING FACTORS (GRFs).

We present insights into the role of miR396 in *Arabidopsis* leaf development. Mir396 regulates seven transcription factors of the GRF family. Overexpression of this miRNA lead to downregulation of GRFs and reduced leaf cell number. Conversely, high levels of GRF2 activity increased cell proliferation in leaves. Using reporter constructs, we determined that miR396 is essential to establish a dynamic spatio-temporal pattern of GRF2 expression that coincides with the activity of cell proliferation.

Analysis of miR396 and its targets in various plants species revealed two different types of divergence of this conserved regulatory module. First, particular miR396 variants are expressed only in monocots. Expression of these versions in *Arabidopsis* indicated that they are stronger repressors than the *Arabidopsis* counterparts. Second, a search for novel miR396 targets revealed that a basic-helix-loop-helix transcription factor is regulated only in a group of Brassicales. The importance miR396-mediated repression of this bHLH for normal *Arabidopsis* development will be discussed.

IUBMB II-S01
TOBAMOVIRUS ALTER miRNAs SYSTEMICALLY INDEPENDENTLY OF VIRUS PRESENCE
Bazzini AA, Manacorda CA, Conti G, Rodriguez MC, Villanueva S, Asurmendi S
Instituto de Biotecnología, CICVyA, INTA, Buenos Aires, Argentina. E-mail: sasurmendi@cnia.inta.gov.ar

Plant virus infections produce a variety of disease symptoms that causes important economically losses in agriculture. The events triggered by virus infection including changes in plant gene expression, metabolism and development. Some of these events may be required for the virus replication and spread, some may be plant responses and others may be just a side effect. Different molecules emerged as candidates to modulate this complex interaction, and a group of them are microRNAs (miRs). miRs are involved in many different cell processes, including development and response to biotic and abiotic stresses. Here we describe a positive correlation between severity of disease symptoms, dynamic spread of infection and alteration of miRs and accumulation of miR-targets in *Nicotiana tabacum* after infection with six tobamoviruses. In a microarray-based study of a time course TMV-U1 infection in tobacco is shown that several of the miRs tested were down-regulated at early stages and up-regulated during late stages of infection. The data suggest that a systemic signal may alter the miR pathway at early times of systemic infection. These results describe a new detail of host-pathogen interactions and highlight the importance of early steps of infection and the relevance of the role of miRs in symptom development.

IUBMB II-S02
ANTIVIRAL IMMUNITY IN *Drosophila*: SYSTEMIC RNAi AND VIRAL SUPPRESSORS
Andino R
Department of Microbiology and Immunology - University of California, San Francisco, USA. E-mail: Raul.Andino@ucsf.edu

Multicellular organisms evolved sophisticated defense systems to confer protection against pathogens. An important characteristic of these immune systems is their ability to act both locally at the site of infection and at distal uninfected locations. In insects, such as *Drosophila melanogaster*, RNA interference (RNAi) mediates antiviral immunity. We showed that effective antiviral immunity depends on the ability of cells to uptake dsRNA. Accordingly, mutant flies defective in this dsRNA uptake pathway are hypersensitive to infection. Inoculating naked dsRNA into flies elicited a sequence-specific antiviral immune response that required an intact dsRNA uptake pathway. Thus, similar to protein-based immunity in vertebrates, the antiviral RNAi response in flies also relies on the systemic spread of a virus-specific immunity signal. As a countermeasure to the RNAi antiviral defense, viruses encode suppressors of RNA silencing to ensure their survival. We have established a direct link between the mechanisms of action of viral RNAi suppressors and the outcome of infection, showing that RNAi suppressors function as virulence factors.

IUBMB II-S03
RNAi, THE KEY PLAYER IN ANTIVIRAL RESPONSE IN INSECTS
Saleh MC
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The term RNA interference (RNAi) or RNA silencing encompasses a type of gene silencing mechanism conserved among various species from different kingdoms. RNAi-related pathways have roles in many, different aspects of cell life, ranging from gene expression, epigenetic modification and regulation of heterochromatin, and resistance to pathogens. One of these pathways, the small interfering RNA (siRNA) pathway, is mainly involved in the defense against parasitic nucleic acids: transposons and viruses. Indeed, in insects, the siRNA pathway is the major antiviral response.
Many insects act as vectors for an increasing number of emerging human viral diseases. A mastery of the insect immune system and antiviral response could lead to a better control over the transmission of disease. In fact, many insect viruses develop a persistent infection during which cells are infected and produce viral particles, yet without clear signs of infection. In this context a key question is how viruses establish a persistent infection in insects. To understand this complex host-pathogen interaction, we are using a safe and powerful model D. melanogaster and a set of (+) strand RNA viruses. Using high throughput sequencing we have showed that the establishment and maintenance of persistence is regulated through multiple RNAi pathways in insects. These and other results will be discussed.

IUBMB II-S04
THE ELUSIVE HUMAN tRNA LIGASE IDENTIFIED
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The processing of pre-tRNA molecules entails removal of 5'-leader and 3'-trailer sequences, base modifications and template-independent addition of nucleotides. In addition, intron-containing pre-tRNAs undergo excision of the intervening sequence in two steps: first, the TSEN endonuclease generates 5'- and 3'-exons with 2', 3'- cyclic phosphate and 5'-OH ends, respectively. In animals, the second step entails direct exon ligation and retention of the 3'-terminal phosphate by an elusive RNA ligase.
We have observed that 3'-P, 5'-OH ended double stranded RNA molecules (dsRNAs) become covalently linked upon incubation in HeLa cell extracts, where the RNA terminal cyclase RTCD1 converts 3'-P ends into 2', 3'-cyclic phosphate resembling tRNA exon termini. We therefore attempted to purify the tRNA ligase using 3'-P, 5'-OH dsRNA as a surrogate substrate mimicking tRNA exons.
RNAi-mediated depletion of a candidate protein inhibited maturation of intron-containing pre-tRNA as well as dsRNA ligation, both *in vitro* and *in vivo*. We were also able to achieve exon half ligation in immunoprecipitates from a stable cell line expressing a myc-tagged wild type, but not a mutant, inactive form of the enzyme.
The discovery of the mammalian tRNA ligase provides a molecular basis for further studies not only focused on tRNA splicing but also on RNA repair and non-canonical splicing events.

IUBMB II-S05

SMALL RNAs REGULATE ALTERNATIVE SPLICING THROUGH TRANSCRIPTIONAL GENE SILENCING

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We found that small interfering RNAs (siRNAs) control alternative splicing (AS) by promoting changes in chromatin structure. When targeting promoter regions, siRNAs trigger transcriptional gene silencing (TGS), by promoting heterochromatin formation. We showed that siRNAs targeting intronic or exonic sequences located close to an alternative exon regulate its splicing. The effect requires RNA:RNA hybridization with endogenous target transcripts, is AGO1-dependent and is counterbalanced by factors favoring chromatin opening or transcriptional elongation. The promotion of heterochromatin marks (H3K9me2 and H3K27me3) at the target site, the need for HP1alpha, and a reduction in pol II processivity suggest a mechanism involving the kinetic coupling, that we called TGS-AS for AS regulated by transcriptional gene silencing. Using ChIp-seq, in collaboration with J. Valcárcel and E. Eyras of Barcelona, we identified 24,000 target regions for AGO1 in the human genome. AGO1 is enriched in exons and promoters and shows preferential overlapping with H3K9me2, H3K27me3 and H3K36me2 histone marks. We have found a list of candidate alternative splicing events to be affected by TGS-AS according to AGO1 binding, the presence of facultative heterochromatin histone marks and/or pol II and antisense transcription.

MI-S01

P. aeruginosa AND CIPROFLOXACIN: BACTERIAL RESISTANCE MECHANISM AND MUTAGENIC EFFECT OF THE DRUG

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Although Ciprofloxacin (CIP) is one of the most effective antibiotics against *P. aeruginosa* infections, resistance to this drug emerges quickly in the clinical setting, and is often linked to cross-resistance to other antibiotics. Resistance of *P. aeruginosa* to CIP is mainly due to mutations in Gyr A and P arC subunits of the DNA topoisomerases, and the expression of multidrug efflux pump MexC D-OprJ by mutations in the transcriptional repressor N fxB. We analyzed the molecular nature of CIP resistance at different drug concentration for the PAO1 wild type and hypermutator strains defective in the Mismatch and 8-oxoguanine Repair Systems (MRS and GO respectively). We report the main resistance pathway and mutational spectrum for each strain, and show that *gyrA* is mainly mutated in cells selected at high CIP concentrations, while *nfxB* mutations are dominant at low drug doses. It was also observed that: a) *nfxB* mutants showed cross-resistance to other antibiotics; b) double mutant *gyrA/parC* cells were generated in hypermutator MRS defective strain at a higher proportion than expected; c) oxidative stress stimulate the mutation frequency in GO defective strains; and d) *nfxB* mutants emerge at a very high frequency when cells are grown at subinhibitory levels of CIP. Different experimental approaches indicated that this last phenomenon is due to a direct effect of CIP on the bacterial cells.

MI-S02

THE MIGHTY TINY. WHY TO STUDY PROKARYOTE SMALL REGULATORY RNAs?

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In order to withstand environmental varying conditions and to carry out cellular differentiation, gene expression must be regulated. Traditionally, regulation of gene expression in prokaryotic cells has been thought to be a major responsibility of protein factors. However, this "dogma" has been shattered in recent years when it was realized that small, non-protein-coding, ribonucleic acids (sRNAs) can also perform subtle and specific regulatory functions. Typically, sRNAs have 50-500 nt, do not encode polypeptides and act on target mRNAs to control gene expression at a post-transcriptional level. In the first period of sRNA research (70's to 2001), a handful of sRNA molecules were characterized in model microorganisms; their regulation, their function, and their mechanism of action were revealed. In the second period (2001 to date), systematic searches to catalogue sRNA genes have been performed in numerous sequenced genomes. Today, next generation sequencing technologies are boosting the discovery of small RNA transcripts. The challenge, still, is to uncover functions of the hundreds of computationally and experimentally identified sRNAs. This presentation will provide an overview of the features of characterized sRNAs, and of the approaches that have been used to search for sRNA candidate genes.

MI-S03

ON HOW _Salmonella_ CONTROLS GROWTH INSIDE EUKARYOTIC CELLS

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Successful bacterial pathogens have evolved strategies to preserve host integrity and ensure their spread to susceptible individuals. The analysis of this 'persistence' phenomenon constitutes a big challenge in mechanistic terms. We are defining how *Salmonella enterica* serovar Typhimurium, a pathogen causing asymptomatic and chronic infections in human and animals, persists in the intracellular niche of eukaryotic cells. A state of limited intracellular proliferation, as reported in fibroblasts, is modeled by multiple virulence factors such as the regulatory system PhoP-PhoQ and the *Salmonella*-pathogenicity islands 1 and 2 (SPI-1 and SPI-2). Remarkably, some of these functions down-regulate the growth rate of the pathogen inside the eukaryotic cell. This 'growth-attenuation' state was shown to occur in vivo in the intestine of infected mice. Other recent studies reveal a delicate role of PhoP-PhoQ in dictating the time at which SPI-2 is activated in non-growing intracellular bacteria. Up-regulation of certain small non-coding RNAs has also been observed in these persisting bacteria. Studies on the host cell also implicate specific signal-transduction cascades activated by non-growing intracellular bacteria. Overall, the data are consistent with the existence of an intricate crosstalk between pathogen and the host cell designed to ensure coexistence of both partners.

MI-S04

AN INTRACELLULAR OPPORTUNISTIC PATHOGEN THAT ALTERS Rho GTPases IN MACROPHAGES

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Opportunistic infections pose a significant threat to human health, especially to those patients who benefit most from advancements in the treatment of genetic diseases, cancer, and organ transplantation, but who become immunosuppressed. *Burkholderia cepacia* complex bacteria (Bcc) and *Burkholderia cenocepacia* in particular are our main model organisms to study the pathogenicity of opportunistic bacteria. Bcc bacteria are a major health risk for children and young adults with genetic conditions like cystic fibrosis and chronic granulomatous disease. Lung and airways infected patients are very difficult to treat given the extraordinary resistance of these bacteria to clinically useful antimicrobials. Therefore, through out research we hope to find novel ways to prevent or ameliorate the effect of these infections in susceptible patients. We discovered that Bcc bacteria survive intracellularly in free-living amoebae and macrophages by altering the maturation of the phagosome, and this research has led to the hypothesis that these cells become a reservoir for the persistence and dissemination of these bacteria in the host. We have also identified a novel secretory system in *B. cenocepacia* that produces effector molecules with the ability to alter the cytoskeleton of infected macrophages and is required for infection in an animal model of chronic lung infection. I will describe our current understanding on the effect of intracellular *B. cenocepacia* on the assembly of the macrophage NADPH oxidase, actin cytoskeleton disruption and cell death, which revolves around the manipulation of host GTPases.

PL-S01
TACKLING THE METABOLISM OF TOMATO FRUITS BY GENETICAL-GENOMICS APPROACHES
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Tomato fruits not only constitute the most produced and consumed vegetable worldwide but it is also considered a central model to understand the photosynthetic and heterotrophic metabolisms "all-in-one" organ. In order to elucidate regulatory points underlying traits of nutritional values we have identified a large collection of genes putatively involved in regulating carbon partitioning, aminoacids and vitamin E contents in tomato fruits. Through substitutions of domesticated alleles by their wild counterparts a vast level of the metabolic variation was exposed. To further investigate the mechanisms underlying this variation, we set out to sequence the genome of *Solanum pennellii*, the closely wild related to the domesticated *S. lycopersicum* species. The first draft version of the assembly is composed by contigs spanning approximately 60 % of the genome. This resource allowed us to map most of the genes of interest and to associate metabolic traits with the collection of candidate genes. Moreover, a gfp-based VIGS system, along with a collection of iRNA transgenic plants, was set as a rapid strategy for testing these candidate crop quantitative metabolic loci. Study cases will be presented and discussed.

PL-S02
BIOGENESIS OF Fe-S- AND HEME- CONTAINING PROTEINS IN PLANTS
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Iron-sulfur and hemeproteins are involved in a wide variety of cellular processes such as respiration, photosynthesis and regulation of gene expression. The assembly of Fe-S and heme groups into polypeptides has become an area of intense research. Particularly, the synthesis of Fe-S proteins involved complex cellular machineries. Many of the genes are conserved in bacteria, yeasts, mammals and plants. We recently characterized mtHsp70-1 and 2, Isu1, HscB, AtNfs1 and AtFH, six mitochondrial *Arabidopsis* genes homologous to others that participate in yeast and mammals Fe-S assembly. We found that AtFH interacts and modulates AtNfs1 catalytic activity, suggesting the involvement of frataxin in early steps of Fe-S cluster formation. AtFH deficiency down-regulates AtNfs1 expression and other genes involved in Fe-S and heme synthesis. We also found an alteration in the activity of different mitochondrial and chloroplastic Fe-S proteins and hemeproteins such as catalase. The results indicate that AtFH, besides their role in protecting bioavailable iron within mitochondria and the synthesis of Fe-S groups, also plays a role in the biogenesis of heme group. Our data substantiate the hypothesis that AtFH would have a major role in heme production as an "iron chaperone" for ferrochelatase also is involved in the biogenesis of plant hemeproteins.

PL-S03
PLANT REGULATORY NETWORKS AND DEVELOPMENT
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Our long-term goal is to investigate the mechanisms by which plants control gene expression and to elucidate the structure and dynamics of the underlying gene regulatory networks (GRNs). We have used a number of cellular processes as "windows" to explore the architecture of plant GRNs in monocots (e.g., maize) and dicots (e.g., *Arabidopsis*). In *Arabidopsis*, we are focusing on how the epidermis differentiates into various cell types, including leaf hairs (trichomes) and pores (stomates). We identified direct targets for several trichome selectors using a combination of ChIP-chip and genome-wide expression analyses. These studies revealed several tiers in a complex network of transcription factor-target gene interactions and provided novel insights on epidermal cell differentiation. Genome-wide location analyses carried out on second tier regulators further exposed unique network motifs involved in the trichome GRN. Combined with the development of two public databases, AGRIS (http://arabidopsis.med.ohio-state.edu/) for *Arabidopsis* and GRASSIUS (www.grassius.org) for maize and other grasses, information on transcription factors, promoters and their interactions is integrated, facilitating the identification and visualization of GRNs.

PL-S04
A METHYL TRANSFERASE LINKS THE CIRCADIAN CLOCK TO THE REGULATION OF ALTERNATIVE SPLICING
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Circadian rhythms allow organisms to time biological processes to the most appropriate phases of the day. Here we show that PROTEIN ARGININE METHYL TRANSFERASE 5 (PRMT5), which transfers methyl groups to arginine residues present in histones and Sm spliceosomal proteins, links the circadian clock to the control of alternative splicing. Mutations in prmt5 impair circadian rhythms in *Arabidopsis* and this phenotype is caused, at least in part, by a strong alteration in alternative splicing of the clock gene PRR9. Furthermore, genome-wide studies show that PRMT5 contributes to the regulation of many pre-messenger-RNA splicing events. PRMT5 expression shows circadian oscillations, and this mediates the circadian regulation of expression and alternative splicing of a subset of genes. Circadian rhythms in locomotor activity are also disrupted in dart5, a mutant affected in the *Drosophila* PRMT5 homologue, and this is associated with alterations in splicing of the clock gene period. Our results demonstrate a key role for PRMT5 in the regulation of alternative splicing and indicate that the interplay between the circadian clock and the regulation of alternative splicing by PRMT5 constitutes a common mechanism that helps organisms to synchronize physiological processes with daily changes in environmental conditions.

CB-S01

KEEPING THE BALANCE OF AMYLOID β WITH PROTEASES ON BOTH SIDES OF THE EQUATION

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The progressive accumulation of amyloid β peptides (Aβs), a major feature of the aged human brain, is deeply exacerbated in Alzheimer's disease (AD), Down syndrome (DS) and hereditary dementias. Regardless of the role of Aβs in the pathogenesis of these diseases, the mechanisms leading to their accumulation are poorly understood. Aβs are released by sequential endoproteolysis of a transmembrane amyloid precursor protein (APP). The aspartyl proteases BACE1 and γ-secretase generate the amino and carboxyl-termini of Aβ peptides, respectively. Within endogenous levels of APP, the activity of BACE1 determines the rate of Aβs generation. After being released, Aβs is purportedly removed by transport to the circulation and in situ proteolysis by Zn^{2+} metallopeptidases such as insulin-degrading enzyme (IDE) and neprilysin (NEP), among others. In sporadic AD, BACE1 expression and activity are increased while IDE and NEP activities are reduced, consistent with an unbalanced process leading to higher steady-state levels of Aβs. We have recently identified possible subcellular sites of Aβs-IDE interactions and a likely mechanism for IDE non-classical secretion implicated in degradation of extracellular Aβs. Understanding how BACE1 and IDE activities are modulated may provide additional insight into Aβs homeostasis regulation in health and disease.

CB-S02

LIPID RAFT TARGETING OF ALZHEIMER'S DISEASE APP SECRETASES

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Alzheimer's disease is characterized by cerebral deposition of β-amyloid peptides derived from sequential proteolytic processing of amyloid precursor protein (APP) by BACE1 and γ-secretase. Cholesterol has been identified as a risk factor for Alzheimer's disease and shown to influence APP processing. A subset of APP and BACE1 localizes to cholesterol- and sphingolipid-rich detergent-resistant membranes (DRM) in cultured cells and brain, whereas γ-secretase subunits and APP-derived C-terminal fragments predominantly associate with DRM. We discovered that two subunits of γ-secretase undergo post-translational S-palmitoylation, a lipid modification that facilitates lipid raft association. Similarly, we have characterized multiple S-palmitoylation in BACE1. Mutagenesis studies indicate that S-palmitoylation is required for DRM association of BACE1, but is not the sole determinant for DRM localization of γ-secretase. Studies conducted in cultured cell lines show that S-palmitoylation does not contribute to catalytic activity of BACE1 or γ-secretase. Nevertheless, S-palmitoylation is a critical modification that regulates BACE1 localization in hippocampal neurons. Moreover, expression of S-palmitoylation-deficient γ-secretase subunits attenuates amyloid deposition in transgenic mice. Thus, post-translational S-palmitoylation and localization of APP secretases in lipid rafts of neurons contribute to Alzheimer's disease pathogenesis.

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CB-S03

FROM STRUCTURAL TO CELLULAR BIOLOGY OF PARKINSON DISEASE: RATIONAL DESIGN OF AGGREGATION INHIBITORS

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The misfolding of proteins into a toxic conformation is proposed to be at the molecular foundation of a number of neurodegenerative disorders including Alzheimer and Parkinson's disease. One common and defining feature of protein misfolding diseases is the formation and deposition of amyloid-like fibrils. The aggregation of the protein alpha-synuclein (AS) is a critical step in the etiology of Parkinson's disease (PD) and other neurodegenerative synucleinopathies. The study of the structural and toxic mechanisms related to AS amyloid formation is critical to advance in the design of a therapeutic strategy. The identification of aggregation inhibitors and the investigation of their mechanism of action are fundamental in the quest to mitigate the pathological consequences of amyloid formation. By the combined application of a battery biochemical and biophysical tools we have addressed structural and molecular unresolved details related to the mechanistic rules that direct the anti-amyloid effect of small molecules on AS amyloid fibril formation.

Lamberto *et al*. (2009) Proc Natl Acad Sci U S A 106: 21057-21062.

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CB-S04

GENOME-WIDE RNAi SCREEN IDENTIFIES NOVEL GENES REQUIRED FOR THE RESPONSE TO HYPOXIA

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The Hypoxia Inducible Factor (HIF) is an alpha-beta heterodimeric transcription factor composed of two helix-loop-helix-PAS (bHLH-PAS) subunits. It is regulated mainly at the level of oxygen-dependent protein stability of its alpha subunit. Whereas in normoxia HIF-alpha is rapidly degraded at the 26S proteasome, in hypoxia the protein is stabilized, and accumulates in the nucleus.

We have conducted an unbiased genome-wide RNAi screen in *Drosophila* cells aimed to the identification of novel genes involved in HIF regulation. After 3 rounds of selection, 35 genes emerged as critical regulators of HIF activity in hypoxia, most of which had not been previously associated to HIF biology. We focused our analysis on components of chromatin remodeling complexes, including Reptin, Pontin and Moira, the transcription elongation factors Spt5 and Spt6 and the miRNA pathway component Argonaute 1. We have validated the requirement of these genes for the activation of a HIF inducible reporter in transgenic flies, and by assessing the expression of 4 dHIF target genes in embryos. Studies carried out both in cell culture and *in vivo* confirmed the physiological requirement of Argonaute 1 and the miRNA machinery for HIF dependent transcription, as well as a role of Pontin in HIF dependent transcription through the modification of chromatin structure at HIF-responsive promoters.

LI-S01

CHOLESTEROL LOSS DURING AGING IN HIPPOCAMPAL NEURONS. CAUSES AND CONSEQUENCES

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In a recent work we demonstrated that cholesterol is gradually lost in hippocampal neurons during aging. This cholesterol reduction is the consequence, at least in part, of increased cholesterol hydroxylation, due to the upregulation of the enzyme cholesterol-24-hydroxylase or Cyp46. Naturally, cholesterol reduction will have a number of consequences, some beneficial and some deleterious. One of these beneficial consequences seems to be increased survival via tyrosine kinase receptors pathway, specifically TrkB. In fact, TrkB activity can be induced in young neurons by the simple reduction of cholesterol. Moreover, impairing cholesterol loss by Cyp46 knock down silenced TrkB activity. Cholesterol loss-mediated TrkB activation appears to be relevant in conditions of stress. Inhibition of Cyp46 does not lead to cell death in normal aged cells *in vitro* or animals but it does under stress conditions. These data imply that in the aged brain, survival strength is guaranteed by the activation of mechanisms that supplement the use of the canonical neurotrophins. Given that Cyp46 mutations have been found in certain cases of Alzheimer's Disease (AD) individuals, the above results suggest that this disease may be due to failure in normally surplus antistress mechanisms. On the other hand, although cholesterol loss has positive consequences in terms of survival, live imaging experiments using single molecule tracking of membrane receptors indicated that lipid changes produced during aging would also result in reduced neuronal performance.

LI-S02

MANIFOLD STRUCTURAL TRANSDUCTION BY MEMBRANE LIPIDS. WHAT ARE LIPOLYTIC ENZYMES DOING?

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Lipid substrate-product mixing is an important factor determining the membrane structural dynamics because segregated phase domains can occur thus changing membrane topology. In turn, this can affect enzymatic activities responsible for the lipid composition and modulate backwards the structural dynamics. In addition, enzymatic reactions and surface topography are sensitive to other independent pathways leading to metabolic-structural cross-talk at the membrane level. These events provide rapid structural information transduction mechanisms that are finely regulated by the lipid-protein composition. I will summarize some recent results on the synergic cross-talk among structural dynamics and surface lipolysis mediated by the activity of *B.cereus* sphingomyelinase, *pp* phospholipase A2 and *Cl. perfringens* neuraminidase. The enzymatically-induced structural changes can be explained by out-of equilibrium compositional changes due to formation of lipid products. Formation of segregated domains due to lipid miscibility/immiscibility results in transient surface shapes and long-range patterns. This is because the lipid mixing and reaction kinetics are superimposed to relaxation processes relieving dipolar repulsion within domains, balanced by line tension. This can also drive topological bilayer/non-bilayer transitions, lipid flip-flop and membrane fusion/fission events.

LI-S03

AUTOTAXIN AN LYSOPHOSPHATIDATE INDUCE CHEMO-RESISTANCE AND RELEASE BREAST CANCER CELLS FROM TAXOL-INDUCED MITOTIC ARREST AND CELL DEATH

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Taxol is a microtubule-stabilizing agent that is widely used to treat breast and other cancers. However, Taxol resistance is common and approximately 33% of women diagnosed with breast cancer die partly because of chemo-resistance and our inability to block metastasis. It is vital to understand the mechanisms that produce this resistance to chemotherapy to improve treatment. We hypothesized that autotaxin, whose expression in tumors is associated with increased growth, angiogenesis and metastasis, would produce chemoresistance. We used cultured breast cancer cells and showed that autotaxin is required for extracellular lysophosphatidylcholine (LPC) to stimulate both cell migration and Taxol resistance. Autotaxin does this by converting LPC to lysophosphatidate (LPA) with subsequent activation of LPA receptors. Autotaxin plus LPC, or LPA alone, antagonize the Taxol-induced arrest of cancer cells in G2/M of the cell cycle, thereby protecting from cell death. This LPA effect depends on phosphatidylinositol 3-kinase activity and a decrease in the Taxol-induced production of the pro-apoptotic lipid, ceramide. LPA also increases the expressions of anti-apoptotic proteins and those that modulate spindle formation and microtubule dynamic instability. We conclude that these signaling effects could also contribute to resistance to the actions of other chemotherapeutic agents.

LI-S04

TRIACYLGLYCEROL METABOLISM IN OLEAGINOUS BACTERIA

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Members of *Rhodococcus* genus are specialist in the accumulation of triglycerides (TAG). Some of them can be considered oleaginous microorganisms since they are able to produce significant amounts of those lipids under certain conditions. The pathways of rhodococcal central metabolism are able to efficiently convert diverse carbon sources to the key metabolic intermediates such as pyruvate, acetyl-CoA and glycerol-3-phosphate to create reducing equivalents that are required by lipid biosynthetic pathways and to produce the necessary energy as ATP. In addition, these bacteria seem to be able to maintain a high carbon flux toward the lipid production pathways. The TAG biosynthesis in rhodococci has been proposed to occur via sequential acyl-CoA-dependent reactions referred to as the 'Kennedy pathway'. The last step of TAG biosynthesis is catalyzed by diacylglycerol acyltransferases enzymes (DGAT). The occurrence of genes coding for DGAT enzymes is highly redundant in rhodococcal genomes. The enrichment of genes and enzymes involved in TAG metabolism in rhodococci suggest an important role of these lipids in the physiology of these microorganisms. Basic knowledge on this field is also relevant for predicting biotechnological applications of oleaginous bacteria in the industry, for example for the production of biofuels, oleochemicals, lubricants, and other manufactured products.

A2B2C-S01
LOOKING FOR STRUCTURE IN GENE EXPRESSION PROFILES
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Typical DNA microarray assays produce a huge amount of information, and clustering techniques are usually employed to reveal common patterns of gene expression across different samples. The rationale behind this approach is a 'guilty-by-association' scenario, where genes with similar profiles of activity are supposed to have related functions or to be regulated by common mechanisms. In this communication we introduce a variant of a clustering procedure rooted in statistical mechanics paradigms: the Super Paramagnetic Clustering. The proposed novel algorithm seeks to find biologically meaningful gene clusters, integrating microarray transcriptional data with biological information about gene functions, as provided by the Gene Ontology database. The new algorithm was applied to several gene expression data sets. We found out that it greatly improves the biological coherence of cluster partitions, compared to the naive SPC implementation, and other traditional clustering methods. In conclusion, using a combined metric, we present a method that can simultaneously mine for meaningful structure in both, expression and functional spaces. The algorithm shows a robust behavior and succeeds in recognize sensible gene clusters that might be used to identify relevant biological process for the phenotypes of interest.

A2B2C-S02
STRUCTURE-BASED PREDICTION OF PROTEIN-DNA BINDING SPECIFICITIES
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The specificity displayed by a DNA-binding protein towards its DNA target sites is an essential feature for the function of any transcription factor in a given genome where an excess of non-specific sites exists. Exhausting all possible combinations for a binding site of length N involves checking binding against 4N DNA sequences, something that is not always achievable experimentally. The aim of this work is to accurately predict DNA binding profiles. Here we present a structure-based computational method that uses FoldX, a protein design software, to predict the specificity of any DNA-binding protein as long as the structure of the complex is available. We compare our results to all available experimental data, obtaining an overall very good agreement. To validate our predictions experimentally we performed comprehensive DNA binding assays using protein binding microarrays and obtained results agreeable with the benchmark set. We suggest FoldX derived structure-based DNA binding prediction as a powerful tool to infer protein binding sites and design new specificities.

A2B2C-S03
LOCALIZING FRUSTRATION IN PROTEIN MOLECULES
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Energy landscape theory does not deny the possibility that conflicting interactions may exist locally in a protein domain. Such “local frustration", being tolerable, would naturally arise from neutral evolutionary drift or in some cases could indicate a functional adaptation, as alternate configurations become accesible and control protein motions. We recently developed a quantitative method for localizing frustration in native proteins (PNAS 104:19819). Here we present our latest applications of these "frustratometer" algorithms for the analysis of proteins' conformational fluctuations. We analyzed a non-redundant set of allosteric proteins and found that the regions that are known to undergo structural transitions are likely to be highly frustrated in either or both conformations. We relate the local frustration distribution to the function of these proteins and explore its relations to the transitions between the conformational substrates. We discuss how methods for localizing frustration can give insights into the functional aspects of the evolution of proteins' energy landscapes.

CB-C01

CALCIUM-DEPENDENT ASSOCIATION OF ARGINYLATED CALRETICULIN WITH STRESS GRANULES

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Post-translational modifications of proteins are important for the regulation of cell functions; one of these modifications is post-translational arginylation. In the present study, we demonstrated that cytoplasmic CRT (calreticulin) is arginylated by ATE1 (arginyl-tRNA protein transferase). We also show that a pool of CRT undergoes retrotranslocation from the ER to the cytosol, because in CRT-knockout cells transfected with full-length CRT, cytoplasmic CRT appears as a consequence of its expression and processing in the ER. After the cleavage of the signal peptide, an N-terminal arginylatable residue is revealed prior to retrotranslocation to the cytoplasm where arginylation takes place. SGs (stress granules) from ATE1-knockout cells do not contain CRT, indicating that CRT arginylation is required for its association to SGs. Furthermore, R-CRT in the cytoplasm associates with SGs in cells treated with several stressors that lead to a reduction of intracellular Ca^{2+} levels. However, in the presence of stressors that do not affect Ca^{2+} levels, R-CRT is not recruited to these loci despite the fact that SGs are formed, demonstrating Ca^{2+}-dependent R-CRT association to SGs. We conclude that post-translational arginylation of retrotranslocated CRT, together with the decrease in intracellular Ca^{2+}, promotes the association of CRT to SGs.

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CB-C02

PROTEASOMAL DEGRADATION OF ARGINYLATED CALRETICULIN (RCRT) IN THE CYTOPLASM

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Posttranslational arginylation of proteins consists in the covalent union of an arginine into an acidic amino acid at the N-terminal by arginyl-tRNA protein transferase. We demonstrated that the calreticulin (CRT) arginylation occurs in the cytoplasm. CRT is a resident of the ER, however it was also found in others subcellular compartments. Arginylation participates in the N-rule pathway, which relates half life of proteins to its N-terminal. Arginylation performs other functions not involved with degradation.

In the present study RCRT and CRT were synthesized in the cytoplasm of CHO cells by a previously established method. Stable cell lines were obtained with the constructions Ub-RCRT-EYFP and Ub-CRT-EYFP. The cleavage of ubiquitin by deubiquitinases was corroborated by Western-Blot (WB). CHO cells that express CRT-EYFP and RCRT-EYFP in the cytoplasm were incubated in the presence of the proteasomal inhibitor MG132 and analyzed by WB, immunocytochemistry and flow cytometry. Treatment of these cells with MG132 induced the accumulation of RCRT. Moreover, CRT showed an increased rate of degradation with respect to RCRT in the presence of MG132.

Thus, these results indicate a proteasomal degradation of RCRT in the cytoplasm and support the idea that posttranslational arginylation of CRT seems to regulate its cytosolic function.

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CB-C03

ROLE OF VINCULIN IN THE PROCESS OF COLLECTIVE CELL MIGRATION OF THE NEPHROGENIC URETERIC BUD (UB)

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In mammals, nephrogenesis is completed posnatally. In this work, we studied the role of vinculin in the process of collective migration of UB cells. We made primary cultures of renal papillary collecting duct/UB cells of 7 days-old rats, and studied the expression of vinculin and actin cytoskeleton by immunofluorescence. We observed isolated cells making contact by filopodia and long membrane prolongations, and colonies of cells with migratory phenotype. Some border cells contained greater amount of vinculin than the rest of cells, and marginal semicircular bundles of actin, recruited by vinculin located in the plasma membrane. A selective Rho-kinase inhibitor abrogated the accumulation of vinculin in border cells. These results indicate that UB cells display a great capacity to form colonies and to migrate collectively. We interpret that vinculin accumulation, and the recruitment of actin filaments, only occurs in those cells whose function is the one to indicate the direction of the collective migration of the UB cells, which is a Rho - dependent mechanism. This behavior could explain the advance of the UB from papilla to renal cortex where takes place the mutual induction between the metanephric mesenchyme (MM) and UB cells, and as a result MM undergoes mesenchymal–epithelial conversion to gives rise to the nephron, and the UB gives rises to the renal collecting duct system.

CB-C04

CONTRACTILE APPARATUS OF SEMINIFEROUS TUBULE MYOID CELLS

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Myoid cells (MC) surround the seminiferous tubules (ST) and express contractile proteins of muscle cells like á-actin and myosin. Previous works postulated an orthogonal display of actin filaments (AF) forming a net.

In this study we analyzed the distribution of AF and myosin II filaments (MF) of MC by confocal microscopy and TEM. Adult rat ST segments were isolated and maintained in control conditions or contracted with 50 nM ET-1. The segments were hatched with anti á-actin-FITC or anti SMMII-IgG-Cy3 antibodies. Other segments were processed by conventional technique for TEM study.

Confocal microscopy images of ST segments showed that MC contain both AF and MF distributed in two independent layers instead of a net: the inner layer with filaments perpendicular to the tubular axis and the external layer with filaments parallel. We confirmed this observation by TEM. In ST segments treated with ET-1, MC have smaller cellular area (1127±77,9 µm ± SEM) and bigger high (4,7±0,4 µm ± SEM) than controls (1823±68,8 µm and 3,3±0,2 µm respectively). TEM showed that MC of contracted segments have folded both nuclei and basement membranes, intertwined AF and MF, and a major anchorage of filaments to the plasma membrane.

The knowledge of the participation of AF and MF in MC contraction is very important to understand the role that MC carry out in the transport of sperm cells in the ST.

CB-C05

LOCALIZATION OF TRANSMEMBRANE-PROTEINS: ROLE OF CYTOPLASMIC MOTIFS AND TRANSMEMBRANE DOMAINS

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Transmembrane (TM) proteins are directed to their target organelles through several mechanisms, of which two will be analyzed in this work: Interaction with protein coats through aminoacidic motifs present in cytoplasmic tails, determining subcellular localization, and organelle-specific properties of TM domains. Neither the universality nor the distribution of cytoplasmic motifs has been analyzed. We have built a database with proteins whose subcellular localization and TM topology is known, which allows us to evaluate the presence of different motifs in the cytoplasmic tails of transmembrane proteins that localize to the Endoplasmic Reticulum, Golgi apparatus or plasma membrane. This same dataset is being analyzed to determine if TM domains belonging to proteins with different subcellular localizations possess distinct asymmetrical distribution of certain amino acids among transmembrane faces, membrane bilayer leaflets and distance to TM borders. Differences in these distributions could determine distinct physicochemical interactions with different lipids that compose membranes, which in turn, could determine lateral diffusion and inclusion into certain types of vesicles. Preliminary results indicate that organelle-specific TM properties exist, and that redefinition of certain cytoplasmic motifs allows us to suggest a novel Golgi retention motif.

CB-C06

MODELING FUSION/FISSION-DEPENDENT INTRACELLULAR TRANSPORT

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Eukaryotic cells have distinct membrane-bound compartments that 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. Hundred of factors have been implicated in intracellular transport by overexpression and knockout experiments; however, how they control and mediate transport is not well understood. By modeling iterative events of organelle fusion and fission we demonstrated that luminal components are efficiently transported when geometric asymmetries between the resulting organelles were programmed. In contrast, transport of membrane-associated components was inefficient. We have extended our model to assess the requirement for membranous marker transport during iterative fusion/fission events in simulations that include recycling and formation of internal vesicles. The results indicate that two basic principles are required: i) only organelles with the same or compatible Rab membrane microdomains (MMD) can fuse, ii) during fission, Rab MMD and the membrane marker distribute asymmetrically in the two resulting organelles. With these rules, membrane markers were directed to “lyososomal” compartments or “recycled” to the extracellular medium according to the tropism assigned to the marker during the fission processes.

CB-C07

SPECIFICITY OF TRANSMEMBRANE PROTEIN PALMITOYLATION

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Palmitoylation is carried out by a family of palmitoyltransferases (PATs) characterized by the presence of a 50-residue long cysteine-rich domain (DHHC-CRD domain). Yeast has 7 members of the family and each of these proteins is thought to be responsible for the palmitoylation of a set of substrates. Substrate specificity of PATs is not yet fully understood. Several yeast PATs seem to have overlapping specificity, and for mammalian cells, it has been proposed that the machinery responsible for palmitoylating peripheral membrane proteins lacks specificity altogether. Here we investigate the specificity of transmembrane protein palmitoylation, which in the yeast S. cerevisiae, is carried out mostly by two PATs, Swf1 and Pfa4. We show that palmitoylation of transmembrane substrates requires specific PATs, since other yeast PATs cannot carry out Swf1 or Pfa4 functions. Furthermore, we find that Swf1 is highly specific for its substrates, as it is unable to substitute for other PATs.
To identify where Swf1 specificity lies, we used a chimeric-gene strategy and carried out a bioinformatic survey to identify amino acids responsible for the determination of specificity (SDPs). Our results indicate that the DHHC domain might be involved in the determination of specificity. Finally, we show that the position of the cysteines in Tlg1, a Swf1 substrate, is important for their palmitoylation.

CB-C08

FATTY ACID SYNTHESIS IS REQUIRED FOR PROTEIN TRANSLOCATION DURING SPORULATION

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During sporulation in *B. subtilis* an asymmetrical division creates two compartments, a larger one (mother cell) and a smaller one (prespore). The σ^E factor is activated only in the mother cell by the membrane protease SpoIIGA which requires SpoIIR, a prespore expressed protein. SpoIIR has a Sec-type signal sequence and is believed to be secreted across the prespore membrane to act as a cell-to-cell signal. We have previously shown that inhibition of fatty acid but not phospholipid biosynthesis prevents σ^E activation. To study the requirement of fatty acid synthesis on SpoIIR secretion, we examined strains bearing a GFP-SpoIIR fusion during sporulation by fluorescence microscopy. The fusion protein was first concentrated along the septum, but later fluorescence signal accumulated in the prespore cytoplasm suggesting SpoIIR processing. Conversely, cytoplasmic fluorescence was not detected in sporulation arrested cultures, implying an interruption in SpoIIR secretion. Disruption of proteolytic processing was confirmed by western blot using anti-GFP antibodies. Fatty acid synthesis is not a general requirement of protein transport since we found that it is dispensable for secretion of AmyE, another Sec-dependent protein. Together, our results strongly suggest a novel role of de novo fatty acid synthesis on protein membrane translocation during a differentiation process.

CB-C09

RT INTRODUCES SPECIFIC cDNA BACKGROUND SYNTHESIS IN RT-PCR ASSAYS: HOW TO WORK IT OUT?

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NMDA receptors (NMDARs) are tetramers formed by two NR1 subunits and different NR2 and/or NR3 subunits. NMDAR were involved in synaptic plasticity, learning, memory and several neuropathologies. We have developed Amplicon vectors derived from herpes simplex virus type1 (HSV-1) to modify NMDAR subunits expression. Amplicon vectors were chosen for: neurotropism, large transgene capacity and ability to maintain transgene expression. We injected a vector codifying a NMDAR-NR1 antisense (AS vector) into rat hippocampus. RT-PCR assays with hippocampal RNA from animals injected either with an AS vector, a control vector or vehicle showed an amplification signal for NR1 antisense. However, only animals injected with the AS vector suffered impairments in learning and memory. RNAse Protection Assay showed that the template for this signal was NR1 mRNA. To quantify the relationship between specific and background amplification, qRT-PCR was performed with primers or without primers at the RT. Analysis of several genes leading us to conclude that a background signal for each mRNA in the RT-PCR assay is always present and is introduced by the RT reaction. Background signal should be identified, quantified and subtracted from the specific amplification signal. Now, we have constructed new Amplicon vectors bearing shRNA against NMDAR subunits to efficiently modify their expression.

CB-C10

QUANTITATIVE STUDY OF MOTHER-DAUGHTER ASYMMETRY IN Ace2 LOCALIZATION IN YEAST

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Saccharomyces cerevisiae reproduces by budding, a process that is intrinsically asymmetric. Mother cells form buds that separate to become daughters. Three asymmetric processes have been studied the most: mating type switching, replicative aging, and Ace2-driven daughter cell-specific gene expression. The molecular mechanism that leads to the activation of the third process is not fully understood.
The transcription factor Ace2 is seen in both nuclei at the end of mitosis, but disappears from the mother´s nucleus soon after. Localization of a protein to the nucleus is the net result of import and export reactions. To gain insight into the mechanism responsible for Ace2 asymmetry, we developed a method based on FRAP that enables us to calculate nuclear transport rates from individual live cells. We found that the ratios between the import and export rates are similar in mothers and in their daughters. This result is surprising as equal ratios imply equal nuclear fractions. However, absolute transport rates are asymmetric: mother cells have significantly slower nuclear shuttling dynamics than their daughters. Yeast maintain this kinetic asymmetry actively since we found regulatory mutations that result in inverted behavior. Our results allowed us to reject previous mechanisms for Ace2 asymmetry and propose a new, dynamic, model.

CB-C11

STUDYING THE LINK BETWEEN CHROMATIN AND SPLICING BY IMAGING APPROACHES

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Recent evidence has shown that chromatin structure is connected to the pre-mRNA splicing process, both at the levels of nucleosome positioning and histone modifications. In addition, modulation of intragenic chromatin results in regulation of alternative splicing. Here, we investigate putative mechanistic links between chromatin structure and pre-mRNA splicing events using living- and fixed-cell imaging. Our working hypothesis is that changes in chromatin structure can lead to differential co-transcriptional recruitment of splicing factors. After treatment of HeLa cells with the hyper-acetylating drug trichostatin A, which results in general chromatin relaxation, we observed a redistribution of several splicing factors from the nucleoplasmic fraction to accumulation in nuclear speckles (sites of storage). Furthermore, we found that the co-localization between the splicing factor SC35 and histone H3 is significantly reduced. By using Fluorescence Recovery After Photobleaching approach, we measured dynamic changes of nucleoplasmic and speckled fractions of splicing factors in response to chromatin modulation. Finally, we measured in vivo the interaction between splicing factors, and between splicing factors and chromatin using Förster Resonance Energy Transfer. Altogether, these techniques will provide new insights in the emerging field of connections between chromatin and splicing.

CB-C12

GENOME-WIDE SEARCH FOR ENDOGENOUS SMALL RNAs AFFECTING ALTERNATIVE SPLICING BY TGS

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We have recently shown that siRNAs targeting sequences located close to an alternative exon are able to regulate its alternative pre-mRNA splicing (AS) in human cells. The effect needs AGO-1 and is abolished or reduced by factors that favor an open chromatin structure or increase transcriptional elongation. The mechanism, named TGS-AS, involves the presence of facultative heterochromatin epigenetic marks (H3K9me2 and H3K27me3) at the target site.
Using ChIP-seq with antibodies to AGO1 protein and different histone marks we identified more than 20.000 binding sites in the genome of the human mammary cell line MCF7. Approximately 50% of the targets map within genes. Moreover, we detected a significant enrichment of AGO1 peaks in promoters and 5' UTRs of highly transcribed genes that it is not associated with histone marks, but shows overlapping with two small RNAs families: PASRs and tiRNAs. On the contrary, inside of genes AGO1 and H3K9me2 are enriched in exons, preferentially in genes with low expression levels.
Several histone marks display from 5% to 20% overlapping when assessed at the whole genome level. However the convergence increases to 55% when only AGO1 target sites are considered.
Consistently, we have found a set of candidate alternative splicing events to be affected by TGS-AS according to AGO1 binding and the presence of facultative heterochromatin histone marks.

CB-C13

ANALYSIS OF ZEBRAFISH nolc1l: THE ORTHOLOGE OF THE HUMAN GENE INVOLVED IN TREACHER COLLINS SYNDROME

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Treacher Collins Syndrome (TCS) is a congenital craniofacial disorder characterized by head and neck anomalies, such as hypoplasia of the facial bones, cleft palate, and ear defects that result in conductive deafness. TCS is caused by autosomal dominant mutations in tcof1 gene. To date, the *in vivo* functions of *tcof1* and its encoded protein, Treacle, are poorly understood. The main goal of this work was to shed light on the *tcof1* role during embryonic development using zebrafish as animal model. The zebrafish *tcof1* gene has not been described, but a homologue to mouse and human *tcof1*, called *nolc1l*, was found by *in silico* genome analysis. The nolc1l cDNA was cloned, and its expression pattern analyzed by semi-quantitative RT-PCR and whole-mount in situ hybridization during embryogenesis. High levels of mRNA were detected in 1-cell stage, indicating its maternal origin. The lowest level was detected at 12 hours post-fertilization (hpf); the expression increased again from that stage reaching a plateau at 24 hpf. Similarly to mammalian *tcof1*, *nolc1l* is ubiquitously expressed until late somitogenesis stages, from which it becomes localized to cephalic regions and the notochord. Finally, by using splicing morpholinos we knocked down *nolc1l* expression by generating dominant negative proteins. This is the first work identifying the sequence and biological behavior of TCS gene in zebrafish.

CB-C14

INSULIN DEGRADING ENZYME CORE PROMOTER IS REGULATED BY NRF-1 AND RELATED-ETS TRANSCRIPTION FACTORS

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Insulin Degrading Enzyme (IDE) is a conserved metallopeptidase that contributes in the in vivo metabolism of cerebral insulin and amyloid â peptide (Aâ) of Alzheimer's disease (AD). IDE mRNA and activity levels are decreased in AD brain, but its transcripts levels increased in cultures of primary rat astrocytes exposed to fibrillar Aâ. Despite its involvement in insulin and Aâ peptide clearance, little is known about the regulation of IDE expression. Aims: To characterize the core promoter of human IDE gene. Methods: in silico analysis; 5'RACE; U87MG and HeLa cell lines transfections; promoter deletions in pGL3-luciferase; site-directed mutagenesis; EMSA. Results: 1-IDE 5' sequence lacks a canonical sequence with promoter function. 2-IDE shows several transcription start sites from -39 to 28 that may lead to the generation of different protein isoforms. 3-The promoter construct -120/59-luc, containing canonical sequences for NRF-1 and ETS family, showed the highest transcriptional activity. 4-Mutation of the putative NRF1-binding site abolished transcription of the reporter gene and mutation of ETS site resulted in a 75% decrease compared to control; 5-NRF1 and Ets1, a member of ETS family, bind to the predicted sequence *in vitro*. Conclusions: the mitochondrial biogenesis regulator-NRF1 and ETS likely drive IDE transcription, modulating IDE expression and subcellular localization.

CB-C15

THE RD1 CLUSTER FROM *Mycobacterium marinum* IS RESPONSIBLE FOR AUTOPHAGY ACTIVATION UPON INFECTION

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Mycobacterium marinum (Mm), is widely used as a model to study pathogenic mycobacteria. In recent studies we have observed a marked recruitment of the autophagic protein LC3 to Mm phagosomes in cells incubated in control (i.e. full medium) or starvation conditions; a feature that is lost with heat-killed bacteria. However, when autophagy was induced pharmacologically by the drug rapamycin (Rap) the Mm phagosome was clearly decorated with LC3, despite bacterial status (i.e. live or heat-killed bacteria). Moreover, Rap was able to deliver LC3 to phagosomes containing an inert particle such as IgG-coated latex beads (IgG-LB). Interestingly, this process required a functional Atg5 protein since it was not observed in MEF Atg5 knock out cells, indicating that it depends on a functional autophagic pathway. We have also observed that Mm infection target LC3 to neighbor IgG-LB phagosomes even in control condition. Finally, we assessed the infection with Mm lacking the virulence cluster RD1 (Mm ΔRD1), which encodes several virulence factors and is deficient in the ESX-1 secretion system. We have found that Mm ΔRD1 was unable to recruit LC3 to its phagosome, but, as expected it acquired LC3 when Rap was used. Thus, our results suggest indicate that a factor secreted by the ESX-1 secretion system is responsible for LC3 recruitment to Mm phagosomes, a requirement overcomes by Rap treatment.

CB-C16

AUTOPHAGIC ACTIVATION BY ALPHA-HEMOLYSIN FROM *Staphylococcus aureus*

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Staphylococcus aureus induces a caspase-independent cell death, with the participation of the autophagic pathway. Autophagy involves the sequestration of cytosolic components, organelles and microorganisms in a vacuole called autophagosome, which finally fuses with the lysosome to degrade the trapped material. We have recently shown that á-Hemolysin (Hla), a pore forming toxin, which is the main virulence factor secreted by *S. aureus* , is able to induce the autophagic pathway in CHO cells. The toxin caused a marked activation of autophagy in a concentration-dependent manner, as assessed by LC3-puncta formation. However, our results indicate that the LC3-positive vesicles generated in response to the toxin are not acidic and non-degradative compartments, suggesting that autophagosome maturation is inhibited by Hla. Interestingly, we have found that Hla-induced autophagy is independent of the PI3K/Beclin 1 complex, since the toxin effect was not prevented by silencing Beclin 1 or by the classical PI3K inhibitors 3-methyladenine and wortmannin. We have recently identified some of the essential molecules of the signaling pathway involved in this "non canonical" autophagic response induced by the toxin.

CB-C17

LYSOSOMES PARTICIPATE IN EARLY STEPS OF TL(III)-MEDIATED APOPTOSIS OF PC12 CELLS

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Previously, we demonstrated that the exposure of rat pheochromocytoma (PC12) cells to Tl(I) or Tl(III) (10-100 ìM) decreased cell viability through the activation of mitochondrial (Tl(I) and Tl(III)), or extrinsic (Tl(III)) pathways of apoptosis. In the present work, we investigated if Tl(III) could be incorporated into cells following the route of iron uptake and damage lysosomes. Tl(III) caused a marked lysosomes acidification, effect that depended on serum or transferrin presence in the medium. Longer incubations (18 h) permanently damaged lysosomes, evidenced from the impairment of acridine orange uptake and the release of cathepsins B and D. Cathepsins mediated the cleavage of pro-apoptotic protein BID, involved in mitochondrial damage and in the triggering of the intrinsic pathway of apoptosis. Cells preincubation with pepstatin A (cathepsin D inhibitor) partially prevented Tl(III)-supported caspase 3 activation, effect that was not observed in cells preincubated with E64d (cathepsin B inhibitor). Caspase 3 activation in Tl(I)-treated cells was not affected by these inhibitors. These experimental results support the role of lysosomal uptake of Tl(III) and its involvement in the early steps of Tl(III)-mediated PC12 cells apoptosis.

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CB-C18

COMPLEX I SYNDROME IN RABBIT HEART SUBMITTED TO ISCHEMIA-REPERFUSION. EFFECT OF ADENOSINE

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Isolated and perfused rabbit hearts were exposed to 15 min ischemia and 5 or 30 min reperfusion. Ischemia-reperfusion (I/R) decreased left ventricle O_2 consumption (46%) and malate-glutamate supported state 3 respiration (32%) with no changes in state 4 respiration. Mitochondrial complex I, but not complexes II and IV activities was 28% lower after I/R. Mitochondrial NO production decreased 28%, with a diminished mtNOS functional activity using malate-glutamate as substrate, without modifications in mtNOS expression. State 4 H_2O_2 production with malate-glutamate was increased by 77% from controls after 30 min of reperfusion. Mitochondrial phospholipid oxidation products (TBARS) were increased (42%), whereas protein oxidation was slightly increased. In addition, there was a 50% increase in tyrosine nitration of the mitochondrial proteins. Rabbit heart I/R leads to a condition of dysfunctional mitochondria, named “complex I syndrome”, with decreased O_2 uptake and complex I activity and increased H_2O_2 production, oxidation products content and protein nitration. Supplementation with adenosine in the Krebs-Henseleit perfusion solution attenuated post-ischemic ventricular dysfunction and protected the tissue from the mitochondrial alterations observed, this could be attributed to preservation of ATP levels associated to a regulation of the cardiomyocytes Ca^{2+} concentration

ST-C01

EFFECTS OF LIPIDS OVER DESK, THE *Bacillus subtilis* THERMOSENSOR

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The DesK thermosensor from *B. subtilis* belongs to the group of histidine kinases (HK) with sensing mechanisms linked to the transmembrane segments (TMS). It is a polytopic protein containing five TMS and controls the phosphorylation state of DesR, which in turn regulates the expression of the cold shock-inducible des gene which codifies the Δ5-Des. As temperature sensing is essential for the survival of living cells, a major challenge is to understand how thermal information is processed by a biological thermometer to restore cellular functions. Since DesK regulatory function is defined by interaction with the bilayer, it represents an ideal system for studying the molecular mechanism of thermodetection and signal transduction.
We analyzed the effect of some aspects of membrane lipid structure including thickness, phase behavior and phospholipids head group on the autokinase activity of full-length DesK. To this end, we reconstituted DesK *in vitro* in a series of phosphatidylcholines containing monounsaturated or saturated fatty acyl chains of different lengths and also in lipids with other head groups. The three membrane aspects analyzed affected the autokinase activity of the HK. Thus, the results presented here suggest that transmission of the signal across the membrane by DesK is regulated by both, the properties of the acyl chains and the polar head of membrane phospholipids.

ST-C02

CHROMATIN RELAXATION TRIGGERS INDUCTION OF CDK INHIBITOR p19INK4d REGARDLESS OF DNA DAMAGE

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It is widely reported that chromatin suffers rearrangements when DNA damage is generated. However, the role of chromatin in the DNA damage response of a cell is not yet well understood. p19INK4d, a member of the INK4 cell cycle inhibitors, is induced after endogenous DNA damage. However, when exogenously DNA damaged molecules are introduced into the cell, this induction is not observed, suggesting that the actual trigger of p19 induction should be something else other than DNA damage itself. Here we show that three different chromatin relaxing agents (chloroquine, trichostatin and hypotonic medium) trigger transcriptional induction of p19, and that this happens even in the absence of DNA damage. Interestingly, this induction was shown to depend upon ATM, Chk1 and Chk2 kinase activity, as is the case for endogenous DNA damage p19 induction. In addition, the induction of p19 elicited by chromatin relaxation is also mediated by the transcription factor E2F1. Notably, when cells were exposed to both situations simultaneously, no increase in p19 mRNA levels was observed with respect to the levels obtained with one condition alone, suggesting that DNA damage and chromatin relaxation share the signaling pathway. The specific induction of p19 by chromatin structure alterations supports a role for p19 as a sensor of DNA damage

MI-C01

CcpA REPRESSES THE DIVERGENT *cit* OPERON OF *Enterococcus faecalis* THROUGH MULTIPLE *cre* SITES

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In *E. faecalis* the genes encoding the enzymes involved in citrate metabolism are organized in two divergent operons, *citHO* and *oadHDB-citCDEFX-oadA-citMG* (*citCL*). Both operons are specifically activated by addition of citrate to culture medium. We observed that the *citHO* and *citCL* promoters are repressed in the presence of sugars transported by the PTS, strongly suggesting a Carbon Catabolic Repression (CCR). Moreover, our results showed that repression was relieved in a ccpA-deficient *E. faecalis* strain indicating that the pleiotropic transcriptional factor CcpA was involved in the regulation. Sequence analysis of the proximal intergenic region of the cit operons revealed the presence of three putative catabolite responsive elements (*cre*). In this work, we analyzed the role of CcpA and *cre* sites in the regulation of citrate metabolism. By gel mobility shift assays (EMSA) we confirmed that *E. faecalis* CcpA is capable of binding to each *cre* site. Furthermore, we analyzed the effect of its cofactor, HPr. The results indicated that unphosphorylated HPr showed a minor increase in CcpA affinity for the *cre* sites, whereas serine-phosphorylated HPr (P-Ser-HPr) produced a significant increase in affinity. Thus, in *E. faecalis* the binding of the P-Ser-HPr-CcpA complex to the *cre* sites repressed the expression of the activator CitO and also inhibited the expression of the catabolic genes.

MI-C02

A UNIQUE *cre* SITE CONTROLS TWO MALATE UTILIZATION OPERONS IN *Enterococcus feacalis*

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Enterococcus faecalis is able to use malate in aerobic and anaerobic conditions. The *mae* locus of *E. faecalis* consist of two putative divergent operons, *maePE* and *maeKR*. The first operon codifies for the malic enzyme (MaeE) and for the malate transporter (MaeP). The second operon encodes a two component system composed by the membrane kinase sensor (MaeK) and the transcriptional regulator (MaeR). In a previous work we showed that inactivation of *maeR* or *maeE* caused complete loss of malate utilization in *E. faecalis*. In this communication we demonstrated that both operons are specifically induced in response to the presence of malate in the growth medium and are repressed by the addition of PTS sugars. We performed transcriptional and mutagenesis analysis and established that a unique *cre* site (*c*atabolite- *r*esponsive *e*lement) located between the -14 and -27 from *maePE* and -76 and -89 from *maeKR* transcriptional start sites governed the glucose repression of both operons.

MI-C03

THE Rcs SIGNAL TRANSDUCTION PATHWAY IS TRIGGERED BY ECA STRUCTURE ALTERATIONS IN *Serratia marcescens*

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S. marcescens is a gram negative enteric bacterium that acts as an opportunistic pathogen. Despite numerous reported *S. marcescens* infections and the emergence of antibiotic-resistant strains, the virulence mechanisms of this organism are unresolved. The enterobacterial common antigen is a highly conserved exopolysaccharide in enteric bacteria which role remains uncharacterized. Previously, we have demonstrated that disrupting the integrity of the ECA biosynthetic pathway imposed severe deficiencies to *S. marcescens* motile capacity. We show that alterations in the ECA structure activate the Rcs phosphorelay, which results in the repression of the flagellar regulatory cascade. In addition, a detailed analysis of *wec* cluster mutant strains, that provoke the disruption of the ECA biosynthesis at different levels, suggests that the absence of the periplasmic ECA cyclic structure could be the signal detected by the RcsF-RcsCDB phosphorelay. We also identify SMA1167 as a member of the Rcs regulon, and show that high osmolarity induces Rcs activity in this bacterium. These results widen the knowledge about the identity of envelope stress signals that induce the Rcs regulatory system. They also provide a new perspective to understand the phylogenetic conservation of ECA among enterobacteria and the basis for virulence attenuation detected in *wec* mutant strains in other pathogenic bacteria.

MI-C04

REGULATION OF UNSATURATED FATTY ACIDS BIOSYNTHESIS BY GROWTH TEMPERATURE IN *Bacillus*

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Bacilli cells respond to a decrease in growth temperature by introducing double bonds into fatty acids by desaturases enzymes. Our results show that *B. cereus* and *B. licheniformis* UFAs proportion in their membrane lipids increase as the growth temperature decrease. To analyze the desaturase expression upon a temperature shift we assayed the â-galactosidase activity of *des-lacZ* fusions in cells of *B. subtilis*. When cultures grown at 37ºC were shifted to 25ºC the levels of â-galactosidase increased for both Ä5-desaturases whereas the Ä10-desaturase of *B. cereus* showed a constitutively expression. Temperature response has been characterized in *B. subtilis*, where the Ä5-Des expression is tightly regulated by DesK-DesR regulatory system. The analysis of the genome of *B. licheniformis* and *B. cereus* reveals the existence of two genes encoding proteins that share a high level of identity with DesK and DesR only in *B. licheniformis*. We investigated the expression of *des-lacZ* fusions in strains of *B. subtilis des-, desK-, desR-*, and we found that the levels of â-galactosidase activity at 25ºC of Ä5-Des of *B. licheniformis* was very low while the expression of the others Des-fusions were not affected. Our results indicate that Ä5-Des of *B. licheniformis* shares a common regulatory mechanism with *B. subtilis* but a different pathway controls UFAs biosynthesis in *B. Cereus*.

MI-C05

CHARACTERIZATION OF THE PhoP/PhoQ SYSTEM IN *S. marcescens* AND ITS ROLE IN PATHOGENESIS

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The opportunistic human pathogen *Serratia marcescens* is a significant cause of hospital-acquired infection. However, few reports have explored in depth the mechanisms that contribute to *S. marcescens* pathogenesis within its host.

In this work, we analyzed the role of the PhoP/PhoQ two component system in *S. marcescens* virulence. We demonstrated that *phoP* mutant exhibited impaired growth in minimal broth limited in Mg^{2+}, in acid pH, and showed increased sensitivity to antimicrobial peptides than the wild type strain. In addition, â-galactosidase assays revealed that *phoP* transcription was modulated by the Mg^{2+} and Polymyxin B levels.

Furthermore, the *phoP* strain was diminished in the survival inside epithelial cells, despite the fact that confocal microscopy analysis revealed that *phoP* mutant was able to enter and multiply into vacuoles in epithelial cells similarly to the wild type strain. Nevertheless, and in contrast to wild type *S. marcescens*, the vast majority of the vacuoles harbouring the *phoP* mutant strain colocalized with the acidic compartment marker Lysotraker, suggesting that they could not evade lysosome fusion and degradation.

Our results indicate that the PhoP/PhoQ system is crucial in *S. marcescens* virulence, regulating processes that are involved in supporting intracellular survival and lysosomal fusion avoidance inside the host epithelial cells.

MI-C06

A NOVEL PATHWAY FOR PROTEIN LIPOYLATION IN *Bacillus subtilis*

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Lipoic acid (LA), a covalently bound cofactor, is essential for the function of several key enzymes involved in oxidative and single carbon metabolism. The current model for protein lipoylation involves two pathways: one in which exogenous LA is transferred to apoproteins in a process mediated by LA ligase A (LplA), and an endogenous one, that involves LipB, which transfers octanoic acid to target proteins. These octanoylated domains are converted into lipoylated derivatives by lipoyl synthase (LipA).

We have previously shown that *B. subtilis* is able to synthesize LA, and if exogenously provided, ligate it to apoproteins. We have also demonstrated that LipL and LipM are essential for the endogenous lipoylation pathway. However, the role of each of these proteins was not clear.

In this work we performed physiological and biochemical characterization of different LA auxotrophic mutants and used *in vitro* assays that allowed us to conclude that *B. subtilis* protein lipoylation is carried out through a novel mechanism. This pathway involves the sequential action of LipM, LipL and the glycine cleavage system H protein, which acts as a previously undescribed lipoyl/octanoyl carrier.

Notably, in *B. subtilis* four proteins, LipM, GcvH, LipL, and LipA, are essential for the endogenous protein lipoylation pathway, instead of the two-protein model of *E. Coli*.

MI-C07

ArgP AND Lrp REGULATE TRANSCRIPTION OF *lysP*, THE SPECIFIC LYSINE PERMEASE OF *E. coli*

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Expression of *lysP*, encoding the lysine specific transporter LysP in *Escherichia coli*, is regulated by the exogenous available lysine concentration. In this study, the LysR-type transcriptional regulator ArgP was identified as activator of *lysP* expression. At concentrations of lysine 25 µM, *lysP* expression was shutoff and phenocopied an *argP* mutation. Purified ArgP-His_6 bound to the *lysP* control region at a sequence containing a conserved T-N_{11}-A motif. Its affinity increased in the presence of lysine, but not in the presence of the other known co-effector arginine. *In vivo* data suggest that lysine-loaded ArgP and arginine-loaded ArgP compete for each other at the *lysP* promoter. Lysine-loaded ArgP could prevent *lysP* transcription at the promoter clearance step similarly as described for the lysine-dependent regulation of *argO* [Laishram, R. S., and J. Gowrishankar. 2007. *Genes Dev*. 21:1258-1272]. DNA-affinity purification identified the global regulator Lrp as a second protein that binds to the *lysP* promoter/control region. An *lrp* mutant had reduced *lysP* expression in absence of external lysine. Electrophoretic mobility shift assays (EMSA) corroborated binding of Lrp to the *lysP* control region. These results fit with a model in which ArgP is a major regulator of *lysP* expression, while Lrp modulates transcription of this gene under lysine-limiting conditions.

MI-C08

ADAPTABILITY TO BIOFILMS IS HASTENED IN *P. aeruginosa* MUTATORS BY PHENOTYPIC DIVERSIFICATION

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The aim of the present study was to investigate the structure, phenotypic diversification and competitiveness of *Pseudomonas aeruginosa* mutator biofilms. For this purpose, the environmental strain Hex1T and its respective *mutS* mutant, Hex1TMS, were grown in flow-cell biofilms and inspected by confocal laser scanning microscopy. Structural analyses revealed that biofilms formed by Hex1TMS strain were similar to those formed by Hex1T strain, displaying the characteristic mushroom-shaped structures of *P. aeruginosa* biofilms. However, mutator biofilms showed significantly increased phenotypic diversity in comparison to wild type biofilms, with a Shannon-Weaver Index *(H)* of 0.31 and 0.03, respectively ($P<0.0001$). Normal, giant, small, wrinkly, transparent and brown pigmented were among the different morphotypes found. Competition experiments between Hex1T and Hex1TMS strains with initial ratios 1:1 and 1:100 showed that the hypermutable strain had increased fitness after 6 days, indicating a potential adaptive advantage of mutators during biofilm mode of growth. The fact that this increased competitiveness was afforded by phenotypic diversification was confirmed by competing the principal evolved morphotypes with the parental *mutS* strain. These results give further evidences of how mutator bacteria can accelerate the speed of evolution of a bacterial population.

MI-C09

A NOVEL *B. suis* ADHESIN IDENTIFIED BY PHAGE DISPLAY MEDIATES BACTERIAL ADHESION TO EPITHELIAL CELLS

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Brucella is an intracellular pathogen responsible for the zoonotic disease called brucellosis. Adhesins are important virulence factors in bacterial infections. However, little is known about these molecules in brucellaceae. In an attempt to identify genes coding for adhesins to some key components of the host extracellular matrix molecules, a phage display library of fragmented genomic DNA from *B. suis* was prepared in pG8SAET vector. Three genes encoding putative Fibronectin-binding molecules were identified by using panning procedures. Binding specificity to fibronectin was confirmed by ELISA and panning experiments for all candidates. One of these candidates, designated BmaC, belongs to the type I autotransporter protein family. Phages displaying fusions to BmaC peptides were able to adhere to culture cells and blocked the binding of *B. suis* to HeLa cells in a dose-dependent manner. A deletion mutant in the *bmaC* gene was obtained, and adhesion capability in this strain was determined. In co-infection experiments the wild type strain out-competed the *bmaC*-mutant and infections of epithelial cells demonstrated that *bmaC* contributes to adherence to the host tissues. Biochemical and microscopy studies suggest that BmaC is a surface-exposed adhesin, localized in the outer membrane of the bacteria. BmaC may have a crucial role in the interaction of *B. suis* with its host.

MI-C10

Rhodobacter sphaeroides* CHEMORECEPTOR, McpH. EXPRESSION AND FUNCTIONAL ANALYSIS IN *E.coli

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Chemoreceptors (MCPs) are transmembrane proteins responsible for sensing environmental stimuli and transducing them to bacterial flagellar motors.
MCPs are classified into seven classes, according to the length of their highly conserved coiled coil cytoplasmic domain. *E. coli* chemoreceptors, belonging to the 36H class, are organized in trimers of dimers composed by dimers of different specificities.
In order to assess whether functional interactions within the signaling complex and higher order MCP organization represent conserved features, we expressed heterologous receptors in E. coli cells.
Here we report cloning and expression of McpH, a putative organic acid receptor from *Rhodobacter sphaeroides* that belongs to the 34H class.
McpH was able to generate clockwise flagellar rotation when expressed as the only receptor in *E. coli* cells, indicating its ability to form active signaling complexes with the histidine kinase CheA and the coupling protein CheW.
Moreover, McpH was able to form mixed trimers of dimers with *E. coli* MCPs as indicated by *in vivo* crosslinking assays.
Taken together, these results suggest that MCPs from different classes share their higher order organization and functional interactions with other chemotaxis proteins.

MI-C11

***Brucella abortus* OXYGEN SENSING: THE PrrB/PrrA AND NtrY/NtrX TWO COMPONENT SYSTEMS**

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Bacterias of the *Brucella* genera are facultative intracellular pathogens that produces abortion and infertility in animals and a febrile illness in humans. The pathogenicity of these bacterias is in part due to its ability to adapt to harsh conditions like microaerobic environments. The aim of this study is to elucidate the mechanisms by which *Brucella* can sense and adapt to low oxygen tension. We report the characterization of two *B. abortus* two-component system mutant strains: *prrB::Km* and *ntrY::Km.* The PrrB/PrrA system has been characterized as a redox-sensing system in other bacterias and is conserved in *Brucella.* The NtrY/NtrX system sense an unknown signal and regulates nitrogen fixation and metabolism in *A. Caulinodams.*
Brucella prrB::Km and *ntrY::Km* strains grow slowly than wild type strain in in vitro culture conditions. Infection of macrophages and mice is affected in the NtrY mutant but not in the PrrB mutant. Moreover, the NtrY expression is increased in anaerobic growth conditions and its expression is diminished in absence of PrrB, showing a correlation between both systems. In agreement, the in vitro growth of a double mutant *ntrY/prrB* genes is severally affected, in particular in microaerobic conditions. These results suggest that in *Brucella* NtrY/NtrX and PrrB/PrrA two-component systems are coordinately acting to adapt to different enviromental conditions.

MI-C12

THE ROLE OF GumE IN POLYMERIZATION OF THE *Xanthomonas campestris* EXOPOLYSACCHARIDE XANTHAN

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Xanthan is a linear cellulosic â(1,4)-D-glucose polymer with trisaccharide side chains. Normal xanthan produced by wild type *Xanthomonas campestris* has an average molecular length of 0.9 ì m (around 2,500 pentasaccharide repeat units). Biosynthesis and assembly of xanthan requires the enzymes GumB to GumM. We have already shown that *gumB*, *gumC*, and *gumE* mutant strains did not produce xanthan, but synthesized the lipid-linked repeat units. Longer xanthan molecules were obtained by overexpression of GumB and GumC. This increase in molecular length correlated with the increased viscosity observed. Here we studied GumE, a 432 amino acid protein. Hydropathy plotting of GumE showed a high degree of hydrophobicity, with ten transmembrane segments and two major hydrophilic loops, similar to other Wzy polymerases. A 507-bp deletion within *gumE*, removing the two hydrophilic loops and the three transmembrane segments between them, abolished xanthan biosynthesis. The full-length *gumE* expressed in trans fully restored xanthan production of *gumE* mutant strain. Overexpression of GumE in the wild-type background caused a significant decrease in xanthan viscosity (around 40-60%) without changing the total amount of polymer produced. This effect was attributed to a shortening of the polymer chains. These results suggest that the levels of GumE, GumB, and GumC modulate the chain-length of xanthan.

MI-C13

INSIGHTS INTO THE CONFORMATION AND FUNCTION OF RapA2 A CALCIUM BINDING ADHESIN FROM *R. leguminosarum*

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R. leguminosarum develops a biofilm in minimal medium that depends on the acidic exopolysaccharide (EPS) and proteins secreted by the PrsDE type I secretion system. RapA1, a substrate of PrsDE, is a 25 kDa Ca^{2+} binding protein. It is composed of two homologous domains proposed as lectin domains that recognize the EPS. RapA1 was termed an adhesin since it can agglutinate rhizobial cells. Our aim was to characterize RapA2 from *R.l.* bv. *viciae* strain 3841 and seek out its role in biofilm formation. We performed homology modelling of RapA2 and found structural similarity to eukaryotic cadherins, involved in Ca^{2+} dependant cellular interactions. This observation was confirmed analyzing RapA2 by CD spectroscopy. RapA2 is composed of â-sheet elements and its thermal stability relies on Ca^{2+} binding. Despite these similarities, we showed by light scattering that RapA2 is not able to dimerize, a property essential for cadherin adhesive activity. Overexpression of homologous RapA1 in strain 3841 disrupted tight cell-cell interactions within the biofilm, which argues against a role in adhesion. Moreover, by means of a modified ELISA assay, we found that RapA2 interacts with the EPS indirectly by binding to EPS-associated proteins. Our results suggest that RapA2 may associate with other protein partners, forming complexes involved in the dynamics of the extracellular matrix of the biofilm.

MI-C14

RHOMBOID PROTEASES DISPLAY UNIQUE DOMAIN COMBINATION AND NOVEL FUNCTIONS IN *Haloarchaea*

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Rhomboids are highly specific intramembrane proteases which are widely conserved in living organisms. Even though they are present in all archaeal genomes sequenced so far, nothing is known on the biology of rhomboid proteases in this domain of life. The aim of our work is to characterize rhomboid proteases in haloarchaea. Mining of haloarchaeal databases showed that all sequenced genomes encode rhomboid protease homologs, some of these contain a canonical six transmembrane segment (TMS) topology whereas others have unique features including extra TMSs or an N-terminal AN-1 Zn-finger motif. In the model haloarchaeon *Haloferax volcanii* the Zn-finger rhomboid homolog is encoded by the *rho2* gene and it is predicted to form and operon with a putative endonuclease V (*nfi*), potentially involved in DNA repair. By means of RT-PCR we observed that *rho2* is expressed at different growth stages in *H. volcanii* and that it is transcriptionally linked to *nfi*. We generated a knock-out mutant of the *rho2* gene which did not show a differential phenotype in standard growth conditions. However, the mutant strain displayed impaired ability to recover from UV irradiation in minimal medium. Altogether these results show that haloarchaea encode rhomboid proteases with conserved as well as unique features which may play novel physiological roles including DNA repair.
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MI-C15

CHARACTERIZATION OF CELLULOLYTIC BACTERIAL STRAINS FROM NATIVE FOREST SOIL FROM MISIONES, ARGENTINA

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The use of lignocellulosic biomass for second generation biofuels requires optimization of the biochemical conversion of cellulose to its fermentable components. The objective of this work was the isolation and characterization of cellulolytic bacterial strains from native forest soil. Soil sample was obtained from a native forest area in Misiones, Argentina. The presence of cellulolytic microorganisms was determined by most probable number assay (MPN). From those samples with higher cellulose degrading activity, bacteria were isolated by successive streak out in defined media with cellulose as sole carbon source. Forty-nine colonies with cellulolytic activity were identified by a degradation halo on carboxi-methylcellulose plates, visualized by congo red staining. Total DNA was obtained from each positive colony and 16s rDNA was amplified with eubacterial specific primers, fD1 and rD1. Amplification products obtained were sequenced and the resulting sequences were compared by Ribosomal Database Project (RDP) and Genebank NCBI databases, identifying mainly *Acinetobacter* followed by *Pseudomonas* and other less represented bacterial families. This study showed the presence of a diversity of cellulolytic microorganisms in native forest soil from Misiones which indicates that this could be an attractive source for the bioprospection of novel enzymes for cellulose degradation.

MI-C16

GENERATION OF THE FIRST AGRICULTURAL SOIL REFERENCE METAGENOME BY NEXT-GENERATION SEQUENCING

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Soil biodiversity is essential for soil nutrition and quality but 90% of soil microorganisms remain unknown. Previous studies have shown that microbial function varies with soil type and responds to soil management. We extracted total DNA from soil collected in four different sites of Argentinean Pampa Region and under three different types of agricultural management, No-Till Farming (NTF), Conventional Tillage (CT) and undisturbed soil (US) to sequence using GSFLX 454. We generated a 4.4 gigabases (Gb) and an over 10 million reads dataset under the name of PAMPA dataset. We created a bioinformatics pipeline to manage large amount of information using FragGeneScan, BlastP and Megan. Simultaneously, we used the online tool MG RAST to process the dataset. Using our reference metagenome, we showed that agricultural soils are enriched with the phylum Acidobacteria when compared with undisturbed soils and present high abundance of metabolisms related with ton and toll transport system, motility and chemotaxis. In contrast, we found that overall NTF and US soils behave similarly at taxonomic and functional level, suggesting that there is no such dramatic impact of agriculture on soil biodiversity as previously thought. To our knowledge, this is the largest soil metagenomic dataset produced and the first study comparing soil biodiversity on NTF, CT and US using Whole Genome Shotgun.

MI-C17

ALKANE MONOOXYGENASE GENE DIVERSITY IN SUBANTARCTIC MARINE SEDIMENTS

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Culture independent methods were used to characterize bacterial populations associated with aliphatic hydrocarbon biodegradation in cold marine environments, using functional genes as targets. Alkane monooxygenase (*alk*B) gene libraries were constructed using DNA extracted from sediments of a chronically-polluted site in Ushuaia Bay, Tierra del Fuego. PCR reactions were carried out using degenerate primers in order to cover a wide range of *alk*B gene types. More than 250 clones were analyzed by RFLP, and representatives of each restriction pattern were sequenced. Phylogenetic analysis revealed a high diversity of *alk*B genes, including novel divergent sequences as well as members of all known variants of *alk*B genes. Particularly, two groups of genes moderately related to the genus *Alcanivorax*, an obligate oil degrading marine bacterium, were highly frequent in libraries from samples obtained in 2006-2008, suggesting that this important group is a stable member of the microbial community at this site. Moreover, these gene variants were related to the ones previously found in *Alcanivorax* isolates from northern Patagonia, indicating they are widespread in the region. Currently, the effect of crude oil exposure on *alk*B gene diversity is being studied using an experimental system, to test the effect of the addition of nutrients, a commonly used bioremediation treatment.

MI-C18

LARGE-SCALE PROTEOMIC ANALYSIS OF SUMOYLATION TARGETS IN *Trypanosoma cruzi*

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SUMOylation is a relevant protein post-translational modification in eukaryotes. The C-terminus of proteolytically-activated SUMO is covalently linked to a Lys of the target by an isopeptide bond, by a mechanism that includes an E1-activating enzyme, an E2-conjugating enzyme, and transfer to the target, sometimes with the assistance of a ligase. The modification is reversed by a protease, also responsible for SUMO maturation. A number of proteins have been identified as SUMO targets, participating in the regulation of cell cycle progression, transcription, translation, ubiquitination, and DNA repair. We have reported that the orthologous genes corresponding to the SUMOylation pathway are present in *T. cruzi*, that the SUMOylation system is functional in the parasite, and defined the requirements for TcSUMO maturation and conjugation. To identify SUMOylation targets and get an insight into their physiological roles we generated transfectant epimastigote lines expressing a double-tagged TcSUMO, and SUMOylated proteins were enriched by tandem affinity chromatography. Over 200 proteins were identified as potential SUMO targets by 2D liquid chromatography-mass spectrometry. Proteomic studies in other organisms have reported that orthologues of *T. cruzi* putative SUMOylated proteins are similarly modified. So far, we have biochemically validated metacaspase 3 as SUMOylation substrate

MI-C19

STUDIES ON HEME TRANSPORT AND METABOLISM BY *Trypanosoma cruzi*

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Trypanosoma cruzi, the etiologic agent for Chagas´ disease, has requirements for several cofactors, one of which is heme. Because this organism is unable to synthesize heme, a prosthetic group for several heme-proteins, it must be acquired from the environment. After that, heme is transported and inserted into the target proteins, which are available throughout different subcellular compartments (included the respiratory chain complexes in the parasite mitochondrion).
We are interested into elucidate as to how heme is imported and distributed in *T. cruzi*, specially focused in its traffic to the mitochondrion. As a first approach to understand heme uptake processes, we designed and optimized conditions to study the biochemical properties of heme transport using a heme fluorescent analog. We observed that heme import is mediated by a membrane active transporter, depending on ATP and sensitive to cation gradients. Once in the mitochondrion, this cofactor has to be inserted into different heme-proteins. We identified and characterized the codifying sequences that encode the *T. cruzi* enzymes involved in heme A biosynthesis, the essential cofactor of cytochrome c oxidase. We analyzed the mRNA level of these cds (*TcCOX10* and *TcCOX15*) and postulated that the observed changes could be a form of regulation reflecting differences in respiratory requirements at different life stages.

MI-C20

Trypanosoma cruzi "HIGH MOBILITY GROUP B" PROTEIN IS A CHROMATIN ARCHITECTURAL FACTOR

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High mobility group B (HMGB) proteins are abundant non-histone chromatin proteins that play important roles in the execution and control of many nuclear functions. TcHMGB is an HMGB family member from *Trypanosoma cruzi*, the causative agent of Chagas disease. TcHMGB has two HMG box domains, like mammalian HMGBs, but lacks the typical C-terminal acidic tail and, instead, bears a 110 amino-terminal domain that seems to be unique of kinetoplastid HMGBs. Despite these differences, TcHMGB maintains HMG box architectural functions: it binds distorted DNA structures like cruciform DNA and it is also able to bend linear DNA. TcHMGB is present in the nucleus in all *T. cruzi* life cycle stages. The protein content, however, is not constant, being higher in replicative forms (amastigotes and epimastigotes) than in the non replicative trypomastigote form. This is consistent with the lower heterochromatin content and higher transcription rates previously reported in replicative forms. In contrast to H1 histone, HMGB proteins cause chromatin to be more relaxed, and concomitantly more accessible to transcription factors, remodeling complexes and other nuclear proteins. In this context, TcHMGB may alter chromatin structure between life cycle stages, but also in different genome regions, taking part in epigenetic control of nuclear processes like transcription, recombination, replication and repair.

MI-C21

SELECTING A *Zygosaccharomyces rouxii* STRAIN WITH HIGH POTENTIAL FOR CONCENTRATE GRAPE JUICE SPOILAGE

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Argentina's grape production is mainly devoted to the industry, where wine and grape juice concentrate are the two major types of commercial products. Because of its high sugar concentrations and low pH, juice concentrates are more stable than other juice products, and therefore they only support the development of a reduced number of microorganisms. Previous works have identified *Zygosaccharomyces rouxii* as the main spoilage yeast present in concentrate musts of Argentina. Nevertheless, using different strains of this same species, we have found a significant variation of the growth behaviour according to the strain analyzed. The aim of this work was to select a native strain of *Z. rouxii* that presents the proper growth parameters, in order to be used for future tests of concentrate grape must self life estimation. Three strains of *Z. rouxii* were evaluated in 68 Brix concentrate grape must. The yeast population was monitored by viable count and turbidity and growth parameters were estimated by the Baranyi model. We found differences for the three assayed strains with respect to the growth rate, lag phase as well as the starting point of the must spoilage (7, 11 and 15 days). Finally, the strain MC9 showed the proper growth parameters (absence of lag phase, and the fastest growth rate) and was therefore selected for the future assays of concentrate grape must self life estimation.

MI-C22

DEVELOPMENT OF A MODEL-WINE MEDIA TO EVALUATE *Dekkera bruxellensis* GROWTH IN MIMIC WINE CONDITIONS

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In recent years, *Dekkera bruxellensis* has caused increasingly severe quality problems in the wine industry associated with volatile phenols production. Thus, a better understanding of *Dekkera*'s growth and metabolism will facilitate its control and manipulation, preventing the development of undesirable flavour changes. To examine the impact of several wine compounds on the growth and the formation of volatile phenols, it is very important to have a chemically defined medium that mimics the wine's composition and that is reproducible under experimental conditions. However, the design of such medium is a challenge, considering that *Dekkera* yeasts have very restricted and not well known requirements for growth. The aim of this work was to optimize the biomass production and to evaluate several culture media of different composition for its ability to support the growth of *D. bruxellensis*. For the biomass pre-inoculum optimization, the best result was obtained in presence of wine. We assayed four different wine-like culture media, which included variations in sugar, vitamin, ethanol and nitrogen content. These cultures were inoculated with two distinct strains (CECT 11045 and B11), previously grown and adapted in two different pre-inoculum media. The culture medium containing trehalose, yeast extract, $MgSO_4$, KH_2PO_3 and vitamins, allowed for the best development of the two strains.

MI-C23

MOLECULAR CHARACTERIZATION OF A BENZOQUINONE REDUCTASE GENE IN THE FUNGUS *Beauveria bassiana*

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Tribolium castaneum, a major pest of stored and processed grains, is the most tolerant beetle to the entomopathogenic fungus *Beauveria bassiana*. Glandular alkyl–1,4–benzoquinones (BQ) are the major components of the defensive secretions of *T. castaneum*. In this work we characterized a 1,4–benzoquinone reductase (BQR) gene of *B. bassiana*, and discussed its function on BQ degradation. We also studied the potential antifungal properties of beetle secretions, in order to elucidate their role in the scarce susceptibility of *T. castaneum* to entomopathogenic fungi. The cDNA of BQR consisted of 947 nucleotides and encoded a deduced protein containing 201 amino acids. Neither glycosylation sites nor signal peptide cleavage sites were found, and PSORT analysis suggested a cytosolic localization. Reduced germination and a significant inhibition on *B. bassiana* growth were detected after fungal incubation in culture media containing *T. castaneum* gland extracts or synthetic BQ. Below minimal inhibitory concentration, the BQR gene expression was significantly induced in BQ–exposed fungi. The largest fold induction was obtained after 1–day incubation; with 50–fold and 12–fold induction for 1 and 0.5 ì g/ì l BQ, respectively. Additional studies on BQR would be needed to understand the effect of these toxic compounds, in order to help develop a fungus–based biological control of these beetles.

MI-C24

FIRST COMPLETE SEQUENCE OF HEPATITIS A VIRUS IN ARGENTINA: RECOMBINATION BETWEEN SUBGENOTYPES IA/IB

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Hepatitis A viruses (HAV), members of the Hepatovirus genus within the *Picornaviridae* family, possess an RNA genome of approximately 7500 nucleotides, translated into a polyprotein of 2227 amino acids.

Six different genotypes are described for HAV. Genotype I is the most prevalent worldwide, and sub-genotype IA is the major HAV population in South America.

In this work, we report the complete HAV genomic sequence and the recombination analysis performed on a stool sample obtained in Santa Fé, in 2006, from a sick child. Phylogenetic analysis placed the isolate within sub-genotype IA. A recombination event with sub-genotype IB was identified, involving a portion of the 2C-3A coding region.

This study constitutes the first report of a full-length HAV sequence in Argentina and the third in South America. Identification of recombination between sub-genotypes IA and IB in an Argentinean isolate is of great relevance as there are no reports of sub-genotype IB strains circulating in Argentina.

IA/IB recombination events detected in Argentina and in Uruguay, and the identification of both sub-genotypes in Brazil, are suggestive of increased co-circulation or introduction of recombinant strains. New HAV complete sequence data are required to establish a more consistent genetic relatedness among isolates and the role of recombination in its evolution.

SB-C01

TWO MOLECULAR ENZYME FORMS AND REACTION MECHANISMS FOR THE GLYCOGENIN AUTO-GLUCOPOLYMERIZATION

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The formation of glycogenin (Gn)-bound α-1,4-oligoglucan primer is required for the *de novo* biosynthesis of glycogen (G). Dimeric Gn, as it exists in solution and in the enzyme crystals, was considered to be the molecular form which synthesizes the primer by intersubunit glucosylation mechanism. We have described however that monomeric Gn is also able to catalyze its intramolecular glucopolymerization. In considering which Gn form actually primes G biosynthesis, this might be determined by the polymerization degree (pd) of the Tyr-linked oligoglucan the Gn form can produce, having the size required by glycogen synthase and branching enzyme for further elongation and branching. A pd of 12 was reported for the oligoglucan produced by Gn dimer; however the auto-glucopolymerization extent capacity of the monomer was unknown. Now we determined the glucopolymerization degree of fully autoglucosylated monomeric and homodimeric Gn and of heterodimers formed by mixing 1) a Gn mutant lacking its tyrosine acceptor with a mutant containing the tyrosine acceptor but lacking glucosylation activity, and 2) the wild type enzyme with a mutant which lacked both, glucosylating activity and tyrosine acceptor. The results show that besides the intersubunit glucosylation of Gn dimer, the intramolecular glucosylation of Gn monomer can produce the oligo-glucopolymer primer for G biosynthesis.

SB-C02

TEMPERATURE-MEDIATED SELF-ASSOCIATION OF RAPESEED 2-CYS PEROXIREDOXIN

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Two-Cys peroxiredoxins (2-Cys Prx) constitute a subfamily of peroxidases that under oxidative stress lower the capacity to reduce peroxides while increase a chaperone activity. Although major redox-dependent conformational changes switch the functions of 2-Cys Prx, little is known about the action of noncovalent interactions on these changes. Therefore, we used biochemical techniques to study the effect of heat on the oligomerization and activities of rapeseed 2-Cys Prx. We found that, at temperatures above 50°C, 2-Cys Prx shifts chromophores to more hydrophobic environments with the concurrent increase of hydrophobic surfaces exposed to solvent. Moreover, gel filtration, static- and dynamic-light scattering indicated that the proportion of the covalently linked dimer decreases while concurrently increases the level of oligomers with higher molecular masses. Notably, visualization of negatively stained specimens in transmission electron microscopy revealed that native toroidal structures of the decamer [(A)2]5 shift to the formation of large fibres when the protein is incubated beyond 50°C. Concomitant with these changes, the temperature-mediated transition switched 2-Cys Prx from peroxidase to molecular chaperone. These results uncover a novel noncovalent perturbation that controls the self-association and the associated functions of 2-Cys Prx.

LI-C01

THIACETAZONE REVEALS SYNTHESIS OF NEW MYCOLIC ACIDS IN *Mycobacterium smegmatis*

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Thiacetazone (TAC), an anti-tubercular drug, inhibits growth of *M. tuberculosis* abolishing cyclopropanation of mycolic acids. TAC does not inhibit growth of fast growing mycobacteria such as *M. smegmatis* on solid media, however we detected a slight growth inhibition in liquid medium. Mycolic acid analysis showed that TAC partially inhibited synthesis of the a1-mycolates sub-family in *M. smegmatis* grown at 37°C in the presence of TAC, with accumulation of a novel mycolic acid species. Paradoxically, a related compound was recently found in *M. smegmatis* growing at low temperature and identified as mono-cyclopropanated mycolic acid. Minimum Inhibitory Concentration assays showed that at 20°C TAC affected colony morphology with an accumulation of an unidentified glycolipid; mycolic acid analysis revealed the accumulation of a novel spot with loss of the synthesis of the putative cyclopropanated mycolic acid, suggesting that both spots may be biosynthetically related members of α-mycolate family.
In conclusion, TAC partially inhibits mycolic acids biosynthesis with a greater effect at low temperature, alters morphology by changes in glycolipids profile and modifies the composition of mycolic acid families in *M. smegmatis*, suggesting a differential susceptibility of the methyltransferases, enzymes involved in the genesis of mycolic acids families in this species.

LI-C02

c-Fos PHYSICALLY INTERACTS AND ACTIVATES SPECIFIC POLIPHOSPHOINOSITIDE SYNTHESIS ENZYMES

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The oncoprotein c-Fos, in addition to its well described function as a transcription factor belonging to AP-1 family, associates with the endoplasmic reticulum (ER) and activates key enzymes involved in the synthesis of phospholipids. To attain an overall activation of phospholipid synthesis, only particular enzymatic activities were positively affected. Herein, it was examined which enzymes of the PtdInsP biosynthetic pathway are activated by c-Fos, In order to understand the mechanism of enzyme activation, analysis of protein-protein interactions between c-Fos and different phospholipid enzymes by co-immunoprecipitation and Fluorescence Resonance Energy Transfer (FRET) were performed. It was found that c-Fos specifically associates to and activates the enzymes CDP-diacylglycerol synthase (CDS) and PtdIns4 Kinase II alpha (PI4KIIα), whereas neither association nor activation of Phosphatidylinositol Synthase (PIS) or PtdIns4 Kinase II beta (PI4KIIß) were observed. Finally, it was observed that depending on the cellular content of c-Fos, the channelling of the common precursor PtdOH could be directed towards the Kennedy or to the PtdIns pathway of phospholipid synthesis, suggesting a new mode to balance between these pathways as a function of the immediate requirements of cells.

LI-C03

C-SRC AND TC45 REGULATE C-FOS TYROSINE PHOSPHORYLATION STATE AND PHOSPHOLIPID SYNTHESIS ACTIVATION

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The expression of the proto-oncoprotein c-Fos is tightly regulated responding rapidly and transiently to a plethora of stimuli. c-Fos heterodimerizes with jun proteins to form AP-1 transcription factors that regulate the expression of target genes such as those involved in DNA synthesis. Although described more than 20 years ago, a precise molecular understanding of the participation of AP-1 in complex processes such as proliferation and growth has not yet been achieved. In our laboratory, we have identified an additional activity of c-Fos independent of its AP-1 activity. This is its capacity to associate to the endoplasmic reticulum (ER) and activate lipid synthesis.
In T98G cells, the association of c-Fos to the ER and, consequently, the capacity of c-Fos to activate phospholipid synthesis is regulated by the phosphorylation state of its tyrosine residues #10 and #30. The small amount of c-Fos present in quiescent cells is tyr-phosphorylated, dissociated from the ER membranes and does not activate phospholipid synthesis. However, upon induction of cells to re-enter growth, c-Fos is rapidly induced, found dephosphorylated, associated to ER membranes and activates phospholipid synthesis.
Herein, using in vivo and in vitro experimental strategies, it was found that c-Src is capable of phosphorylating c-Fos tyr residues whereas the phosphatase TC45 (TC-PTP) dephosphorylates them.

LI-C04

Fe^{2+} INITIATED PEROXIDATION OF SONICATED LIPOSOMES MADE WITH RETINAL LIPIDS: EFFECT OF INDOLEAMINES

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Melatonin and its structural analogues display antioxidant activity *in vivo* but their activity in model membranes is not very well known. We have investigated the antioxidant effect of melatonin, 5-methoxytryptamine, 5-OH-tryptophan and N-acetylserotonin on Fe^{2+}-initiated peroxidation of sonicated liposomes made with retinal lipids. Three *in vitro* assays were used: i) conjugated dienes production, determined at 234 nm; ii) TBARS formation, and iii) changes in fatty acids analyzed by gas chromatography–mass spectrometry. All the compounds were evaluated against butylated hydroxitoluene (BHT) which was chosen as a reference because of its high antioxidant capacity. After the addition of Fe^{2+} prompt conjugated dienes production was observed. In presence of increasing concentrations of BHT the start of the reaction was delayed and initial reaction rate was lowered but these factors were not modified in the presence of melatonin or related indoleamines. TBARS starts immediately after addition of Fe^{2+}. BHT delays the emergence of TBARS. Melatonin and its structural analogues have not effect on time of appearance of TBARS. Polyunsaturated fatty acids PUFAs disappear completely after incubation of retinal liposomes with Fe^{2+}. BHT protects PUFAs against oxidative damage. Our data indicate that melatonin and its structural analogues do not possess antioxidant action in this system.

LI-C05

GLYCEROL-3PHOSPHATE ACYLTRANSFERASE2 IS EXPRESSED IN SPERMATOGONIA AND UNDIFFERENTIATED CANCER CELLS

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The first step in the *de novo* glycerolipid biosynthesis is catalyzed by glycerol-3-phosphate acyltransferase (GPAT). Four genes encoding GPATs have been cloned; *Gpat2* encodes a protein mainly expressed in testis. We have previously shown that *Gpat2* overexpression in CHO-K1 cells increased triacylglycerol synthesis and storage, as well as cell proliferation. We quantified *Gpat2* expression by qPCR in 3T3-L1 cells differentiating to adipocytes. Unlike the other GPAT isoforms, *Gpat2* was not induced upon adypocite differentiation. This result is consistent with the *in silico* analysis of transcriptomes, which showed that *Gpat2* was downregulated in different models of cell differentiation. *In situ* hybridization showed that *Gpat2* mRNA was expressed exclusively in mouse spermatogonia and the expression dramatically declines when cells enter meiotic and differentiation phases. *Gpat2,* included in cancer-testis gene database, was detected by RT-PCR in MDA-MB 231 cells, a breast cancer undifferentiated cell line. In *Gpat2* overexpressing cells incubated with 14C-arachidonate we observed an increase in radioactive TAG and a decrese in radioactive PC and PE after 3 h incubation. After 6 and 24 h there was a significant increase in radioactive lysophosphatidylcholine. Our results suggest that some metabolites derived from GPAT2 activity may contribute to cell proliferation and survival.

LI-C06

ROLE OF C/EBP AND SP1 IN THE REGULATION OF CKα AND CCTα EXPRESSION DURING NEURONAL DIFFERENTIATION

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Neuronal differentiation is characterized by neuritogenesis and neurite outgrowth, processes which are dependent on membrane biosynthesis. We previously demonstrated that the production of phosphatidylcholine (PC), the major membrane phospholipid, is stimulated during RA-induced neuronal differentiation in Neuro-2a cells. PC synthesis is promoted by an ordered and sequential activation of choline kinase alpha (CKα) and choline cytidylyltransferase alpha (CCTα). Early after RA stimulation, the increase in PC synthesis is mainly governed by the biochemical activation of CCTα, later, the transcription of CKα- and CCTα–encoding genes is induced.

To elucidate the mechanism by which the expression of CKa and CCTα is induced during RA-induced neuritogenesis, we used promoter reporter assays and EMSAs. These analyses allowed us to postulate that C/EBP induces CKα expression, and Sp1 is involved in the regulation of the basal CCTα expression and in its induction during RA-induced neuritogenesis.

PL-C01

Mutator TRANSPOSON ACTIVATION INDUCED BY UV-B IN *Zea mays*

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The *Mutator* (*MuDR/Mu*) DNA transposon family in maize includes diverse elements; they all share similar ~215 bp Terminal Inverted Repeats (TIRs), but each has unique internal sequences. *MuDR* is the regulatory element for the entire system, and the presence of transcriptionally active *MuDR* elements is required for transposition of the nonautonomous *Mu* elements. *MuDR* encodes two genes, *mudrA* and *mudrB*. *mudrA* encodes the transposase, MURA, and *mudrB* encodes a protein with unknown function. In a previous work, we demonstrated that both *mudrA* and *mudrB* transcripts are expressed at higher levels after an 8h-UV-B treatment, in both *Mutator* active and silencing plants. This transcript increase is accompanied by an increase in histone H3 acetylation and by a decrease in DNA and histone H3 methylation. No changes in the siRNAs were detected. To further evaluate the role of UV-B in activating *Mutator*, we performed Chromatin Immunoprecipitation (ChIP) analysis using polyclonal antibodies specific for MURA to assess the *in vivo* binding capacity of the transposase to its target site in the *Mu* TIRs. We found that UV-B irradiation induces the binding of MURA to *MuDR* TIR in both active and silencing plant leaves. In addition, using plants containing a modified transposon *RescueMu*, we found that at least in *Mutator* active plants, novel excision events occur after 8h UV-B treatments.

PL-C02

Ostreococcus tauri ADP-GLUCOSE PYROPHOSPHORYLASE: INSIGHTS INTO THE ACTIVATOR BINDING SITE

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ADP-glucose pyrophosphorylase (ADPGlcPPase) controls the synthesis of starch in plants and algae. The enzyme from photosynthetic organisms is a heterotetramer, composed of two small (S) and two large (L) subunits, and is mainly activated by 3-phosphoglycerate (3PGA), the first intermediary of the carbon fixation pathway. To study the evolution of structure and function of subunits in the ADPGlcPPase family, we synthesized the genes encoding for the S (*Ota*S) and L (*Ota*L) subunits of the unicellular alga *Ostreococcus tauri*, one of the most ancient eukaryotic species in the green lineage. An interesting feature of the *Ota*L subunit is that a highly conserved Lys residue, present in the *Ota*S subunit, demonstrated to be important for 3PGA binding in the *Anabaena* ADPGlcPPase, is replaced by Arg. In this work, we constructed the mutants *Ota*S_{K443R} and *Ota*L_{R466K} and co-expressed them together or with the corresponding wild type subunits. 3PGA kinetics showed $A_{0.5}$ values of 0.22, 0.58, 1.2 and 2.9 mM for *Ota*S/*Ota*L_{R466K}, *Ota*S/*Ota*L, *Ota*S_{K443R}/*Ota*L_{R466K} and *Ota*S_{K443R}/*Ota*L, respectively. In addition, *Ota*S_{K443R}/*Ota*L displayed the lowest activation fold, thus confirming the importance of the mutated Lys residue for 3PGA activation. Our results suggest that both subunits evolved independently and adopted divergent activation properties associated with metabolic differences present in each organism.

PL-C03

ON THE REGULATION OF TRIOSE-PHOSPHATE METABOLISM BY PROTEIN PHOSPHORYLATION

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Posttranslational modifications, as protein phosphorylation, regulate different processes in plants. In this work we analyzed phosphorylation of two plant cytosolic Ga3P dehydrogenases: the phosphorylating (Ga3PDHase, EC 1.2.1.12), and the non-phosphorylating (np-Ga3PDHase, EC 1.2.1.9) enzymes; which respectively derive triose-P to the synthesis of ATP and NADH or NADPH. We found that, in wheat endosperm, a protein kinase from the SnRK1 family is involved in modification of both Ga3PDHases. Phosphorylation occurs on residues Ser404 (np-Ga3PDHase) and Ser205 (Ga3PDHase), which localize in respective conserved domains that were described as preferred sites for SnRK1 phosphorylation. We purified and characterized the wheat endosperm SnRK1 kinase. The enzyme required Mg^{2+} or Mn^{2+} (but not Ca^{2+}) for activity and it was allosterically inhibited by nearly physiological concentrations of Rib5P and to a lesser extent by Fru1,6bisP and 3PGA. Glc6P (the main effector of spinach leaf SnRK) produced little or no effect. MALDI-TOF analysis evidenced that sucrose synthase copurified with SnRK1, the former also being a target of the kinase action. We hypothesize that phosphorylation of both Ga3PDHases by SnRK1 would occur within a mechanism (specific of heterotrophic tissues) that coordinates carbon metabolism during the stage of reserve carbohydrates accumulation in developing wheat seeds.

PL-C04

PROTEOMIC AND METABOLOMIC ANALYSIS OF ORANGE FRUIT AFFECTED BY HEAT TREATMENT DURING POSTHARVEST

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Heat treatment or curing is a common procedure prior to long time storage of fruits to prevent fungal rots as those caused by *Penicillium digitatum*. In this study we have characterized changes induced by curing on the proteome and general metabolism of orange fruit (*Citrus sinensis* L. Osbeck var. Valencia late). 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 proteins were found to be differentially expressed along the storage period. Identification of these proteins showed 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. GC-MS analysis in sacs and flavedo showed that many metabolites were significantly altered by curing. In flavedo, major differences were found in acid levels while the sugar content found in heat treated samples was higher in both tissues. By this approach, it was possible to define some important points of the global response to curing, providing the first analysis at the molecular level of citrus fruit responses to heat treatment.

PL-C05

STRUCTURE AND FUNCTION OF THE EXON JUNCTION COMPLEX IN *Arabidopsis thaliana*

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The exon junction complex (EJC) is deposited onto mRNAs during splicing and directs post-transcriptional processes in the cytoplasm. In this work, we studied three EJC components in *Arabidopsis thaliana*: AtMago, AtY14 and AtPym. Plants that contain the β-glucuronidase (gus) gene under the control of the respective promoter sequences show expression mainly in meristematic regions. A bioinformatic search allowed the identification of site II motifs (TGGGCC/T) that profoundly affected the expression of the genes when mutated and were recognized by proteins present in nuclear extracts. In addition, over-expression of AtMago or AtY14 in plants produced an increase in the amount of AtPym, as indicated by western blots. In order to determine if these proteins were modified post-translationally, we carried out both in vitro and in vivo phosphorylation assays and we detected that AtY14 and AtPym could be phosphorylated by proteins present in plant extracts. Pull-down assays showed that this modification plays an important role in regulating complex formation between these proteins. Transient transformation assays indicated that the expression of each of the proteins under study produces an increase in the expression of genes that require an intron for maximal expression, suggesting that the exon junction complex participates in intron-mediated enhancement of expression in plants.

PL-C06

ROLE OF NITRIC OXIDE AND PHOSPHOLIPASE D IN STOMATAL CLOSURE AND DROUGHT STRESS

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Nitric oxide (NO) promotes stomatal closure and increases plant tolerance to drought stress. Previously, we have demonstrated the involvement of phospholipase D (PLD) and its product, the lipid second messenger phosphatidic acid in NO signalling during stomatal closure. PLDä is one of the 12 Arabidopsis PLDs which has been involved in dehydration stress responses. However, the role of PLDä in stomatal movement is unknown. In this work we used *pldδ* knock-out null mutants (*pldδ*) to show that PLDä is required for NO, H_2O_2 and abscisic acid (ABA) induced stomatal closure. NO and H_2O_2 production upon ABA treatments in guard cells of *pldδ* plants was comparable to the levels in wild-type (wt). These data suggest that PLDä is downstream of NO and H_2O_2 in ABA-induced stomatal closure. The transcript levels of *RD29A* were analyzed. *pldδ* plants showed higher *RD29A* transcript level compared to wt plants under normal growth conditions. Plants resistant to drought stress have higher level of *RD29A* transcript. In concordance, *pldδ* were resistant to drought as it is indicated by its high relative water content and its low ion leakage compared to wt plants. These results allow us to hypothesize a dual role of PLDä during drought. At early drought stage PLDä participates of the signalling cascade leading to stomatal closure. However, at later stages PLDä participates in membrane degradation.

PL-C07

MECHANISTIC LINKS BETWEEN *KNOXI* AND *CUC* GENES DURING ARABIDOPSIS DEVELOPMENT

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In Arabidopsis, the KNOXI class of transcription factors comprises STM, KNAT1, KNAT2 and KNAT6. These proteins are required to establish and maintain the shoot apical meristem, while the meristem boundaries are determined by CUP SHAPED COTYLEDON (CUC) transcription factors. In many species, these genes interact also during leaf morphogenesis and sculpt organs with different shapes and are regulated by microRNA miR164. While much is known about *CUC1* and *CUC2*, and their quantitative regulation by miR164, mechanistic information about their connection with KNOXI transcription factors is still missing. In this work, we demonstrate that the induction of *STM* caused the up-regulation of *CUC* expression in *Arabidopsis thaliana*. Continuous expression of *STM* in the leaf primordia causes the activation of *CUC1-3*, as well as microRNA MIR164a, which provides a negative feedback-loop by post-transcriptionally regulating *CUC1* and *CUC2*. Detailed promoter studies allowed us to pinpoint relationships between these genes. Furthermore, we found that precise expression of miR164 targets inside the *STM* expression domain is necessary for organ separation during Arabidopsis inflorescence development.

PL-C08

AN ESSENTIAL ROLE FOR NITRIC OXIDE IN AUXIN SIGNALING PATHWAY

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Nitric oxide (NO) is a second messenger implicated in many plant cell signaling events. In particular, NO is a key molecule that operates in the auxin-regulated signaling cascade contributing to root morphogenesis during plant growth and development. S-nitrosylation is emerging as a specific posttranslational protein modification for the transduction of NO bioactivity. Recently, we demonstrated that NO production is induced in IAA-treated Arabidopsis roots. NO is also required for the auxin-induced gene expression and auxin-induced degradation of Aux/IAA repressors. Moreover, NO controls TIR1/AFB2-Aux/IAA protein-protein interaction leading to Aux/IAA degradation and auxin-dependent gene expression. Here, we demonstrate that the auxin receptor TIR1 is S-nitrosylated. Mutations of cysteine residues, Cys140 (C140A) and Cys480 (C480A) in TIR1 protein disrupted its interaction with Aux/IAA repressors. Arabidopsis transgenic lines overexpressing TIR1 protein in *tir1-1* background rescued the normal root sensitivity to auxin. However, when TIR1 C140A was overexpressed, TIR1 functionality was not recovered and seedlings remained resistant to auxin suggesting that S-nitrosylation of this cysteine residue may be critical for auxin signaling. These results open an exciting new field in the physiological regulation of auxin signaling by NO.

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NS-C01

CELL CYCLE INHIBITOR, p19INK4d, AS A POTENTIAL NEURONAL SURVIVAL FACTOR

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The early and wide expression of p19INK4d in the brain and its role in DNA repair suggest that it might be involved in protecting neurons from cell death. The aim of this work was to examine the role of p19 in differentiated SH-SY5Y cells and primary cultures of rat hippocampal neurons subjected to genotoxic agents such as â-amyloid peptid (âA) and neocarzinostatin (NCS). âA and NCS induced p19 expression and phosphorylation. These effects were recapitulated by treatment with a Ca^{2+} ionophore and by increasing the extracellular K^{+} concentration, suggesting that they both involve intracellular Ca^{2+} mobilization. p19 phosphorylation following genotoxic stress was impaired by downregulation of CDK5 by antisense and reduction of its activity through inhibition of calpain, a CDK5 stimulator. This indicated that this process is mediated by CDK5. Experiments using E2F decoy oligonucleotides and reporter assays showed that E2F1 is responsible for p19 induction upon DNA damage. p19 overexpression stimulated DNA repair in neuronal cells treated with âA or NCS, whereas its downregulation had the opposite effect. Consistently, p19 ablation reduced cell viability and caused an increase in caspase 3 activity in hippocampal neurons following genotoxic stress.
These results support a role for p19 as a survival factor that protects neurons from apoptosis induced by genotoxic agents.

NS-C02

MELANOPSIN X DISPLAYS A RADICAL TEMPORO-SPATIAL SWITCH IN EXPRESSION DURING CHICK RETINAL DEVELOPMENT

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Two melanopsin (Opn4) orthologs have been identified, Opn4m and Opn4x. We examined expression patterns of photodetection molecules in chicken retina to define potential roles for both genes. Retinas from embryonic (E) day 4 to 19 or post-hatch day 7 (P7) chicks were used for western blotting (WB) or RT-PCR. WB used Opn4m/x antibodies and RT-PCR was done for the transcription factors Pax6, Brn3 and CRX, the G transducin proteins Gq and á transducin, opsins Opn4m and 4x, rhodopsin and red opsin. Immunohistochemistry was done on E8-19 and P7 retinas using Opn4m/x and cell-specific antibodies. We observed two waves of transcription, one at E4 with expression of Pax6, Brn3, Gq and Opn4m; the second at E7-12 with CRX, á-transducin, rhodopsin and red opsin. Strikingly, Opn4x did not appear till E8. WB confirmed mRNA data for Opn4m/x. Immunolocalization of Opn4 proteins showed that Opn4m was restricted to the ganglion cell layer (GC) at all ages. By contrast, at E8 Opn4x was also limited to the GC, but by E15 its expression was mainly confined to cells in the outer retina, presumably horizontal cells. Results suggest that initial wave of expression could confer early photosensitivity to cells of GC while Opn4x switches expression to horizontal cells that play a role in receptive field construction. Moreover, the data suggest the existence of a new type of photosensitive retinal cells.

BT-P01

EVALUATION OF AN ARTIFICIAL microRNA-MEDIATED STRATEGY AGAINST FOOT-AND-MOUTH DISEASE VIRUS (FMDV)

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FMDV, an icosahedral (+) ssRNA virus, is the etiological agent of the highly contagious foot-and-mouth disease. Current vaccines require 7 days to induce protection, and new tools of control are needed. Our aim was to determine the antiviral potential of an artificial microRNA (amiR)-mediated strategy against FMDV. A 21-nt sequence within 3D region of FMDV-A (3D1A) was chosen as target. BHK21 cells were stably transfected with a plasmid encoding the precursor of an amiR against 3D1A and transgenic cell lines were selected and cloned. Expression of amiR3D1A in cell lines was demonstrated by RT-real time PCR and sequencing. Two amiR$^+$ cell lines were transfected with plasmids pRLUC/3D1A (encoding 3D1A sequence downstream of *Renilla* luciferase) and pFLUC (encoding firefly luciferase). Twenty four h post-transfection, normalized luciferase activity was significantly lower in cells transfected with pRLUC/3D1A than in cells transfected with a control plasmid, whereas it did not decrease in cells transfected with pRLUC/3D1O carrying an homologous FMDV-O target sequence. A growth curve of the highly virulent FMDV/A/Arg2001 strain, as determined by quantitation of intracellular FMDV genomes by RT-qPCR and intracellular virus titration, was similar in amiR$^+$ and BHK21 cells. Although functional, the sole expression of amiR3D1A does not suffice to control replication of FMDV/A/Arg2001 in transgenic cells.

BT-P02

PRODUCTION OF AN INSECTICIDE BACULOVIRUS IN INSECT CELL CULTURES IN TWO BIOREACTORS

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Anticarsia gemmatalis Hübner (Lepidoptera: Noctuidae) is one of the main insect plagues for soybean crops in South America. Its larvae are susceptible to the natural infection with occlusion bodies (OBs) of the *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV), which is also used as a biological insecticide to control that plague. The saUFL-AG-286 cell line has shown to be a potential candidate to develop a process to produce AgMNPV OBs in insect cell cultures, because its susceptibility to AgMNPV infection, adaptability to grow and replicate the virus in low-scale suspension cultures, and ability to proliferate and produce very high yields of AgMNPV OBs in a low-cost serum-free medium. However, the capability of saUFL-AG-286 cells to proliferate and to produce OBs in AgMNPV infected cultures in bioreactors has not been yet evaluated. The aim of this work was to evaluate the growth of saUFL-AG-286 cell cultures in the low-cost serum-free UNL-10 medium in both a mechanically stirred reactor and a concentric airlift reactor. Cultures grew in both reactors with maximum growth rates of 0.029 h^{-1} in the airlift and 0.024 h^{-1} in the stirred reactor. Maximum cell densities were also higher in the airlift reactor. Despite differences in culture behavior and kinetics production, cultures infected with AgMNPV in both bioreactors yielded similar mounts of OBs ($2x10^8$ Obs ml^{-1}).

BT-P03

BACULOVIRUS PRODUCTION IN FETAL BOVINE SERUM DEPRIVED CELL CULTURES

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Anticarsia gemmatalis is a pest in South America`s soybean crops, which could be controlled by the Multinucleopolyhedrovirus of *A. gemmatalis* (AgMNPV). Currently, its commercial production is based on infected larvae. However, the possibility of using modified baculoviruses in Integrated Pest Management programs has stimulated an interest to develop alternative multiplication processes. This study evaluated the AgMNPV production in UFL-Ag-286 cells previously deprived Fetal Bovine Serum. Culture media containing 1% FBS during the previous 48 hours achieved a synchronized condition where 90% of cells were found in G0/G1 stage, showing the presence of non-filamentous actin. All characteristics were estimated from cellular viability tests, cell actin detection trials and flow cytometer cell cycle analysis. AgMNPV production was tested by transcript studies and budded viruses and occlusion bodies yield quantitation. Results showed that the transcript analyses in synchronized cells produced 10 to 50 times more specific transcripts, and the productivity was 9.8 times more in BVs and 3.8 times more in OBs with respect to non-treated cells. UFL-Ag-286 cells previously deprived in FBS showed to be a better host for AgMNPV propagation, improving baculovirus production for its different possible application: bioinsecticide, recombinant protein expression system or gene delivery in mammals.

BT-P04

BACULOVIRUS CORE GENES: TOWARD DEFINING THE MINIMUM BACULOVIRUS GENOME

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Baculoviridae is a large family of insect viruses, mainly investigated because they have a great potential as pest bioinsecticides. Also, they are used in other applications such as protein expression and as vectors for gene delivery in mammals. These viruses have double-stranded, circular and supercoiled DNA genomes (80-180 kbp), encoding between 90 to 180 genes. *Baculoviridae* is subdivided in four genera: *Alphabaculovirus* (Lepidoptera-specific NPVs); *Betabaculovirus* (Lepidoptera-specific GVs); *Gammabaculovirus* (Hymenoptera-specific NPVs); and *Deltabaculovirus* (Diptera-specific NPVs). All members share a common set of genes known as Core Genes, whose amount varies as more baculovirus genomes became available. Currently, there are 57 sequenced genomes which encourage a further revision in the number estimation of *Core Genes*.

Performing comparative computational analysis, we found a common set of genes that seem to be baculovirus key factors for some of the major required biological functions. In addition, we identified the common orthologous genes shared between viruses that infect Lepidoptera, and the equivalent information for those that infect Hymenoptera or Diptera. The phylogenetic relationships for each *Core Gene* were also established. All this information provides the predictive basis for thinking in the generation of baculovirus artificial genomes with minimum gene content.

BT-P05

DEVELOPMENT OF A REVERSE GENETIC SYSTEM FOR THE STUDY OF BACULOVIRUS P74 PROTEIN

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Baculoviridae is a virus family containing members that infect insects from different orders: Lepidoptera, Hymenoptera and Diptera. These pathogens are excellent candidates for biological control of agriculture pests because they specifically infect and kill the host. The large cccdsDNA genomes (80-180 kbp) encode between 90-180 proteins. During infection exist two phenotypes: BVs (Budded Viruses), responsible to systemic infection; and OBs (Occluded Bodies), containing a protective matrix and responsible of per os infection. Each phenotype has different proteins involved in specific host cell recognition: F or GP64 in BVs and PIFs (Per Os Infectivity Factors) in Obs.
P74 protein is one of the 5 recognized PIFs, a transmembrane polypeptide located in virions derived from OBs. With the aim to study its function we designed and developed a reverse genetic strategy based on Bac to Bac system. In particular, we modified the bacmid of AcMNPV by the replacement of p74 gene for polyhedrin; and then, we introduced gfp and 16 p74 chimeric genes constructed from different species viruses: AcMNPV, SeMNPV, HaMNPV and AgMNPV. On the other hand, we constructed fusions between p74 and dsRed to infer the P74 topology. The different recombinant viruses generated in this work were studied by microscopic observations, immune trials and bioassays highlighting the role of each structural P74 domain.

BT-P06

EFFICIENT PRODUCTION OF A KDEL-TAGGED DENGUE VIRUS PROTEIN IN PLANT CELL SUSPENSION CULTURES

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Dengue virus envelope glycoprotein (DV-E) is the antigen associated with immunity induction and it is an effective candidate for the development of a subunit vaccine and a promising antigen for diagnostic kits. As a part of a project to develop a plant-made dengue virus vaccine, we explored the ability of plant cells to produce DV serotype 2 (DV-2) E protein in *Nicotiana tabacum* and *Morinda citrifolia* cell suspension cultures. DV-E cDNA was cloned with a signal peptide at its 5' end and with and without the addition of KDEL endoplasmic retention sequence at its 3' end to analyze its influence in recombinant protein accumulation levels. The expression cassette was sub-cloned into pCAMBIA 1305.2 binary vector and the cell suspension culture transformation was carried out using *A. tumefaciens* LBA4404. The maximum accumulation levels (0,71 ± 0,06 mg DV-E/L) were obtained by tobacco cells at 5 days of culture when KDEL tetrapeptide was fused. It represents 0,3% of the total soluble protein. Its integrity was confirmed by western blot. The recombinant protein was reactive with anti-E monoclonal and polyclonal antibodies. Our results demonstrate for the first time that plant cell *in vitro* cultures represents a low cost expression system suitable for the production of recombinant DV-E protein in which biosafety conditions are guaranteed.

BT-P07

HUMORAL IMMUNE RESPONSES AGAINST FOOT-AND-MOUTH DISEASE VIRUS INDUCED BY HETEROLOGOUS VIRAL VECTORS

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Foot-and-mouth disease virus (FMDV) is the etiological agent of one of the most transmissible diseases of livestock, causing severe outbreaks and important economic losses worldwide. Herpes Simplex type 1 (HSV) amplicons and type 5 recombinant adenovirus (Ad) vectored vaccines were used for the induction of specific FMDV immune responses in mice. BALBc mice were vaccinated with Ad and HSV vaccines expressing FMDV structural proteins and the 3C viral protease. Three groups of mice received two sequential immunizations with HSV or Ad vectors, or 1ì g of inactivated FMDV, respectively. Homologous HSV/HSV or Ad/Ad prime/boost immunizations induced long-lasting FMDV specific antibodies. Mice immunized with HSV amplicons generated mixed IgG1/IgG2a immune responses, while immunization with Ad induced IgG2a as the predominant isotype. Challenge of mice vaccinated with HSV amplicons with a high dose of live virus, resulted in partial protection, with a significant reduction of viremia when compared to mice immunized with Ad. Heterologous vaccination regimens, priming with Ad and boosting with HSV, induced higher levels of FMDV specific antibodies than homologous prime/boost regimes. We demonstrated that Ad and HSV vectors encoding FMDV structural proteins induce potent humoral immune responses when administered either in homologous or heterologous prime/boost immunizations.

BT-P08

OPTIMIZED LOVASTATIN PRODUCTION BY SOLID-STATE FERMENTATION WITH *Aspergillus terreus* WILDTYPE STRAIN

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Statins can inhibit the *de novo* cholesterol biosynthesis at the rate limiting step of HMG-CoA reductase catalysis. For this reason, statins are clinically used as effective drugs for hypercholesterolemia treatment. Objective. This work was aimed at optimizing and simplifying medium composition and fermentation conditions at shake-flask scale. In a second stage, these results were subsequently applied for the massive lovastatin production by Solid Substrate Fermentation (SSF). Methodology. *Aspergillus terreus* MEC was cultured by submerged fermentation (SF) in lactose-yeast extract medium, in shake flasks at 250 rpm and 25ºC during 14 days. Optimization included different C- and N- sources and the decrease of salts and trace elements concentration. Additionally, the influence of soy flour incorporation and the replacement of lactose by milk whey powder were evaluated. Different solid supports (sugarcane bagasse, wheat straw, soy flour) for SSF were assayed. Extracted lovastatin was analyzed by RP-HPLC with a Diode Array Detector. Results and conclusions. A lovastatin production of 63 mg/L could be reached with optimized liquid medium. Production was significantly increased, up to ~1000 mg/L, by SSF including cheap and readily available substrates such as whey milk adsorbed on textured soy flour, highlighting the relevance of fermentation strategies for secondary metabolite production.

BT-P09

CULTURE MEDIUM FACTORIAL DESIGN OPTIMIZATION FOR FIBRINOLYTIC ENZYMES PRODUCTION BY *Bionectria sp.*

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Thrombotic diseases can be clinically treated with fibrinolytic enzymes and many attempts have been made at laboratory level to increase fibrinolytic enzymes production from microbial sources and to reduce the process cost, including culture medium design, optimization of environmental conditions, and over expression with genetically modified strains. In this contribution we present the optimization of culture medium composition and incubation temperature for fibrinolytic enzyme production by *Bionectria* sp., a selected fungal strain from Las Yungas (Tucumán). Optimization was carried out at Erlenmeyer scale (100-mL working volume) via factorial design methodology. All trials included a common mineral base (%, w/v: NaCl 0.2, KH2PO4 0.05, MgSO4•7H2O 0.05). According to four factorial designs it could be demonstrated the convenience of using soy peptone as N-source, glucose as C-source, and the possibility to eliminate starch, meat peptone and meat extract from original medium composition, whilst 25°C was selected as the optimal incubation temperature. Results showed that culture medium could be successfully optimized by factorial design, achieving a reduction in the production process costs by means of a decrease in culture medium components, the improvement in culture broth rheology, mycelial morphology and mass/energy transfer, and the subsequent two-fold enhancement in productivity.

BT-P10

CORN INDUSTRY SUBPRODUCTS FOR BIOMASS PRODUCTION OF AN ATRAZINE DEGRADING BACTERIAL CONSORTIUM

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Bioremediation is a well known tool used to degrade or transform contaminants. Bioremediation processes can be carried out both by biostimulation and/or bioaugmentation. The last process consists in the introduction of specific competent degrading strains or consortia of microorganisms. In a previous work, in order to produce high biomass concentration of an atrazine degrading bacterial consortium, traditional carbon sources as glucose or citrate were used. Furthermore, water of maceration (MA) and syrups mixture (SM) are low cost corn industry subproducts that represent an economical alternative for cultivation of these microorganisms. The aim of this work was to study the biomass production of an atrazine degrading bacterial consortium isolated from soils of the Argentinean Humid Pampa using these subproducts as carbon and energy source. Shake flasks experiments using factorial design were carried out in 250 ml Erlenmeyer flasks using atrazine as nitrogen source and MA or SM as carbon and energy source. Biomass production and atrazine consumption were monitored over time. SM was able to replace traditional carbon sources in culture media for support consortium growth and maintain atrazine degrading capacity, being a potential substrate for biomass production useful for bioaugmentation processes.

BT-P11

PROTEOMIC STUDY OF *Rhodotorula mucilaginosa* RCL-11 REVEALED DIFFERENTIAL PROTEINS EXPRESSION UNDER COPPER STRESS

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Organisms subjected to metal exposures in their natural environments generally have had develop resistance mechanisms. *Rhodotorula mucilaginosa* RCL-11, yeast isolated from a copper filter plant at the province of Tucumán, Argentina, has the ability of supporting high amounts of copper metal by a slow down in its growth rate. In order to understand the mechanism involved in RCL-11 resistance to copper it was conducted a proteomic approach. Results of atomic absorption spectroscopy showed that copper concentration in the medium decreased from 0.5 to 0.2mM 48 h later inoculation occurred. Analyzing by mono-dimensional gel electrophoresis the crude cells extracts, it was observed differential bands expressions between cells with or without copper. Further, with the aim of studying these differences, two-dimensional electrophoresis analyses was carried out. Gels were silver-stained, scanned and analyzed with Image Master Program. 2-D analysis of RCL-11 revealed that 48 h copper exposure produced an over-expression of 20 proteins; some of them increased their expression according to the time of copper exposure (16, 24 and 48 h). The results obtained in the present work show that when exposing *R. mucilaginosa* RCL-11 to 0.5mM copper concentration, it is produced a differential protein expression probably involved in cell resistance mechanisms.

BT-P12

DYNAMICS OF A MARINE-MICROBIAL COMMUNITY DURING BIODEGRADATION OF BILGE WASTE HYDROCARBONS

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The aim of this study was to identify the dominant taxons of a hydrocarbon-degrading microbial community enriched from bilge waste oily phase, and to assess its population dynamics during bilge waste biodegradation. Samples were retrieved from an aerated batch bioreactor with 2.1 g/L total hydrocarbons in seawater medium during a 14-day biodegradation experiment. Total DNA was analyzed by PCR-DGGE, and the relative intensity of each dominant band calculated. Members of the genus *Marinobacter* were dominant in the enrichment, and *Pseudomonas*, *Shewanella* and *Halomonas* were also detected. During the biodegradation experiment, the exponential-growth phase agreed with n-alkane depletion and an increase in the prevalence of *Pseudomonas* and *Shewanella*. Only after emulsification, biodegradation of other more recalcitrant hydrocarbons found in an unresolved complex mixture (UCM) occurred, associated with a predominance of *Marinobacter* and *Shewanella*. Cluster analysis from DGGE fingerprints showed shifts in the microbial community structure which matches with the pattern of sequential hydrocarbon biodegradation found (n-alkanes-UCM). *Shewanella*, a genus which can use diverse electron acceptors such as metals and colonizes emulsified oil from spills, showed high prevalence during both n-alkane and UCM biodegradation of bilge wastes, suggesting a promising potential for bioremediation.

BT-P13

BIOTRANSFORMATIONS CATALYZED BY LIPASES FROM SPORE-FORMING MICROORGANISMS

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It is generally accepted that lipase-catalyzed hydrolytic activity does not always correlate with its esterification or transesterification activity. Therefore, the selection of a proper lipase for a given application is required. Thus, the aim of this study was to investigate the reactivity of 64 lipases from spore-forming microorganisms towards hydrolytic and transesterification reactions by using p-nitrophenyl palmitate as a chromogenic acyl donor substrate. Specific hydrolytic activities were not highly correlated with the corresponding specific transesterification activities using ethanol (r=-0.113, P=0.375), starch (r=0.226, P=0.073), low-methoxyl (LM) pectin (r=0.622, P<0.001) and high-methoxyl (HM) pectin (r=0.533, P<0.001) as acyl acceptor. The highest specific hydrolytic activity (52.73±1.68 U/mg) was exhibited by lipase from strain 5. Lipases from strains 30, 47 M, 5 and *Brevibacillus agri* E12 gave the highest transesterification activities in the presence of ethanol (26.36±2.55 U/mg), starch (0.31±0.04 U/mg), LM pectin (1.25±0.22 U/mg) and HM pectin (3.12±0.56 U/mg), respectively. Under our assay conditions, no hydrolytic activity was detected with lipase from strain 30.

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BT-P14

IMPROVED EXPRESSION AND CHARACTERIZATION OF HUMAN PROINSULIN FUSED TO THIOREDOXIN IN *E. coli*

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Native proinsulin (PI) belongs to the class of the difficult-to-express proteins in *E. coli*, due to its small size, a high proteolytic decay and the necessity to reproduce the native disulfide pattern. Large amounts of properly folded PI are needed to test PI autoantibody of populations at risk for type 1 diabetes. The aim of this study was to recover high yield of properly folded human PI as a fusion to thioredoxin (TrxPI) from inclusion bodies (IB) of *E. coli*. TrxPI was solubilized from IB, refolded *in vitro* and purified by anion exchange chromatography. It was subjected to mass spectometry analysis and to proteolitic digestion using *S. aureus* protease V8. To study the immunochemical behaviour of TrxPI compared to standard PI, dose-response curves were performed with specific anti-proinsulin polyclonal sera. TrxPI recovered from IB yielded 10 mg/L culture, and its purity was confirmed by a single peak in RP-HPLC. The product identity and integrity were verified by mass analysis (22,173.5 Da) and mapping with V8. Dose-response curves showed parallelism and identity between TrxPI and standard PI. In conclusion, it was possible to obtain high yield of purified human PI as a fusion protein in *E. coli.* Proper PI folding was confirmed by biochemical and immunochemical assays indicating integrity of the chimera and the epitopes involved in the interaction with antibodies.

BT-P15

OPTIMIZATION OF MEGALOMICIN ANALOGS PRODUCTION IN *E. coli*

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Previously we have demonstrated that substrate flexibility of the sugar transferases MegDI/DVI allowed production of two new megalomicin analogs, megosaminyl-erythromycin A and megosaminyl-azithromycin. Antibacterial activity of these new macrolides did not change notably compared to original molecules. However, megosaminyl-erythromycin A showed a significant increase in potency against *P falciparum.* In order to optimize the production of megalomicin analogs in *E. coli* we carried out metabolic engineering of two endogenous pathways that consume the common deoxysugar intermediate, TKDG in *E. coli* BL21 strain. Higher intracellular levels of TKDG increase the biosynthesis of TDP-L-megosamine, which resulted in improved production of megalomicin analogs by bioconversion experiment. Each protein of megosamine operon was expressed and analyzed by Western Blot determining which of them is limiting the efficiency of the bioconversion experiments. New megosamine operons were constructed with alternative gene order and different promoters: pT7, pBAD, pT5, pLacUV5, pC24 and pTAC. Production of megalomicin analogs were evaluated by bioconversion experiment. In addition, bioconversion experiments using a fed-batch bioreactor allowed us to produce megalomicins analogs in minimal medium using different carbon sources. The best amount of bioconversion was obtained using glycerol as carbon source.

BT-P16

DEVELOPMENT OF ELECTROCHEMICAL AND OPTICAL IMMUNOBIOSENSORS FOR DIAGNOSIS OF CHAGAS DISEASE

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Access to appropriate diagnostic tools is an essential component in the evaluation and improvement of global health. Most diagnostic tools currently available have been developed to be implemented in laboratories highly or moderately equipped; so, largely inadequate for meeting health needs in remote or impoverished settings with no or limited infrastructure. To solve this problem, point-of-care (POC) diagnostic platforms based on immunobiosensors emerge as an alternative. Immunobiosensors are detection systems that transduce a biological signal based on the antibody-antigen recognition into a measurable signal through physical, chemical, optical or electronic transducers. In this work, we have developed optical and electrochemical immunobiosensors for the diagnosis of Chagas disease. Recombinant antigens Ag1, Ag13, Ag36, SAPA and TSSA were purified as GST fusion proteins by affinity chromatography and immobilized on carboxi-modified magnetic beads. Functionalized micro-beads were incubated with sera from infected and non-infected patients and the immune complexes were detected using rabbit anti-human IgM/G Cy5 or peroxidase conjugate antibodies. Our results indicate that the immunobiosensors here developed can be use for the diagnosis of Chagas disease and reveal the potential of such biosensors for the development of POC diagnostic platforms for multiple infectious diseases.

BT-P17

BIO-LAYER INTERFEROMETRY USING OPTIC-BASED BIOSENSOR FOR SCREENING OF AFFINITY LIGAND FOR rhEPO

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Bio-layer interferometry is used in optic-based biosensors (ForteBio OCTET RED) for protein-ligands interactions analysis.
Short peptides are ideal to be used as affinity ligands for protein purification. Divide-couple-recombine (DCR) method allows obtaining a library with all possible combinations of the amino acids in the form of "one bead-one peptide". An octapeptide library XXXOXXOO (O=fixed positions and X =Ala, Asp, Glu, Phe, His, Leu, Asn, Pro, Ser y Thr) was synthesised.
After the library screening 60 peptides with affintiy for rhEPO were selected. To study the peptide affinity for the rhEPO we used a BLI biosensor. rhEPO was labeled with biotin and it was immobilised on a super streptavidin biosensor tip (SSA). The running buffer was phosphates 20 mM, pH 7.0, Tween 20, 0.05%. The reference biosensor tip was a SSA with immobilised biocitin. Peptides with the highest affinities were immobilised on agarose. All the peptide-agarose matrices showed affinity for rhEPO. Also we evaluated the affinity of the peptide-agarose matrices for bovine seroalbumin, usually present in the culture supernatant. Those peptides with the highest selectivity were selected for future development of a rhEPO purification method.

BT-P18

FUNCTIONALIZATION OF CARBON NANOTUBES WITH BIOMOLECULES FOR NANOBIOSENSORS TO DETECT CANCER MARKERS

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The aim of this study was to functionalize carbon nanotubes (CNTs) with antibodies or oligonucleotides to produce nanobiosensors for tumor markers detection. For this purpose, first CNTs were oxidized by incubation with a mixture of acids. Then oxidized CNTs (CNTs-COOH) were covalently bound to antibodies or modified oligonucleotides with an amine group at 5' end by incubation with 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC) and N-hidroxysuccinimide (NHS). The product of this reaction is an amide bond between a carboxyl group of CNTs-COOH and an amine group present in the proteins or in the modified oligonucleotides. The products of both steps of this functionalization were characterized by scanning electron microscopy (SEM), Raman spectroscopy and thermogravimetric analysis (TGA). SEM images and Raman spectra showed the characteristic differences on morphology and Raman peaks between CNTs and CNTs-COOH. TGA analysis demonstrated the presence of the organic molecules (protein or DNA) and the degree of functionalization in the samples of CNTs-protein and CNTs-DNA. Thus, these techniques allowed the characterization of CNTs functionalization with biomolecules to be used in the development of electronic nanobiosensors devices for specific cancer markers detection.

BT-P19

BIOTECHNOLOGICAL STRATEGIES FOR PHYTOREMEDIATING CHLORINATED HERBICIDES IN THE HUMID PAMPA

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In agricultural producer countries, the use of chlorinated herbicides (atrazine), associated to intensive farming practices, overflows the ecosystems natural attenuation capacity. Thus, herbicide residues contaminate the soils and water sources, increasing the risk for human health and wildlife. Humid Pampa is the main agricultural region in one of the world's largest herbicide consumer countries; hence, technologies to remediate its residues are needed. In this context, phytoremediation is an effective, environmental friendly and publicly accepted option. Accordingly, the objective of designing biotechnological strategies for phytoremediating chlorinated herbicide residues in an integrated approach to the ongoing Humid Pampa agricultural trend, in is raised. In this way, strategies according to the edaphic, topogeographic and farming practices characteristics of this region where establish. Those strategies include the adequate selection of plant species. For that purpose, atrazine tolerance assays were performed in simple (semisolid agar medium) and complex (microcosms) experimental systems implanted with *Lolium multiflorum*, *Lotus sp* and *Quenopodium quinoa*. *L. multiflorum* was selected and assessed via atrazine dissipation assays with soil, achieving statistically significant attenuation levels. According to the observed results, the strategy scale up to field conditions is planned.

BT-P20

HYBRID SOL-GEL SCAFFOLDS FOR SAOS-2 CELLS

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Sol-gel hybrid materials are being used in the biomaterials field, due to their higher biocompatibility and mechanical properties than organic or inorganic materials alone. When immobilizing cells, the chosen material should be able to maintain its viability as well as provide a suitable platform for support and proliferation. SAOS-2 are osteoblast-like cells which possess several osteoblastic features and whose activity could be easily monitored for viability and morphology assessment. Moreover, silicon materials could be manipulated to render scaffolds of similar compressive strength and modulus to that of bone itself, with possible applications in the bone engineering field. In this work, hybrid sol-gel materials were obtained using as a sol-gel precursor tetra-ethyl-ortho-silicate (TEOS) and as the organic counterparts polyvinyl alcohol (PVA), polyethylene glycol (PEG) and gelatin. Each gel contains an organic/inorganic mass ratio of 1,5. Higher concentrations of PVA, PEG and gelatin led to loss of homogeneity within hydrogels. SAOS-2 cells were added to the gel´s surface in order to evaluate adhesion and proliferation. After 24 hours viability was assessed. Results showed that cells within PEG-TEOS and Gelatin-TEOS hybrids had the highest viability. PVA-TEOS hybrids scored 80% in comparison to TEOS-PEG gels and TEOS alone showed the lowest viability.

BT-P21

IMMOBILIZATION OF OVARIAN FOLLICLES FOR ALTERNATIVE HORMONAL REPLACEMENT THERAPIES

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Cell immobilization in sol-gel matrices is a useful tool for design biomaterials avoiding the immune rejection risk. Hormone replacement therapy [estradiol (E) and progesterone (P)] is used to treat menopausal symptoms, although controversial due to risk of tumor emergence. The aim was to evaluate the viability and functionality of new systems of hormonal delivery.
Preovulatory follicles from prepubertal rats primed with 25 U of PMSG (SC) were encapsulated using two sol-gel precursors: TEOS and THEOS and cultured for 1, 3, 6 and 9 days. Cell viability was determined by MTT and secretion of E and P (relative to active cells) by RIA. Immunohistochemistry for P450c17 and aromatase was used to reveal cell structure conservation. Follicles encapsulated in TEOS (FTEOS) and THEOS (FTHEOS) were viable at all days tested, although unencapsulated follicles (UEF) showed higher MTT values ($p<0.05$). P from FTHEOS was higher than from UEF at day 1 ($p<0.001$). E from FTEOS were higher than from UEF at days 1, 3, 6 and 9 ($p<0.01$). FTHEOS only showed differences in E from UEF at days 1 and 3 ($p<0.01$). Positive P450c17 and aromatase staining of UEF, FTEOS and FTHEOS demonstrated conserved cell structure.
The sol-gel matrices tested, especially TEOS, may be eligible for bioencapsulation of ovarian follicles with preserved cell viability and function, allowing a more controlled hormonal delivery.

BT-P22

SOL-GEL IMMOBILIZED TIOPHILIC LIGANDS AND PROTEIN A IN THE RECOVERY OF THE γ FRACTION OF HUMAN SERUM

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Interest in monoclonal and polyclonal antibodies has increased as more applications in biotechnology such as immunoaffinity chromatography, immunodiagnostic, drug targeting and biosensors are found for these versatile molecules. For all these purposes homogeneous antibody preparations are needed. Conventional purification methods using Protein A are expensive to operate due with. None-specific methods like hydroxyapatite or ion exchange chromatography possess low capacity or yield antibodies contaminated with albumin and transferrin. The use of tiophilic ligands shows selective binding of immunoglobulins in the presence of structure forming salts. The most common sorbent structure derives from divinylsulphone coupled with â-mercaptoethanol. For the purpose of comparing both techniques, glass slides and glass beads were functionalized with APTES, which provide $–NH_2$ groups available for reaction with glutaraldehyde (needed for Protein A attachment) and divinylsulphone (needed for the coupling with â-mercaptoethanol). The adsorption of ã- fraction from human serum is being evaluated, using capillary electroforesis, as a ã-fraction:albumin ratio change. Desorption of the adsorbed proteins and reutilization of the derivatized glass slides is also being evaluated.

BT-P23

CONTROL OF RED FLUORESCENT PROTEIN EXPRESSION BY TETRACYCLINE IN AN ADENOVIRAL BIVECTORIAL SYSTEM

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We built a tetracycline-responsive system (Tet-Off) consisting of two recombinant adenoviral vectors (RAd's), one harbornig a sequence coding for a tetracycline-responsive transactivator protein, and another coding for the transgene for the red fluorescent protein DsRed2 under control of a minimal promoter containing the tetracycline-response element. We characterized the functionality of the system in cell cultures which were co-transduced with both RAd's. In order to assess vector's regulatability, cells were cultured with doxycycline (DOX) 1µg/ml. High expression of DsRed2 was observed (red fluorescence) in cells grown without DOX, but only minimal fluorescence levels were detected in cells treated with DOX. In addition, to study the system performance *in vivo*, rats were allotted to 2 groups which received stereotaxic injections of both RAd's in the brain. One group received regular tap water (Control group) whereas the other one received water containing 1mg/ml DOX (DOX group). On day 2 post injection, brains were removed and coronal sections prepared. Transgene expression was assessed by fluorescence microscopy. In the control but not in the DOX group, numerous DsRed2 fluorescent cells were observed. We conclude that our Tet-Off system is suitable for implementing gene delivery to the brain and subsequently regulating transgene expression by administration or removal of DOX.

BT-P24

Z PROTEIN-INDUCED VLPs AS AN ANTIGEN PRESENTATION SYSTEM

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Viral matrix proteins are involved in the formation of mature virions as budding particles during viral infection. Z protein of Junín virus, expressed without any other viral component in mammal cells was capable to induce budding of virus like particles (VLPs). In this study we propose to use this property to obtain VLPs carrying potential antigens fused to Z.
To test this system, the immune response against eGFP was evaluated. For this, a mammalian expression vector comprising the Z protein gene fused to eGFP gene (ZeGFP) was constructed. This plasmid was transfected on 293T mammalian cells, and VLPs were purified from the supernatant. We show that VLPs have sequences from Z and eGFP proteins inside them, and evaluated the immune response induced against eGFP after inoculation of Balb/c mice with VLPs. A humoral immune response anti-eGFP was detected when the mice were inoculated with whole ZeGFP-carrying VLPs but not when were inoculated with triton X-100 disrupted ZeGFP-carrying VLPs or with eGFP alone, using similar protein amounts.
These preliminary results indicate that VLP structure stimulates the immune response against eGFP. Future studies will require the optimization of the immunization protocol, and will test the use of Z fused to different viral antigens, including Measles virus Nucleoprotein and the group A rotavirus VP6 proteins, as heterologous antigen models.

BT-P25

IMPROVEMENT OF AROMA IN TRANSGENIC POTATO AS A CONSEQUENCE OF IMPAIRING TUBER BROWNING

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Sensory analysis studies are critical in the development of quality enhanced crops, and may be an important component in the public acceptance of genetically modified foods. It has recently been established that odor preferences are shared between humans and mice, suggesting that odor exploration behavior in mice may be used to predict human olfactory preferences. We have previously found that mice fed diets supplemented with engineered "nonbrowning" potatoes consumed more potato than mice fed diets supplemented with wild-type potatoes (WT) (Llorente *et al*., 2010. Plant Biotechnol J. DOI: 10.1111/j.1467-7652.2010.00534.x).This prompted us to explore a possible role of potato odor in mice preference for "nonbrowning" potatoes. Taking advantage of neuroscience paradigms, several experiments were conducted with mice and humans on the basis of olfaction alone. These experiments showed that "nonbrowning" tubers, in addition to their extended shelf life, maintain their odor quality for longer periods of time than WT potatoes. Taken together, these findings also suggest that our previous observations might be influenced, at least in part, by differential odors that are accentuated among the lines once oxidative deterioration takes place. To our knowledge this is the first report on the use of an animal model and neuroscience paradigms applied to the sensory analysis of a transgenic crop.

BT-P26

SiO_2 IMMOBILIZED POLYPHENOLS FROM YERBA MATE (*Ilex paraguariensis*) AS HEAVY METAL BIOSORBENT

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The control of industrial effluents and polluted waters has motivated the development of assorted solutions. The low cost sorbents are an alternative to the traditional and more expensive methods, such as ion exchange resins. Low cost sorbents are those that require little or non processing and are typically abundant industrial secondary products or wastes. The biosorbents are low cost sorbents obtained from a biological source, such as saw dust or rise husk. Some vegetable biosorbents have the capability of adsorbing heavy metals. This is related to polyphenolic compounds that interact with the metals within the plant. In this work, by means of the Sol-gel chemistry, a polyphenol extract had been immobilized within a SiO_2 network. The SiO_2 polymers give the biosorbent a mechanical support for its application and removal from polluted waters. As source of polyphenols, the residual dust of yerba mate (*Ilex paraguariensis*) production was used. Tetraetoxy silane was chosen as SiO_2 precursor and glutaraldehide has been used as the crosslinking agent. The biosorbent obtained were able to remove Pb(II), Cr(III) and Cr(VI) from contaminated solutions at acidic pHs were it is know that the polyphenol-metal complex is stronger. A kinetic and adsorption capacity analysis (Langmuir, Freunlich, Dubinin-Radushkevich) are presented with the characterization of the material (SEM, BET).

BT-P27

SCREENING OF DECOLOURIZING YEASTS WITH DYE ASSIMILATION ABILITY FROM LAS YUNGAS RAINFOREST

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Environmental pollution by textile-dye containing wastewaters is a matter of worldwide concern. Yeasts bioremediation, a poorly explored and less exploited methodology, stands out as an interesting approach supported on their metabolic diversity and high growth rates. In this work, a screening protocol based on dye tolerance and dye assimilation ability on solid media was performed with a 2000 mg/L dye mixture. Thirty-nine yeast isolates from "Laurel del Monte" underlying soils from "Las Yungas" (Tucumán, Argentina) were obtained. Based on decolourization haloes and colony dyeing, 15 isolates showed the highest decolourization ability on agar plates containing Vilmafix® Blue RR-BB, Vilmafix® Red 7B-HE, Vilmafix® Black B-V and Vilmafix® Yellow 4R-HE, either alone or as a mixture. Screening on agar plates with synthetic media supplemented with each dye as sole C or N source, led to the selection of 10 isolates. Microsatellite RAPD analysis enabled the discrimination of these isolates into six groups. Following tests consisted on the analysis of growth and decolourization kinetic profiles, along with the production of Manganese peroxidase, laccase and tyrosinase activities in dye-supplemented (200 mg/L) liquid media. Time-dependent supernatant spectra, growth curves and enzymatic activities led to the selection of the two most promising isolates for future experiments.

BT-P28

CHARACTERIZATION OF NEW ACTIVE TRANSPOSONS ISOLATED FROM INSECTS

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Transposons are mobile DNAs spread in most organisms including some viruses. The ability of these sequences in mobilizing from one physical position to another can be a decisive factor in evolution processes due to genomic rearrangements (gene interruption, deletions, inversions, translocations, etc.). In Eukarya, transposable elements (TEs) are a significant percentage of genomes showing a great diversity in gene content, size and mechanism of transposition. According to the above, TEs are classified into two main groups: Class I (retrotransposons) and Class II (DNA transposons). Insect cells are the best system to study and produce baculoviruses, a pathogen used as bioinsecticide, protein expression system and gene therapy or vaccine vectors. These viruses have big dsDNA genomes and structural mutations produced by transposition processes could be the main force to their evolution. With the aim to discover and characterize new active transposons from insects, we transfected prokaryotic plasmids in insect cell lines (Sf9 and Sf21 from *Spodoptera frugiperda*, Hi5 from *Trichoplusia ni* and UFL-Ag-286 from *Anticarsia gemmatalis*) and then we recovered modified plasmids with DNA insertions by *Escherichia coli* transformation.

The proposed strategy has allowed isolating and sequencing 4 TE´s never before described and Piggybac, a transposon with many applications in biology.

BT-P29

rhGH-AFFINITY-PEPTIDE SELECTION USING COMBINATORIAL LIBRARIES

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Growth hormone (hGH) is used in medicine to treat children's growth disorders and adult growth hormone deficiency. High purity is necessary for its parenteral administration. Thus, downstream processing should be optimized to obtain higher yields and low cost processes. Affinity Chromatography (AC) is considered an efficient method for protein purification in complex mixtures. Short peptides are ideal ligands for industrial separations because of its low cost, high stability and easy manufacture.

We synthesized a peptide combinatorial library XXOXXOOO (O=fixed and X=variable positions) using Divide-couple-recombine (DCR) method that allows obtaining a library with all possible combinations of amino acids in the form of "one bead-one peptide". Peptide ligands can be selected from library screening. We have developed a rapid and non-expensive strategy for peptide identification contained on positive beads, by using MALDI-TOF-MS, and 4-hydroxymethylbenzoic acid. We selected more than 50 peptides with rhGH affinity using this strategy. The peptides showed consensus sequences. Those peptides could be used not only as affinity ligands for new strategies development based on AC rhGH purification but also as diagnostic-rhGH-kits ligands. This kind of systems could improve cost/benefit ratio obtaining economically competitive products.

BT-P30

A REGULATABLE ADENOVECTOR FOR SIMULTANEOUS EXPRESSION OF THYMULIN AND GFP GENES IN MUSCLE AND BRAIN

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Thymulin (FTS) is a thymic peptide possessing hypophysiotropic activity and antiinflammatory effects in the brain. In order to regulate FTS expression tightly in gene therapy studies, we built a two-adenovector Tet-Off regulatable system in which transgene expression can be turned off at the transcriptional level by doxycycline (DOX). It consists of two adenoviral vectors: RAd-$(GFPtreFTS)_{bd}$, expressing the genes for green fluorescent protein (GFP) and FTS under the control of a bidirectional regulatable promoter, and RAd-tTA, constitutively expressing the transactivator protein tTA. We also constructed a control system expressing only GFP. Different cell lines were co-transduced with both RAd's. High expression of GFP and FTS was observed, whereas both genes were silenced by addition of DOX. Male rats received ICV injections of either both RAd's or RAd-$(GFPtreFTS)_{bd}$ alone (control). After 48 hs thymulin levels rose in the CSF (24±4.6 fg/ml) of animals injected with both RAds relative to controls (4±0.3 fg/ml). This system was also administered i.m. to rats. Initially, serum FTS levels rose (384±74 fg/ml), but fell to basal levels (102±16 fg/ml) after addition of DOX to the drinking water. This regulatable gene expression system constitutes a suitable tool to further investigate the role of thymulin in the maturation of the neuroendocrine system during postnatal life in rodents.

CB-P01

ANALYSIS OF THE INTERFERENCE OF HUMAN PAPILLOMAVIRUS E6 ONCOPROTEINS WITH CELL POLARITY REGULATION

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Human Papillomavirus (HPV) E6 oncoproteins bind PDZ-domain proteins involved in cell junction organization. For investigating the interference of HPV E6 proteins with tight junction structures, important for the maintenance of cell polarity, we are analyzing the level and distribution of tight junction proteins in HPV E6-positive cells. We observed, by inmunofluorescence assays, that Par-3 protein, a key element of this kind of junctions, localizes at cell-cell interaction in epithelial cells, and high risk HPV E6 protein appears to alter Par-3 cell localization. Cell polarity also implies the correct distribution of membrane Phosphoinositide (PIP) lipids. It has been demonstrated that some PDZ proteins interact with these lipids and this interaction is relevant for PIP correct distribution. We want to address if E6 HPV proteins could disrupt the interaction PIPs-PDZ, interfering with PIPs localization and tight junction complexes. For this purpose we analyzed the differences in PIP distribution in the presence and absence of E6 proteins using a PIP biosensor fused to GFP. Preliminary results have shown that E6 expression induces PIPs mislocalization, reducing their presence at the cell borders. Moreover, cancer HPV-positive cells showed a completely altered pattern of PIP localization with a punctuated cytoplasmic distribution.

CB-P02

MACROPHAGES TUMOR ENVIRONMENT INDUCE BREAST CANCER PROLIFERATION VIA NF-κB AND c-FOS

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The tumour microenvironment plays vital roles in cancer but the molecular basis underlying these tumour-promoting effects is yet not fully understood. The NF-êB and growth factor signalling pathways are implicated in resistance to endocrine treatments in breast cancer. Here, we report that macrophage tumor associated induces proliferation of endocrine sensitive breast cancer cell lines in the absence of estradiol, or in the presence of tamoxifen. Macrophages-Breast Cancer Cells cocultured induced a sustained release of TNFá and IL-6 from both cell types, leading to activation of NF-êB, STAT3 and ERK in the breast cells. Furthermore this cells-crosstalk induces hyperphosphorylation of the estrogen receptor (ER) that rendered it constitutively active. The formation of a novel NF-êB/STAT3/phospho-ER complex at the Cyclin D1 gene induced proliferation regardless of ER ligand status, demonstrating that the tumor microenvironment can rapidly activate signalling pathways implicated in endocrine resistance.

CB-P03

COLON CANCER CELLULAR MODEL FOR STUDYING RADIORESISTANCE MODULATION

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Tumor radioresistance is one of the main causes that lead to radiotherapy failure. The aim of this study was to evaluate the intrinsic radiosensitivity of colon human cancer cell lines to further study the modulation of radiation response by silencing radioresistant associated genes. Survival curves and DNA damage induced by gamma radiation were evaluated in HT-29, Caco-2 and LoVo cells. Survival curves were fitted to the linear-quadratic model $S=\exp-(\acute{a}D+\hat{a}D^2)$, showing significant increasing values of á parameter: HT-29 (á=0.47+/-0.07) Caco-2 (á=0.86+/-0.17) and LoVo (á= 1.41+/-0.13) and the loss of the quadratic component (â=0) for this last cell line. To evaluate DNA damage and repair induced by gamma radiation (2 Gy), phosphorylated histone H2AX (ãH2AX) was determined by immunohistochemistry as a measure of DNA double strand breaks (DSBs) at 0.5 and 6 h post-irradiation. The number of foci per nucleus decreased significantly at 6 h post-irradiation: 65% for HT-29, 40% for Caco-2 and 20% for LoVo cells. The evaluation of initial damage by analysing foci size at 30 min post-irradiation showed a significant increase of 33% in HT-29, 66% in Caco-2 and 132% in LoVo.
These results show that these cells have different degree of radioresistance to gamma radiation (HT-29>Caco-2>LoVo) and they may be a useful model to study the modulation of radioresistance.

CB-P04

Fra-1 AND c-FOS SUPPORT BREAST TUMOR GROWTH BY ACTIVATING PHOSPHOLIPID SYNTHESIS

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c-Fos, in addition to its AP-1 transcription factor activity, also activates phospholipid synthesis thus supporting membrane biogenesis required for cell growth. The basic domain (BD) of c-Fos is essential for activation. Fra-1, another c-Fos family member, has a BD almost identical to c-Fos and conserves its lipid activating capacity.
Recently, Fra-1 overexpression was reported in human breast cancer cells. Consequently, we studied if Fra-1 participates in cancer cell growth by activating phospholipid synthesis. We found Fra-1 overexpressed in growing MDA-MB 231 and MCF7 cells which co-localizes with endoplasmic reticulum (ER) markers. Stripping membranes of associated proteins (0.1 M KCl treatment) results in quiescent cell phospholipid synthesis rates which are restored to initial activated rates when recombinant Fra-1 or c-Fos are added to the stripped membranes. Similar results were verified in human breast tumor samples as compared to normal tissue. Phospholipid synthesis was significantly higher in tumors as compared to normal tissue but was significantly reduced when subjecting tumor samples to KCl treatment. Addition of recombinant Fra-1 or c-Fos restored phospholipid synthesis activity to initial rates, confirming our previous findings. Our results indicate that both Fra-1 and c-Fos support breast tumor growth by activating lipid synthesis.

CB-P05

HYPOTHYROIDISM SENSITIZES ACTIVATION AND NET FORMATION IN NEUTROPHILS

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Upon activation with chemotactic compounds neutrophils or polymorphonuclear leukocytes (PMN) form extracellular traps (neutrophil's extracellular traps, NETs). This event depends on the activation NADPH oxidase-2 (NOX-2). We think that hypothyroidism (H) sensitizes activation of PMN. To test this we used a model of H induced with 6-n-propylthiouracil (PTU) in female Wistar rats. Rats received drinking water without (control, CO) or with 0.1 g/L of PTU (hypothyroid, HT) for 30 days. Rats were euthanized by decapitation and blood was collected for PMN isolation by gradient density centrifugation. PMN were cultured 24 h in the presence or not of 10 µM fMLP. NOX-2, myeloperoxidase (MPO) and NET formation in PMN, were determined by immunocytochemistry and confocal imaging in single plan images. The MPO activity was measured by spectrophotometry using o-dianisidine as substrate. Nitric oxide (NO) levels in culture supernatant were measured as nitrites with the Griess reagent. Our results show an increased NOX-2 and MPO content in PMN of HT. Overall MPO activity remained unchanged. Activated PMN from HT rats showed increased fMLP-induced formation of NETs. Nitrite production was higher in PMN from HT rats. Our results suggest that hypothyroidism sensitizes neutrophils to activation and NET formation. This research will contribute to the understanding the impact of HT on inflammation.

CB-P06

HYPOTHYROIDISM, THYROID HORMONE RECEPTORS AND INFLAMMATION IN THE HEART

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The superfamily of nuclear receptors includes a number of transcription factors, which activity regulates inflammation, oxidative stress and metabolism. However, the effect of hypothyroidism on heart inflammatory profile is unclear. Herein, we sought to determine whether hypothyroidism affects nuclear receptors expression and inflammation in the heart. To accomplish this aim, a hypothyroidism model was produced in female virgin Wistar rats (150-180 g body weight) by treating them with (hypothyroid, HT) or without (euthyroid, ET) 100 mg/L of 6-n-propyl 2-thiouracil in their drinking water. After 30 days of treatment HT and ET rats were sacrificed and their heart excised for the determination of PPARã, TNF-á, TRá, TRâ and â-actin (internal control) mRNA by RT-PCR. The protein level of cyclooxygnease-2 (COX-2), inducible nitric oxide synthase (iNOS) and endothelial NOS (eNOS) was determined by Western blot. Confocal imaging of the heart tissue and RT-PCR data are consistent with a reduced expression of TR-á1 and TR-â1, whereas increased COX-2 and iNOS proteins, in the heart of HT with respect to ET rats. However, we observed no changes in eNOS, PPAR-ã mRNA and TNF-á. Our results suggest that hypothyroidism affects the inflammatory profile in the heart. These results impact our understanding of hypothyroidism-associated heart diseases.

CB-P07

THE COLD AQUEOUS EXTRACT OF *Baccharis articulata* STIMULATES APOPTOSIS PROCESS IN HUMAN LYMPHOCYTES

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The cytotoxic action of *Baccharis articulata* (Asteraceae) has not been studied. The aim was to determine the toxic effects of cold aqueous extract of *B. articulata* (Ba-CAE) on human lymphocytes. Lymphocytes separated from peripheral blood of healthy volunteers by density gradient were exposed to Ba-CAE (10, 20, 40, 80, 160, 320, 640 and 1280 ìg/mL) for 18-24 h. Cell viability was determined by staining of trypan blue exclusion and MTT reduction. Apoptosis was determined by Hoechst 33258 staining, TUNEL and DNA fragmentation analysis by agarose gel electrophoresis. A dose-dependent toxicity of Ba-CAE was demonstrated by staining of trypan blue exclusion (CC50=150 ìg/mL). By MTT assay could not quantify this effect, since Ba-CAE was able to reduce tetrazolium salt in absence of cells. Hoechst staining, showed apoptotic figures (fragmented nuclei, kidney shaped nuclei and small nuclear blebs) in cells treated with Ba-CAE (80 to 1280 ìg/mL). The percentage of TUNEL-positive per 400 cells was: cell with media alone: 7+1%, cells treated with Ba-CAE: 10ì g/mL (8+1%), 20ì g/mL (9+3%), 40ì g/mL (43+11%)*, 80ì g/mL (58+18%)*, 160ì g/mL (62+13%)* 320ì g/mL (68+20%)**, 640ìg/mL (73+10%)**, 1280ìg/mL (88+13%)**; *p<0.01; **p<0.001. Agarose gel electrophoresis showed typical DNA laddering in cells treated with Ba-CAE (40 to 1280 ìg/mL). Ba-CAE stimulated apoptosis in human lymphocytes.

CB-P08

EVALUATION OF THE ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF DIPHENYLDISELENIDE IN MACROPHAGES

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There has been an increasing interest in organoselenium chemistry and biochemistry since several of these compounds possess antioxidant activity. Amongst them, diphenyl diselenide (DPDS) has been the focus of this study. We evaluated the antioxidant properties of DPDS in cultured macrophages (Mph). First, we determined the toxicity of DPDS by means of the trypan blue exclusion assay. We found that DPDS diminished the percentage of viable cells in non-stimulated Mph at concentrations higher than 5ìM, whereas it did not induce any change in the viability of the LPS stimulated cells. Then, we evaluated whether DPDS inhibited the production of nitric oxide (NO), an inflammatory mediator which can be harmful to the host tissue. We observed that the addition of DPDS decreased the production of NO in a dose dependent manner in both conditions tested. Next, we analyzed the enzyme iNOS that produces NO from arginine. We found that DPDS induced a diminution of iNOS in the cells in basal conditions but not in the cells activated with LPS, suggesting that another regulation mechanism could exist. Finally we observed that DPDS affected the antigen presentation capacity of Mph since DPDS induced a diminution of the percentage of MHCII+ and CD86+ cells. These results suggest that DPDS could be an interesting anti-inflammatory compound to implement in the therapy of autoimmune inflammatory diseases.

CB-P09

PROTEIN MALNUTRITION ACTIVATES CASPASE-INDEPENDENT APOPTOSIS IN MOUSE LIVER. METHIONINE PROTECTION

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We have previously reported that 5 days under a diet depleted of amino acids (protein and amino acids free diet, or PFD) changes the content of several mouse liver proteins involved in intracellular detoxification and recognized as precancerous, cancerous, and senescence markers. Since deregulation in apoptosis is known to represent a pro-tumorigenic principle, this work examined whether feeding PFD, PFD supplemented with Met (PFD+Met), and PFD followed by 24 h of normal diet (PFD+N) change the content and activity of apoptosis markers in Balb/c mouse liver. Thus, caspase-3, apoptosis inhibitory protein (XIAP), and apoptosis inducing factor (AIF) were evaluated. For this, mitochondrial and cytosolic fractions were subjected to Western blot and enzymatic tests. Compared to controls under normal diet, the liver of mice fed with PFD showed a decrease in caspase-3 activity, an increase of cytosolic XIAP, and a decrease of mitochondrial AIF. These results suggest that PFD inhibits the caspase-dependent apoptosis but activates caspase-independent apoptosis. On the other hand, feeding mice with PFD+Met partially prevented the effects of PFD on the features analyzed. The hepatoprotector effect of Met was similar to that found in PFD+N.

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CB-P10

Pin1 PROMOTES THE ACQUISITION OF AGGRESSIVE TUMOR PHENOTYPES BY ENHANCING MUTANT p53 FUNCTION

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Mutations in the TP53 tumor suppressor gene are among the most frequent genetic lesions in human cancers. The TP53 gene is most frequently hit by missense mutations that lead to the expression of point mutants which accumulate in tumor cells. P53 mutants may acquire new oncogenic functions and concur to the development metastatic phenotypes. In spite of extensive studies, it is not fully understood how mutant p53 acquires oncogenic function. To dissect the mechanisms underlying mutant p53 function, we focused on factors that might link cancer-related signaling with mutant p53 activity. We identified the prolyl isomerase Pin1 as a critical regulator of mutant p53. We show that Pin1 interacts with p53 point mutants and that Pin1 is necessary for different mutant p53 activities such as the cooperation with cell transformation and the enhancement of tumor xenografts proliferation in nude mice. The analysis of the impact of Pin1 and mutant p53 on tumorigenesis in vivo showed that Pin1 enhances tumorigenesis in mutant p53 knock-in mice, that model the Li-Fraumeni syndrome. The cooperation between Pin1 and mutant p53 also affects the behaviour of human tumor cell lines, were Pin1 enhanced migration and invasion through amplification of mutant p53 function. We also characterized different molecular mechanisms underlying the cooperation between Pin1 and mutant p53.

CB-P11

CONDUCTING POLYMER NANOPARTICLE MEDIATED PHOTOHERMAL TUMOR THERAPY

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Since living tissue absorbs little light between ca. 700 and 1200 nm, light in this wavelength region could penetrate several centimeters into de the body to be absorbed by a suitable mediator. Gold nanoparticles and carbon nanotubes have been used in photothermal tumor therapy. Conducting polymer (e.g. Polyanniline) nanoparticles have a broad absorption band, due to free carrier absorption, with a maximum in the NIR range. Polyaniline nanoparticles can be easily synthesized by nucleation and growth polymerization, using poly(vinylpyrrolidone) (PVP) as stabilizer. The stabilized PANI nanoparticles form a stable suspension at physiological pH. Tests using a NIR (780 nm, 100 mW) laser show a clear photothermal heating. The intake of PANI nanoparticles into the cells was followed by UV-vis spectroscopy, using the characteristic spectra of PANI. Then immobilized cells are incubated in PBS buffer containing the dispersed nanoparticles. It is found that, below a concentration threshold, PANI nanoparticles are innocuous to the cells in the dark. It was found that the cytotoxicity of the particle depends on the molecular weight of the PVP used. Irradiation of the cells with NIR light produces cell death. It is envisaged that, being made of organic polymers, the nanoparticles could be more easily biodegraded in the body than carbon nanotubes or gold nanoparticles.

CB-P12

CYTOTOXICITY AND OXIDATIVE STRESS INDUCED BY A COMPLEX OF Cu (II)-VALSARTAN IN OSTEOBLAST CELL LINES

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Several metal compounds show pharmacological properties. Cu(II) exerts essential functions in bone and in the control of inflammatory processes underlying different illnesses (cancer, hypertension, etc). The complexation of metals with pharmacological compounds favors the use of lower doses by synergism of their pharmacological properties. We report the effects of a complex of Cu(II)with the antihypertensive valsartan (Cu-val) in two osteoblastic cell lines (MC3T3-E1 and UMR106). Cu-val showed dose dependent cytotoxicity in both cell lines. It caused a significant decrease in the crystal violet assay from 75 and 25 µM in UMR106 and MC3T3-E1, respectively ($p<0,001$). A decrease in Neutral Red uptake was detected in MC3T3-E1 from 50 µM ($p<0,001$). Reduction of MTT was observed from 50 and 25 µM in UMR106 and MC3T3-E1, respectively. On the other hand, Cu-val caused a ROS increment from 10 and 25 µM in UMR106 and MC3T3-E1, respectively ($p<0.001$). Morphological studies showed important nuclear and cytoplasmic alterations. However, studies on plasmidic DNA cleavage were negative. Altogether these results suggest that the antiproliferative effect of Cu-val is exerted, at least in part, through an increment in the oxidative stress. This complex is an interesting compound to be further evaluated in animal models of cancer since it may offer a different alternative for tumor chemotherapy.

CB-P13

CHLORPYRIFOS PRODUCES DISSIMILAR EFFECTS ON PROLIFERATION IN BREAST CANCER CELLS

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A large body of literature suggests that environmental chemicals are involved in breast carcinogenesis. It has been postulated the use of the organophosphorus chlorpyrifos for replacing the current pesticide for fruit-bearing trees treatment in Alto Valle de Rio Negro and Neuquen. The aim of the present study was to evaluate the effect of chlorpyrifos on cell proliferation of human breast cancer cell line, MCF-7. Proliferation was evaluated by clonogenic assay and bromodeoxyuridine (BrdU) incorporation; cell viability by MTT assay. Reactive oxygen species (ROS) were determined using DCF fluorescent staining and flow citometry. Protein expression was assessed by Western blot. Our results indicate that high concentrations of chlorpyrifos (50 ìM) decreased 40% clonogenic proliferation and 18% cell viability. The inhibitory effect on proliferation was associated to an increase of 32% in the content of ROS and 48% p27 protein expression. On the other hand, exposure of cells to low concentrations of chlorpyrifos (50 nM) stimulated BrdU incorporation in 88%. This effect was related to an enhanced expression of proliferating cell nuclear antigen (PCNA) and cyclin E. We conclude that chlorpyrifos modulates proliferation in a dose dependent way, increasing proliferation at low doses while decreasing it at high concentrations.

CB-P14

ROLE OF PTH AND PTHrP IN THE REGULATION OF CELL CYCLE IN COLON ADENOCARCINOMA CELLS

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Parathyroid hormone (PTH) functions as a major mediator of bone remodeling and as an essential regulator of calcium homeostasis. Parathyroid hormone-related protein (PTHrP) was initially identified through its role in humoral hypercalcemia of malignancy and is able to interact with PTH receptor type 1. In this study, we investigated the role of PTH and PTHrP in the regulation of the cell cycle in human Caco-2 intestinal cells. First, the nuclei were stained with propidium iodide and the DNA content was measured using a flow cytometer. The results revealed that PTH treatment (10^{-8} M, 24 h) increases the number of G0/G1 phase cells and diminishes the number of S phase cells respect to control. In addition, analysis by western blot showed that the hormone induces the expression of the inhibitory proteins p27 and p15 and diminishes the expression of cyclin D1 and D3. However, the amount of p21 was not different in the absence or presence of PTH. By contrast, and although they share the same receptor, there was no change in the expression of these proteins after exposure of Caco-2 cells to PTHrP (10^{-8} M). Taken together, our results suggest that PTH induces changes in the expression of proteins involved in cell cycle regulation and produces G0/G1 phase arrest of Caco-2 intestinal cells.

CB-P15

MANNOSE-6-PHOSPHATE/INSULINLIKE GROWTH FACTOR II RECEPTOR IN DIABETIC RAT MAMMARY CARCINOGENESIS

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We developed mammary tumors in normal and diabetic rats (streptozotocine induced diabetes) by three ip. injections of N-Nitroso-N-Methylurea (NMU) at 50, 80 and 110 days of animals life. The aim of this work was to study the expression of insulin-like-II receptor (IGF-IIR) in mammary gland during carcinogenesis in relation to proliferation markers PCNA and Ciclin D1. Four groups of rats were employed: 1) control; 2) NMU-injected; 3) diabetic; 4) diabetic-NMU injected. Mammary tissue was processed at 60, 90, 120 and 180 days of animal life.

IGF-IIR expression, determined by inmunoblot and immunohistochemistry, was high in group 1 and 3 at all times while in group 2 the expression was reduced with mammary tissue transformation and was significant since 90 days of life. In diabetic-NMU injected animals only a very slight decrease in IGF-IIR expression was evident. Proliferation markers, PCNA and Ciclin D1 expression, were inversely correlated to IGF-IIR expression. When tumors were analyzed, IGF-IIR expression was clearly higher in those developed in diabetic animals. Also, PCNA and Ciclin D1 expression were lower and related to a slower growth rate. These malignant lesions showed a more differentiated histological pattern and longer latency periods than tumors of non diabetic rats. Our results are in correlation with the idea that IGF-IIR acts as a tumor suppressor gene.

CB-P16

EFFECT OF OLIGOELEMENTS AND *Lachesis muta* ON RAT COLONIC CHEMICAL CARCINOGENESIS

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We have previously reported the antitumoral effect of the combination of oligoelements Se, Zn and Mn (4 µg/ml each, Merck) plus Lachesis Muta (4 ng/ml whole venom, Sigma Co) (O-Lm) on experimental mammary and pancreatic carcinomas (Int J Cancer, 2002; Anticancer Res 24:3434-35, 2004; Biocell 29(Supl)102,2005. In the present work we investigated whether O-Lm could prevent rat colonic carcinogenesis induced by 1,2-dimethylhydrazine (DMH).

Two month-old male rats (n=40) received 20 subcutaneous (s.c.) weekly injections of DMH (30 mg/Kg). Half of the animals received a daily s.c. O-Lm injection (0,5 ml) starting 10 days before the first DMH administration; this treatment continued during the entire experimental period. Animals were sacrificed at 12, 18, 21, 24, 28 weeks and histopathological (H&E and PAS staining) and immunohistochemical analyses of PCNA, Ciclin E, Bax, Bcl-2, and antioxidant enzymes were performed in complete colonic mucosa. Results show that O-Lm treatment significantly reduced the number of colonic DMH-induced tumors. This effect was associated with a decrease in PCNA expression, a modulation of pro and antiapoptotic proteins and antioxidant enzymes along the carcinogenic process.

We conclude that O-Lm is a preventive agent of colon carcinogenesis in this experimental model through the modulation of the apoptotic and proliferation process.

CB-P17
IS CALRETICULIN PRESENT IN THE CELL MEMBRANE ARGINYLATED?
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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 which is mediated by arginyl-tRNA protein transferase (Ate 1). We have demonstrated the posttranslational arginylation of calreticulin (CRT) in vitro and in living cells. The arginylated form of CRT (R-CRT) is present in the cytoplasm, in contrast to the non arginylated CRT, resident of the endoplasmatic reticulum. In this work we show by immunocytochemistry in non permeabilized cells that R-CRT is also present at the cell membrane. There is ample evidence of a role for CRT in the cell surface which includes its involvement in thrombospondin-induced disassembly of focal adhesions and a participation in immunogenic cell death. We have previously demonstrate an implication in cell adhesion so we explored the role of RCRT in the modulation of cell adhesion from the membrane by performing focal adhesion disassembly assay. Moreover, we show by flow cytometry that cells lacking of Ate1 enzyme are significantly resistant to apoptosis compared to WT cells using different apoptostic inducers.
This results indicate that changes in protein arginylation regulates protein fate and cell survival.
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CB-P18
ENDOPLASMIC RETICULUM CALCIUM REGULATES *T. cruzi* CALRETICULIN RETROTRANSLOCATION TO THE CYTOSOL
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For most secretory pathway proteins, crossing the endoplasmic reticulum (ER) membrane is an irreversible process. However, in some cases this flow can be reversed. For instance, misfolded proteins retained in the ER are retrotranslocated to the cytosol to be degraded by the proteasome. This mechanism, known as ER associated degradation (ERAD), is exploited by several bacterial toxins to gain access to the cytosol. Interestingly, some ER resident proteins can also be detected in the cytosol or nucleus, being calreticulin (CRT) the most studied. Here, we show that in *Trypanosoma cruzi* a minor fraction of CRT localized to the cytosol. ER calcium depletion, but not increasing cytosolic calcium, triggered the retrotranslocation of CRT in a relatively short period of time. Cytosolic CRT was subsequenntly degraded by the proteasome. Interestingly, the single disulfide bridge of CRT is recuced when the protein is located in the cytosol. The effect exerted by ER calcium was strictly dependent on the C-terminal domain (CRT-C), since a CRT lacking it was totally retained in the ER, whereas the localization of an unrelated protein fused to CRT-C mirrored that of endogenous CRT. This finding expands the regulatory mechanism of protein sorting and may represent a new crossroad between diverse physiological processes.

CB-P19
STOICHIOMETRY AND DYNAMICS OF GOLGI GLYCOLIPID GLYCOSYLTRANSFERASE COMPLEXES
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Glycolipid glycosyltransferases (GT) are Golgi resident, type II transmembrane proteins, with a N-terminal domain (Ntd), constituted by a short cytoplasmic tail, a transmembrane, and a lumenal stem region, linked to a large C-terminal catalytic domain facing the lumen. They concentrate selectively in different sub-Golgi compartments, in an overlapped manner, acting in succession in the transfer of a sugar moiety from an activated donor onto ceramide-linked oligosaccharide acceptors. GT are known to exist as monomers, or to form a variety of enzyme complexes, comprising homodimers, heterodimers or oligomers, through their different domains. Up to now, the stoichiometry of reported complexes is unknown. To approach this issue, we performed Förster resonance energy transfer (FRET) microscopy experiments in living CHO-K1 cells to elucidate the spatio-temporal relationships between GalT2 Ntds and between GalT2 and GalNAcT. We found that GalT2 NTds are able to form homo-complexes in the Golgi membranes. Also, we determined that the stoichiometry of association in the hetero-complex GalNAcT/GalT2 in the Golgi apparatus is 1:2. This molar ratio of two enzymes acting in succession in the pathway of synthesis of glycolipids may have functional implications in determining the composition of glycolipids in cellular membranes.

CB-P20
NEOBIOSYNTHESIS OF GLYCOSPHINGOLIPIDS BY PLASMA MEMBRANE-ASSOCIATED GLYCOSYLTRANSFERASES
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Sialoglycosphingolipids (gangliosides) are synthesized in the Golgi complex before being moved via vesicular transport to the plasma membrane (PM), becoming components of the external leaflet. Glycosphingolipid catabolic enzymes (glycohydrolases) have been found to be associated with the PM, where they display activity on the membrane components. By biochemical and cell biology techniques, we explored the expression and activity of ganglioside glycosyltransferases at the PM of epithelial and melanoma cells. Both ectopically and endogenously expressed CMP-NeuAc:GM3 sialyltransferase (Sial-T2, GD3 synthase) were found to be able to sialylate GM3 at the PM (cis activity) using both the exogenous and endogenous donor (CMP-NeuAc) and acceptor (GM3) substrates. Next, we investigated whether ecto-Sial-T2 is capable to display a trans activity in living cells. It was demonstrated that ecto-Sial-T2 sialylates GM3 both absorbed into the plastic surface of microwells and expressed at the PM of neighbor cells. Thus, the relative interplay between glycohydrolases as well as *cis* and *trans* ecto-Sial-T2 activities emerges as a potential level of regulation of the local glycosphingolipid composition in response to different external and internal stimuli.

CB-P21

GLUCOSIDASE II REGULATES THE EXIT OF MISFOLDED GLYCOPROTEINS FROM THE ENDOPLASMIC RETICULUM

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Glucosidase II (GII) and UDP-Glc:glycoprotein glucosyltransferase (GT) are key players in glycoprotein biogenesis in the endoplasmic reticulum (ER). GII is a heterodimer composed of GIIá and GIIâ subunits that catalyzes the sequential removal of the two innermost Glc residues from the $Glc_3Man_9GlcNAc_2$ glycan transferred to nascent proteins. GII also removes the single Glc unit added to folding intermediates by GT. *In vitro* studies have shown that GII and GT recognize fully mannosylated N-glycans and that both de- and reglucosylation activities decrease together with the N-glycan Man content. Using a series of fission yeast mutants transferring truncated N-glycans (G2M9, G2M7, G2M6 or G2M5) to nascent proteins we found that also *in vivo* GII activity decreases with the Man content and that glycan recognition is mediated by the GIIâ subunit man6P receptor homologous domain. On the other hand, we observed in a set of yeast mutants that transfer deglucosylated N-glycans M9, M7, M6 or M5 that *in vivo* GT activity does not depend on the N-glycan Man content. Mannosidase I is an ER enzyme that trims more Man residues from glycoproteins that stay longer in the ER (misfolded species). The difference between GII and GT activities toward demmanosylated glycoproteins may be a key regulating checkpoint for the exit of glycoproteins from the folding quality control calreticulin/calnexin cycle

CB-P22

EXPRESSION AND CHARACTERIZATION OF FUNCTIONAL GLUCOSIDASE II β SUBUNIT

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Glucosidase II (GII) is a key player in glycoprotein biogenesis in the endoplasmic reticulum (ER). It is a heterodimer composed of a GIIá subunit that catalyzes the sequential removal of the two innermost Glc residues from the $Glc_3Man_9GlcNAc_2$ glycan transferred to Asn residues in nascent proteins, and a GIIâ regulatory subunit. GIIâ is responsible for GIIá retention in the ER and for N-glycan *in vivo* recognition by GII through its mannose 6 phosphate receptor homologue domain (MRH). Direct binding of the complete isolated GIIâ subunit to N-glycans has never been reported. Here we report the expression, purification and characterization of the *Schizosaccharomyces pombe* GIIâ subunit. Circular dichroism spectra of the affinity purified subunit showed that the protein has a predominantly á helix secondary structure. Purified GIIâ resulted active in a functional complementation test as mixing the microsomal fraction of a *S. pombe* mutant expressing only GIIá in the ER with the purified GIIâ subunit restored the ability of the catalytic subunit to efficiently hydrolyze the physiological substrate G1M9. This is the first report of the expression of an active isolated GIIâ subunit. The protein will be used to study its structure by NMR and to asses its binding specificity toward N-glycans

CB-P23

Rab24 MODULATES THE LAST STAGE OF MITOSIS

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Rab24 is an atypical member of the Rab GTPase family whose distribution in interphase cells has been characterized. In this study, we have analyzed the distribution of Rab24 throughout cell division. Rab24 was located at the mitotic spindle during metaphase and anaphase and in the midbody during cytokinesis. We have also observed co-localization of Rab24 with survivin, a protein involved in the regulation of mitosis. Interestingly, more than 90% of transiently transfected cells with Rab24 presented abnormal nuclear connections. These atypical connections were not observed in cells transfected with other Rab proteins. In addition, HeLa cells overexpressing Rab24, analyzed for up to 15h by time-lapse microscopy, remained connected by a chromatin bridge and did not undergo abscission. In contrast, cells overexpressing only GFP undergo a normal cytokinesis event and separate after 120 min. Furthermore, in CHO cells stably transfected with GFP-Rab24wt, we observed a large percentage of binucleated and multinucleated cells. These cells presented an extremely large size compared to stably transfected cells overexpressing GFP alone or a Rab24 mutant S67L. A marked increase in binucleated, multinucleated and multilobulated nucleus was also observed in HeLa cells silenced for Rab24. These findings suggest that Rab24 participate in a pathway involved in a late stage of the mitosis.

CB-P24

IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF *C. elegans* ER Gts

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Quality control mechanisms ensure that newly synthesized glycoproteins reach their properly folded conformation. The UDP-Glc glycoprotein glucosyltransferase (GT) which functions as a conformational sensor is the key element in this mechanism. The human genome encodes two GT homologues, HUGT1 and HUGT2, but only HUGT1p is active. The *C. elegans* genome encodes two GTs: F48E3.3 (CeGT 3.3) and F26H9.8 (CeGT9.8). We determined that *C.elegans* has a ten fold lower GT activity than rat liver and it shares common features with the GT of other organisms. Bioinformatic analysis showed that CeGT3.3p is around 34% identical to HUGT1p and HUGT2p while CeGT9.8p is 25% identical to them. Fold recognition methods showed that in the N-terminal region there are three consecutive thioredoxin like domains (with an á subdomain insertion) of the DsbA-like type in the first 800 residues. The C-terminal region contains a glyco-transf-8 domain responsible for the catalytic activity. We expressed CeGT3.3p and CeGT9.8p in *alg6-gpt1- S. pombe* cells and only the GeGT3.3p was active. Addition of a C-terminal HA epitope to both proteins abolished CeGT3.3 activity and CeGT9.8 became fully proteolysed. We expressed both proteins in *S. cerevisiae* but both lacked GT activity when labeled with HA epitope. These results suggest that CeGT3.3 could be the ER GT while CeGT9.8p may play another biological role in *C. Elegans*.

CB-P25
ROLE OF Rab1b IN COPII DYNAMICS AND FUNCTION
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In eukaryotic cells, proteins destined to be exported are translocated to the endoplasmic reticulum (ER) and are selectively sorted in specialized sites called "ER exit sites" (ERES). Selection and incorporation of the proteins in ERES are performed by Coat protein complex II (COPII). This coat is assembled by recruitment of Sec23/24 and Sec13/31 by Sar1 GTPase (activated by its GEF, Sec12). A further component, Sec16, acts as a platform for COPII assembly at ERES and it appears to stabilize Sar1-GTP. After vesicle budding, COPII is exchanged by COPI complex, a crucial step for ER-Golgi transport. Rab1b GTPase is essential to recruit COPI.
We have previously shown that Rab1b interacts with the COPII component Sec23. Furthermore, FRAP experiments co-expressing Sec13 and Rab1Q67L indicated that Rab1b activity affects Sec13 membrane association-dissociation kinetics at the ERES. Here we show that *in vivo* Rab1b also interacts with Sec24 and Sec31. In agreement, immunofluorescence assays show that Rab1b colocalizes with these COPII structures. Moreover, Rab1b inhibition delays cargo sorting at the ERES. In contrast with Sec13, Sec16 dynamics is not affected by Rab1Q67L, consistent with the fact that Sec16 acts upstream of Sar1. Our data suggest that Rab1b can interact and modulate dynamics of COPII components acting downstream of Sec16.

CB-P26
ROLE OF Rab1b GTPase IN A THYROID SECRETORY CELLLINE
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Rab1b GTPase is essential for protein transport between the endoplasmic reticulum (ER) and the Golgi complex. Rab1b is ubiquitously expressed and in some tissues with high secretory activity (like thyroid, placenta and bronchial epithelial cells) Rab1b mRNA levels are significantly augmented. The impact of Rab1b increase in secretory tissues has not been analyzed. In this work we aim to analyze the role of high Rab1b levels in a thyroid secretory cell line (FRTL-5). In these 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). GFP-Rab1bwt construct was transfected in FRTL5 cells incubated with and without TSH and NIS expression was analyzed by immunofluorescence and flow cytometry. Our results show that Rab1b overexpression increase NIS protein levels even in absence of TSH. This data suggest that Rab1b modulates NIS expression in absence of TSH stimulation. Finally, we analyze the impact of Rab1b level changes on regulation of NIS promoter activity by using luciferase reporter assays in FRTL5 cells. Our study indicates that Rab1b modulates NIS expression, suggesting a new Rab1b role in secretory tissues

CB-P27
MOLECULAR CHARACTERIZATION OF THE INTRACELLULAR TRAFFIC OF LRP1 AND ALPHA 2-M/LRP1 COMPLEX
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The LDL receptor-related protein 1 (LRP1) is an endocytic receptor involved in the á2-Macroglubulin (á2M*) internalization. Previously we demonstrated that LRP1 mediated the á2M*-induced intracellular signaling activation. However, the molecular regulation of the LRP1 signaling and endocytosis activity are not well established. In this work we tried to characterize the LRP1 intracellular traffic with Alexa-Fluor á2M* using pull-chase experiments at 37 °C (0 to 60 min) with a previous binding step at 4 °C (30 min). The intracellular localization of Alexa-Fluor á2M* was examined by confocal microscopy using specific fluorescent antibodies against intracellular vesicles. The clatrin-mediated endocytosis of LRP1 and á2M*/LRP1 complex was compared with transferring receptor (TfR), using Alexa-Fluor Tf, and specifically blocked by a negative Eps15 mutant (EÄ95/295). Our data demonstrated that á2M* is clatrin-dependent internalized by LRP1, since it was fully blocked in cells transiently expressing EÄ95/295. Then, we show that LRP1-á2M complex is localized in early endosomes at 10 min of ligand internalization. After this time, Alexa-Fluor á2M* is localized in late endosomes and lysosomes, whereas LRP1 is in recycling endosomes. Our data suggest that the signaling activity of LRP1 induced by á2M* occur in the plasmatic membrane and/or in early endosomes.

CB-P28
CHOLESTEROL LEVELS DETERMINE THE ENDOCYTIC ROUTE FOLLOWED BY THE ACETYLCHOLINE RECEPTOR
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Stability of the nicotinic acetylcholine receptor (AChR) at the cell surface is critical to the correct functioning of the cholinergic synapse. Cholesterol (Chol) is an essential lipid that modulates AChR. We have studied the endocytosis of AChR in CHO-K1/A5 cells, a cell line heterologously expressing murine muscle adult-type receptor under different Chol membrane contents. Contrary to the norm, endocytosis of cell-surface AChR is accelerated by depletion of membrane Chol. This acceleration is no longer operative when membrane Chol levels are restored. We explored the possible mechanism involved in receptor loss in Chol depleted cells (Chol-). Under such conditions the AChR is internalized by a ligand-, clathrin- and dynamin independent mechanism, which does not involve the presence of the AChR-associated protein rapsyn. The small GTPase Rac1 is required: expression of a dominant negative form of Rac1, Rac1N17, abrogates receptor endocytosis. The accelerated internalization of AChR proceeds even upon disruption of the actin cytoskeleton and is furthermore found to require the activity of the small GTPase Arf6 and its effectors Rac1 and phospholipase D. Thus, membrane Chol appears to act as a key homeostatic regulator of cell-surface receptor levels, determining not only the rate but also the mechanism of AChR endocytosis.
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CB-P29

Rab11 IS PHOSPHORYLATED BY CLASSICAL AND NOVEL PKC ISOENZYMES UPON SUSTAINED PMA STIMULATION

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Bioinformatic analysis showed that Rab11 has high probability of being phosphorylated by different PKC isoforms. In this work, we present evidences that Rab11 is phosphorylated by cytosolic kinases upon phorbol esters stimulation using an *in vitro* assay. Several purified PKC isoforms directly phosphorylated Rab11 and the results indicate that Rab11 was an adequate substrate for classical PKCá and PKCâII but not PKCâI isoenzymes. In addition, the novel PKCå and PKCç but not PKCä isoforms phosphorylated *in vitro* Rab11 upon PMA activation, whereas the atypical PKCî isoenzyme was unable to phosphorylate this GTPase. Rab11 was also phosphorylated *in vivo* in PMA-treated HeLa cells. Interestingly, overexpression of both Rab11 and the different PKC isoforms did not alter transferrin recycling in unstimulated cells. However, a sustained PMA-induced activation resulted in the translocation of the classical PKCá and PKCâII to the endocytic recycling compartment enriched in Rab11 and in the transferrin recycling inhibition. This is the first report showing that Rab11 is differentially phosphorylated by distinct PKC isoenzymes and, that this post-translational modification could be a mechanism for the regulation of intracellular trafficking pathways controlled by Rab11.

CB-P30

***Chlamydia trachomatis* USURPS HOST Akt KINASE NETWORK FOR ITS BENEFIT**

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Chlamydia trachomatis, an obligate intracellular pathogen, is the most frequent sexually-transmitted bacterial disease worldwide. This bacterium usurps host cell components to generate its intracellular replicative vacuole called inclusion. Chlamydial inclusion diverts from the endocytic pathway but interacts with the biosynthetic/exocytic via. Different host cell lipids including sphingolipids are essential for *Chlamydia* development. We have shown that Rab14, a key regulator of vesicular transport between the Golgi apparatus and early endosomes, is recruited to the inclusion and is involved on sphingolipids delivery from Golgi to the inclusion. On the other hand, it has been described that Akt, a Ser/Thr kinase, phosphorylates AS160, a GAP (GTPase Activating Protein) of Rab14. The phosphorylation of AS160 results in the inhibition of its GAP activity, leaving Rab14 in its active state bound to GTP. We analyzed the effect of specific Akt inhibitors in HeLa cells overexpressing GFP-Rab14wt infected with *C. Trachomatis*. Akt inhibitors decreased chlamydial inclusion size and bacterial multiplication. Furthermore, Rab14 was not recruited to the inclusion and the transport of sphingolipids from the Golgi to the inclusion was significantly diminished. These results suggest that *Chlamydia trachomatis* selectively uses Akt pathway to activate Rab14-mediated sphingolipid transport.

CB-P31

THE RhoA GTPase AND THEIR EFFECTORS mDia1 AND ROCK ARE INVOLVED IN *Coxiella burnetii* PHAGOCYTOSIS

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Phagocytosis is an important defense mechanism against pathogens that is regulated by actin cytoskeleton. GTPases of Rho family (Cdc42, Rac1 and RhoA) are the mainly regulators of actin cytoskeleton. mDia1 and ROCK kinase are effectors of RhoA that participate in actin dynamics. *Coxiella burnetii* (*Cb*) is an intracellular pathogen that induces host actin reorganization during internalization by an unknown molecular mechanism. To analyze if *Cb* internalization is regulated by Rho GTPases, HeLa cells were transfected with plasmids encoding negative forms of these GTPases and then infected. We observed that negative forms of Rac1 and, in less extent, RhoA inhibited *Cb* internalization. To assess the participation of mDia1 in bacterial internalization, HeLa cells were infected after transfection with pEGFPmDia1-full length, -ÄN3 (FH1-FH2 domain) or -N1 (GBD domain). We observed that *Cb* internalization decreased in cell over-expressing the mDia1-N1. This result suggests that actin polymerization activity of mDia1 (FH1-FH2 domain) is important for *Cb* internalization. To test if ROCK is involved in the internalization, cells were incubated with Y-27632 (ROCK inhibitor) prior infection. The inhibitor diminished the number of *Cb* internalized what suggests the ROCK participates in this process. These results suggest that Rac1 and RhoA, through ROCK and mDia1 play role in *Cb* phagocytosis.

CB-P32

***Coxiella burnetii* INFECTION TO THE HOST CELL NEEDS Arf6 AND ITS INTERACTION PROTEINS EFA6 AND PIP5K**

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It is known that remodeling of host actin cytoskeleton is needed for phagocytosis of *Coxiella burnetii* (*Cb*) nevertheless the molecular mechanism is poorly characterized. The GTPases Arf6 is implicated in phagocytosis regulating actin cytoskeleton through PIP5 kinase and phospholipase D (PLD) activation. GTPases need to be loaded with GTP by a guanine-exchange factor (GEF) to become active. To determine if EFA6, an Arf6 GEF, is involved in *Cb* phagocytosis, HeLa cells over-expressing EFA6 WT or its inactive mutant R386E were infected and processed for indirect immunofluorescence to quantify the internalized bacteria. We observed that the EFA6-R386E inhibited *Cb* internalization. This result agrees with the previous one observed in cells over-expressing an Arf6 negative mutant. We study PIP5K activation during *Cb* internalization determining the formation of PI(4,5)P2, the product of this kinase. To this end, cells over-expressing GFP-PH, a PLCä-PH domain which binds PI(4,5)P2, were infected for different times. We observed that after 15 min infection GFP-PH accumulated at the membrane in close contact with the bacteria. To evaluate PLD participation in *Cb* infection we inhibited PLD with 2-butanol cell treatment. We observed no effect in the *Cb* internalization. In summary our results suggest that host cell infection with *Cb* is regulated by Arf6 and their interacting proteins EFA6 and PIP5K.

CB-P33

CORTACTIN PHOSPHORYLATION AND Src KINASE ACTIVITY ARE INVOLVED IN *Coxiella* b*urnetii* PHAGOCYTOSIS

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Cortactin is an actin interacting protein that regulates ruffles and lamellipodia formation during cell migration. It has been also involved in phagocytosis of *E.coli*, *Chlamydia*, *Shigella* and *Listeria*. Cortactin has domains to interact with the actin dynamic machinery and its function is regulated by Tyr and Ser phosphorylation catalyzed by Src and Erk kinases, respectively. *Coxiella burnetii* (*Cb*) is an intracellular pathogen that enters to host cell by a poorly characterized mechanism. We study early steps of *Cb* phagocytosis related to actin cytoskeleton. We have observed that Tyr dephosphorylation of cortactin is required for *Cb* phagocytosis. To study if Src is involved in this process, HeLa cells overexpressing cortactin mutants were treated with SU6656, a Src inhibitor, before infection. We estimate phagocytic activity measuring internalized bacteria by indirect immunofluorescence and confocal microscopy. We observed that SU6656 affected *Cb* phagocytosis. On the other hand, we quantified the Src and cortactin phosphorylation stage by SDS-PAGE and Western Blot in lysates obtained from HeLa cells infected for different times. We detected highest phosphorylation levels at 15 min of infection for Src and 60 min for cortactin. These results suggest that Src activation and cortactin phosphorylation could be important at the early and late steps of *Cb* internalization, respectively.

CB-P34

TRANSLATIONAL CONTROL OF *reaper*, A PRO-APOPTOTIC GENE OF *Drosophila*

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Translational control is a key step in gene expression regulation during apoptosis. Proapoptotic genes of *Drosophila*, *reaper(rpr), hid, grim* and *sickle*, are downregulated by the miRNA family miR-2/6/11/13/308. miRNAs can act on different mRNAs and the same mRNA can be target of different miRNAs. In this complex scenario the differences in target recognition could result in different regulators recruited for different mRNA fates. We aim to the identification of regulatory factors controlling the cap-independent translation initiation of *rpr* mRNA. In this study we present the action of miRNAs on *rpr* mRNA translation. We designed a mini-*rpr*-Myc tagged gene for biochemical and functional studies in the natural context of the mRNA 5' and 3'UTRs, avoiding the use of reporter genes with tandem copies of miRNA targets. For translational regulation assay, S2 *Drosophila* cells were transfected with the synthetic mRNAs in two different conditions, with or without miR2 pre-hybridized. ELISA results have shown a down regulation effect for the *in vivo* translation of minigen transcript linked to miR2. Proteomic analysis has allowed identify several factors assembled in the 5' UTR of *reaper*. iRNA assay was designed to determinate feasible roles in the regulation machinery. Recent proteomic analysis with mini-transcripts provides interesting data about miRNA modulators and regulation of translation.

CB-P35

THE v-SNARE VAMP7 IS INVOLVED IN THE DEVELOPMENT OF THE *C. burnetii* REPLICATIVE VACUOLE

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Coxiella burnetii, the etiological agent of Q fever, is a Gram-negative obligate intracellular bacterium. It has been previously described that both the endocytic and autophagic pathways contribute to *Coxiella* replicative vacuoles (CRV) maturation, generating a large parasitophorous niche that shares characteristic of a phagolysosome-autophagolysosomal compartment. We have recently demonstrated that the early secretory pathway also contributes to the *Coxiella*-vacuole development, since disruption of this pathway alters vacuole development. VAMP7 is a v-SNARE involved in both the secretory and endocytic pathways, which particularly participates in the heterotypic fusion between late endosomes and lysosomes. In the present work, HeLa cells transiently overexpressing GFP-VAMP7 were infected with *C. burnetii* for different periods of time. By confocal microscopy, we observed an interaction between GFP-VAMP7 and *C. burnetii* at different times after infection. The endogen protein was also detected at the CRV. siRNA against VAMP7 and a truncated mutant (VAMP7 NT) were used in order to evaluate the role of this protein in the CRV development. Our results indicate that both the knockdown of the protein as well as overexpression of the VAMP7 truncated mutant affected the development of the *Coxiella*-vacuole suggesting that VAMP-7 mediated fusion events are required for the process.

CB-P36

FUNCTIONAL ANALYSIS OF P- BODIES COMPONENTS IN *Drosophila melanogaster*

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The regulation of mRNA translation involves the remodeling of active ribonucleoprotein complexes (mRNPs) into an inactive state. Part of this process occurs in cytoplasmic granules known as processing bodies (P-bodies), which contain factors required for mRNA turnover and translational repression. The aim of this work is to study the components of P-bodies in *Droshopila melanogaster* and the interactions involved in mRNP remodelling. We have cloned several components of P-bodies as fluorescent protein fusiones. S2 and HeLa cells were transfected with the constructs and analyzed for P-bodies formation. The proteins Me31b, Lsm1, Bruno and eIF4E were found in P-bodies. In vivo analysis of interactions was studied by fluorescence resonance energy transfer (FRET). We determined the interacting pairs eIF4E and Me31b. RNAi was performed to dilucidated changes in the complex stability to establish a model for sequential remodeling.

CB-P37

CHARACTERIZATION OF A MAMMALIAN PALMITOYLTRANSFERASE THAT MODIFIES TRANSMEMBRANE SNARES PROTEINS

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Protein palmitoylation or S-acylation is a post-translational modification affecting a large number of proteins. The enzymes responsible for this modification are characterized by the presence of a DHHC-Cysteine Rich Domain. The yeast *S. cerevisiae* has 7 members of this family in its genome, and there are 23 in mammals. Several transmembrane SNAREs, are palmitoylated both in yeast and mammals. In yeast, this modification is carried out by the Swf1 protein. The mammalian orthologue, has not been identified. Although these proteins display low conservation outside the DHHC domain, sequence analyses point to DHHC4 as a putative candidate.

To investigate if DHHC4 is the enzyme responsible for mammalian SNAREs palmitoylation, we generated a shRNA against the DHHC4 sequence, to knock-down the protein expression levels in HEK 293 cells. The efficiency of the knock-down was assessed using specific antibodies against the DHHC4 C-terminus, generated in our laboratory. This antibodies are also being use to study the intracellular localization of this protein.

Initially, we used DHHC4 silenced HEK 293 cells, to assess the palmitoylation status of the yeast SNARE Tlg1. We have found that Tlg1 palmitoylation is severely reduced in these cells, suggesting that DHHC4 might indeed be the mammalian SNARE palmitoyltransferase. Several mammalian SNAREs are currently being tested in the same manner.

CB-P38

CHO CELLS EXPRESSING THE P5-ATP13A2 ARE MORE SENSITIVE TO THE TOXIC EFFECTS INDUCED BY PARAQUAT

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The P-type ion pumps are membrane transporters energized by hydrolysis of ATP. They have been classified into five subfamilies termed P1-P5. Five genes named ATP13A1-5 that belong to the group of P5-ATPases have been identified in humans. Mutations of the ATP13A2 gene underlie a form of parkinson (PD). The ion transported by the P5-ATPases is not known; it was suggested that were Ca^{2+} transporters, nevertheless we have shown that this enzyme is not a Ca^{2+} transporting pump. It was recently reported that the deletion of the closely related P5-ATPase CATP-5 of C. elegans is responsible for the tolerant phenotype seen in the presence of the toxic polyamine analog norspermidine.

We investigated the effect of ATP13A2 overexpression on the viability of CHO cells to the toxic polyamine analog paraquat (1,1´-dimethyl-4,4´-bipyridinium) by measuring the activity of the endogenous enzyme hexosaminidase. CHO cells expressing the ATP13A2 were eight times more sensible to paraquat treatment than CHO cells transfected with the empty vector. The quantization of paraquat-induced production of cellular reactive oxygen species (ROS) measured with the 5(6)-carboxy-2,7-dichlorodihydrofluorescein diacetate probe showed a significant increase in CHO cells expressing the ATP13A2 pump. These results favor the idea that the P5-ATPase ATP13A2 is involved in polyamine transport.

CB-P39

ALDOSE REDUCTASE ACTIVATION AND NA+,K+-ATPase INHIBITION INDUCED BY HIGH GLUCOSE

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The association of tubulin with the membrane induced by glucose and sorbitol was determined in COS, CAD and human erythrocytes cells. When these cells was exposed to high levels of glucose or sorbitol was observed: a) association of tubulin to the membrane, b) formation of a complex acetylated tubulin/Na+,K+-ATPase and inhibition of the enzimatic activity, c) microtubule formation, and d) aldose reductase (AR) activation in a dependent-manner of microtubule formation. These effects triggered by high glucose are present in erythrocytes of diabetic patients and different tissue with non-insulin dependent glucose transporters. Na+,K+-ATPase (NKA) activity is reduced by 50 % in membranes of human erythrocytes from diabetic individuals whereas AR activity was increased in a % and the membrane tubulin increase more than 200 % in these individuals. Immunoprecipitation analysis showed that acetylated tubulin forms a complex with NKA in erythrocyte membranes of diabetic humans. These results suggest that glucose trigger a synergistic effect of tubulin and sorbitol, leading to AR activation and NKA inhibition by microtubules formation.

CB-P40

DECREASED ERYTHROCYTE DEFORMABILITY IS INDUCED BY AN INCREASE IN MEMBRANE TUBULIN

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In hypertension numerous alterations in blood rheology have been described such as red cell deformability. We previously shown that erythrocytes from hypertensive patients have 200% more membrane tubulin. In addition, we determined that acetylated tubulin forms a complex with Na^+,K^+-ATPase (NKA) and inhibits the enzimatic activity. Now, we determine the effect of tubulin content on the red cell deformability. For this, normotensive and hypertensive human erythrocytes were incubated with taxol and nocodazole respectively, in order to increase or decrease the content of membrane tubulin. Then, we determined the membrane tubulin content, NKA activity and deformability. The results show that increases in the membrane tubulin content induces a decreased in NKA activity and deformability. On the other hand, Wistar and SHR rats were injected with the same drugs. Results shown that a decreased in membrane tubulin content is related with increases in deformability and conversely, an increased in the membrane tubulin content is related with a decreased deformability. These results show that content of tubulin membrane, both in vitro and in vivo, affects the deformability of erythrocytes. Since erythrocytes of hypertensive patients contain more tubulin membrane, the increase in the content of tubulin could be the cause of the low deformability of erythrocytes of hypertensive patients.

CB-P41

SIGNALING PATHWAYS INVOLVED IN TNF-α INHIBITION IN PBMC BY *Lactobacillus reuteri*

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Lactobacillus strains have the capacity to modify cytokines production in different cells. However, the molecular mechanisms involved in this effect are not yet well understood. Previous investigations in our laboratory showed that *L. reuteri* CRL 1098 reduced TNF-á production in human peripheral blood mononuclear cells (PBMC), and this effect depended on membrane rafts integrity. The aim of this work was to investigate the signaling pathways involved in the decreased TNF-á production induced by *L. reuteri* CRL 1098 in PBMC. Using Western blot techniques it was demonstrated that when PBMC were co-incubated with *L. reuteri* cell-free supernatant, Lck protein migrated from rafts to non-rafts regions of the membrane. Also, different signaling proteins were modified after the incubation with the bacterial supernatant: increased levels of PI3K, pERK ½, and p-p38 were observed. Citoplasmic fraction of p65 NF-êB decreased while nuclear fraction of p65 NF-êB increased, indicating that this transcription factor could be partially responsible for TNF-á diminution. We postulate that *L. reuteri* could interfere with the ability of NF-êB to bind to DNA targets.
These results provide clues to understand the mechanisms by which *L. reuteri* reduces TNF-á production in PBMC and demonstrate for the first time, interaction between a non-pathogenic bacterium and membrane rafts.

CB-P42

INHIBITION OF MITOCHONDRIAL COMPLEX III BY NITRIC OXIDE

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NO is a physiological regulator of mitochondrial function: NO inhibits cytochrome oxidase activity competitively with O_2 and electron transfer between cyt. b and c. Little is known about how NO interacts with the NO-reactive component of the ubiquinone-cyt. b area of the mitochondrial respiratory chain. The aim of this work was to study the NO inhibitory effect on electron transfer between cyt. b and c. Succinate-cytochrome c reductase activity, and H_2O_2 and O_2^- production rates were measured using beef heart submitochondrial particles and S-nitrosoglutathione (GSNO; 0-750ì M) as NO donor. NO inhibited succinate-cytochrome c reductase activity with a maximal effect (55%) at 500ì M GSNO (6ì M NO) being this inhibition independent on [O_2]. The effect of NO on respiration chain produced a hyperbolic increase in O_2^- and H_2O_2 production with a maximal rate at 500ì M GSNO (1.1±0.2nmolO_2^-/min.mgprotein; 0.55±0.05nmol H_2O_2/min.mgprotein). The ratio O_2^-/H_2O_2 was 2.0 in accordance to the stoichiometry of the O_2^- dismutation reaction. At 150ì M GSNO (2ì M NO) succinate-cytochrome c reductase activity was inhibited by 33% while O_2^- and H_2O_2 productions were increased by 36% and 31%, respectively. These preliminary results show that the interaction of NO with complex III leads to the inhibition of mitochondrial electron transfer and an enhancement of O_2^- production by ubisemiquinone autooxidation

CB-P43

PLEIOTROPIC EXPRESSION OF GENES INVOLVED IN NEUROTRANSMITTERS METABOLISM IN FLIES

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The acylation of neurotransmitters is important for the homeostasis of neural system but also gives rise to important metabolites in insects. The best known are N-â-alanyldopamine (NBAD), the main sclerotization precursor of insect brown cuticles, and carcinine (N-â-alanylhistamine), an essential compound of the visual system. Key enzymes of this metabolism are NBAD-synthase (NBAD-S) and NBAD-hydrolase (NBAD-H) which synthesizes and hydrolyzes â-alanyl derivatives respectively. These enzymes seem to function together in a system regulating the levels of NBAD and dopamine during cuticle sclerotization and of neurotransmitters in nervous tissue. Our aim was to study the expression of NBAD-S and NBAD-H in different tissues during the life cycle of *Ceratitis capitata* and *Drosophila melanogaster*. We also attempt to decipher the role of â-alanyl derivatives. We studied protein expression by western blots, immunohistochemistry and enzymatic activities. Mutant flies were subjected to behavioral studies. We found that the two enzymes are expressed during the whole life cycle, from embryo to adult. NBAD-S is expressed periodically in epidermis whereas NBAD-H is active in a constitutive manner. Both enzymes are expressed constitutively in neural tissue. Our results show that NBAD-S and NBAD-H are significant during the development of insects and for the physiological balance of neural tissue.

CB-P44

MITOCHONDRIAL PROTEIN MODIFICATIONS IN DOXORUBICIN-TREATED erbB4-KNOCKOUT MOUSE

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Neuregulin (NRG) growth factors through cognate tyrosine kinase receptors erbB2/erbB4 are required for the prevention of dilated cardiomyopathies.
Combined therapy with antibodies blocking NRG signaling and anthracycline derivatives produces objective tumor regressions. However, the combined treatment displays a undesired effect by increasing the incidence of severe cardiomyopathy in 30% of patients.
Our laboratory demonstrated that ventricular-specific erbB4 deletion (erbB4-KO) lead to dilated cardiomyopathy in adult mice.
We aimed to investigate the molecular modifications underlying the synergistic cardiotoxicity of anthracycline derivatives and the substantial blockade of the NRG pathway. The treatment of erbB4-KO mouse with doxorubicin (15 mg/kg, three times within a week) induces hypertrophic cardiac growth, and ventricular dilation with increased expresion of fetal cardiac genes. Then, we analyzed ventricular protein extracts by bidimensional electrophoresis (2D-PAGE), protein phosphorylation and further mass spectrometry on peptide digest to identify modified proteins in the erbB4-KO-doxo compared KO. Our comparative analysis of proteins in erbB4-KO-doxo versus WT/WT-doxo/erbB4-KO revealed differences in protein content and/or in the phosphorylation level of mitochondrial proteins.

CB-P45

PARSING THE MOLECULAR CODE FOR TWO NEURONAL ENHANCERS THAT UNDERWENT CONVERGENT EVOLUTION

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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. Hypothalamic POMC expression is driven by two different enhancers named nPE1 and nPE2. Although these two enhancers are evolutionarily unrelated, they are both able to drive *Pomc* expression to the same group of hypothalamic neurons. Expression studies in transgenic mice led us to the identification of a 140 bp region within nPE1 that is necessary and sufficient to drive reporter gene expression to POMC neurons. A comparative analysis of this critical nPE1 region with those previously identified in nPE2 evidenced a small number of short sequences that are present in both enhancers. To test the hypothesis that these shared sequences are critical for enhancer function, we designed transgenic constructs carrying a set of mutations that simultaneously eliminated all the identified sites. These transgenes failed to drive reporter gene expression to hypothalamic neurons demonstrating that the mutated sites play a fundamental role in the neuronal-specific regulation of *Pomc*. Further ongoing studies are aimed at determining the relative importance of each of the identified elements shared by nPE1 and nPE2.

CB-P46

Akt/GSK3β SIGNALING DURING IRON-INDUCED NEUROTOXICITY IN HT22 HIPPOCAMPAL NEURONS

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Akt and glycogen synthase kinase 3â (GSK3â) are key components of signaling pathways involved in synaptic plasticity and neuronal survival. The specific aims of this work were: i) to characterize an iron-induced neurodegeneration model and ii) to study the participation of Akt and GSK3â in the signaling events triggered during neuronal oxidative injury. For this purpose, a cell line of murine hippocampal neurons, HT22, was exposed to increasing Fe^{2+} concentrations (25, 50, 100 and 200 ìM) for 24 h. Cell viability, morphology and lipid peroxidation were evaluated for determining the extent of neuronal injury. Cell viability, measured as MTT reduction, was significantly reduced in the presence of 50, 100 and 200 ìM Fe^{2+}. HT22 cell morphology, evaluated by phase-contrast microscopy, showed evident morphological alterations including rounded cell body with a decreased number of cell projections. The decrease in MTT reduction strongly correlated with the changes in cellular morphology. Based on these data we defined that 24-h exposure to iron (25 and 50 ìM) caused a mild oxidative injury. Under these experimental conditions both Akt and GSK3â phosphorylation were increased. We conclude that iron-induced neurotoxicity activates Akt promoting GSK3â inhibition under mild oxidative injury in hippocampal neurons.

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CB-P47

PARTICIPATION OF c-Fos IN THE BIOLOGY OF NEURAL STEM/PROGENITOR CELLS OF THE CENTRAL NERVOUS SYSTEM

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c-Fos, a transcription factor involved in proliferation of normal and tumor cells, also activates phospholipid synthesis. An increased expression of c-Fos in brain tumors promotes growth by increasing the rate of lipid synthesis; in a mouse model of Neurofibromatosis type 1 (NPcis) that spontaneously develop tumors, no tumors develop in the absence of c-Fos. Cancer can be considered a disease of unregulated cell self-renewal in which mutations convert normal pathways of stem cell self renewal into engines for neoplastic proliferation. So, it results important to study stem cell biology and particularly that of c-Fos to understand tumor development. We observed that mice $c\text{-}Fos^{-/-}$ have three times less neural stem cells in the SVZ than mice $c\text{-}Fos^{+/+}$ (% stem cells/total cells: c-Fos ($^{-/-}$) 0.76%; $c\text{-}Fos^{+/+}$ 2.3%). In addition, after 28 days of culture, neural stem/progenitor $c\text{-}Fos^{-/-}$ cells show less proliferation in TH3 assays ($c\text{-}Fos^{-/-}$ 13693±3993 cpm; $c\text{-}Fos^{+/+}$ 41073±2224 cpm) and increased apoptosis as determined by annexinV *(*$c\text{-}Fos^{-/-}$ 3.05%; $c\text{-}Fos^{+/+}$ 1.47%). EMSA studies showed no changes in AP-1 content in both conditions, with activated synthesis of phospholipids in $c\text{-}Fos^{+/+}$ animals. These results evidence that c-Fos is involved in controlling self-renewal of neural stem cells and that it affects the comportment of neural stem/progenitor cells.

CB-P48

REGULATION OF Akt ACTIVITY BY SUMO CONJUGATION

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A cell generates complex responses upon a variety of stimuli that it receives within a multicellular organism. Our lab studies the molecular mechanisms by which different extracellular cues activate signaling pathways that control splicing factor activity at different steps of gene expression regulation. Based on our previous results demonstrating that: i) Pi3K/Akt pathway regulates alternative splicing; ii) Akt phosphorylates SR proteins, in particular SF2/ASF; and iii) SF2/ASF regulates SUMOylation, we proposed to explore a possible regulatory feedback loop among the components of the Pi3K/Akt/SR protein axis.We found that Akt1 and 2 are SUMOylation targets, as demonstrated by purification of SUMOylated proteins from His-SUMO overexpressing cells by Ni^{2+} affinity chromatography. We evaluated the effect of over-expressing different SUMO E3 ligases on Akt SUMOylation. Interestingly, SF2/ASF is capable of regulating Akt SUMOylation and phosphorylation levels. We are further dissecting the mechanism of Akt SUMOylation, its cross-talk with other Akt post-translational modifications, as well as the consequences on this kinase activity. Akt pathways are involved in many cellular functions and their dis-regulation associates with cancer, thus the understanding of Akt activity regulation is not only relevant for cell biology but may also help designing antitumoral therapeutic strategies.

CB-P49

EFFECT OF HO-1 INDUCTION ON LEYDIG CELL PROLIFERATION

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Heme oxygenase (HO) catalyzes the degradation of heme into three biologically active products: CO, biliverdine and Fe^{2+}. The HO system provides cellular protection through the concerted action of its derivatives involving anti-apoptotic, anti-inflammatory and anti-oxidative mechanisms in many injury and disease models. HO-1 (HO inducible isoform) expression responds to different stressors, pathophysiological states and it is interestingly increased in tumors. We previously reported that HO-1 induction modulates Leydig cell (LC) steroidogenesis and we suggested a cytoprotective effect on these cells.

In this study we aimed to evaluate the effects of HO-1 on LC proliferation. In order to achieve this, we tested different concentrations of hemin (HO-1 inducer) on MA-10 Leydig tumor cells and 33-day old Sprague-Dawley rat LC. Proliferation was assessed by MTT and ^{3}H-Thymidine incorporation assays. Cell viability was tested by Trypan blue exclusion method.

The results indicated that HO-1 inhibits LC proliferation in both experimental models when treated with hemin, in a concentration-dependent manner. Cytotoxic effects were not observed.

We suggest that HO-1 would be exerting one of its cytoprotective mechanisms by truncating cell cycle progression through mechanisms still to be elucidated.

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CB-P50

ANTIPROLIFERATIVE EFFECT OF HISTAMINE IN THE HUMAN ADRENOCORTICAL CARCINOMA CELL LINE H295R

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Histamine is a biogenic amine associated with heterogeneous functions carried out through the activation of four types of G protein coupled receptors. The current knowledge about this molecule points towards its ability to modulate proliferation in a wide variety of cell types.

The aim of this work was to evaluate the effect of Histamine on the proliferation of a human adrenocortical carcinoma cell line, H295R, and to elucidate the molecular mechanisms involved.

Proliferation was assesed by tritiated thymidine incorporation and MTT assays, using Histamine and specific receptor agonists and antagonists. Second messengers were also measured.

Here we report that Histamine inhibited proliferation in H295R cells. This effect was reproduced by specific Histamine receptor subtype 1 (HRH1) agonist and was prevented by the HRH1 antagonist. Competitive binding assay and immunofluorescence staining confirmed the presence of HRH1 in this cell line. Histamine did not affect cyclic AMP levels, whereas IPs were significantly increased by this amine.

In summary, the antiproliferative action of Histamine in the human adrenocortical cell line H295R would be mediated through the activation of the histamine receptor HRH1, leading to an increase in the intracellular IPs levels as the first step in this signalling pathway.

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CB-P51

***CISD1* GENE EXPRESSION MEDIATED BY CFTR ACTIVITY**

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Using Differential Display (DD), we have observed that *CISD1* gene expression was decreased in a Cystic Fibrosis cell model. The first objective was to corroborate the DD results. We have studied the *CISD1* expression levels by semi quantitative RT-PCR and RT followed by real-time PCR in the presence of CFTR inhibitors or activators in different cell models. The next objective was to determine the subcellular localization of CISD1. We have used the expression of a chimera bound to EGFP and confocal microscopy. It was observed that *CISD1* encodes a protein with mitochondrial localization. The function of this protein is unknown but other authors reported a possible role in oxidative phosphorylation and electron transport chain. We also study whether mitochondrial complex l (mCl) activity was affected by the differential *CISD1* expression. We have measured the *in gel* mCl activity by using Blue Native-PAGE (BN-PAGE), in mitochondria isolated from different cell models. We observed that the reduced mCl activity in CF cells can be restored by ectopic expression of CISD1, while CISD1 protein mutated in the binding site 2Fe2S interfered with normal mCl activity of non-CF cells. These results suggest that CISD1 might has a role in regulation of mCI activity and also, a role in the CF phenotype.

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CB-P52

TESTOSTERONE INHIBITS THE INTRINSIC APOPTOTIC PATHWAY INDUCED BY H_2O_2 IN C2C12 SKELETAL MUSCLE CELLS

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Apoptosis occurs in response to environmental or developmental cues, cellular stresses and specific cell death signals. Mitochondria trigger the initial events of the intrinsic apoptotic pathway via release of proapoptotic proteins. In previous work we demonstrated that testosterone protected against H_2O_2-induced apoptosis in C2C12 cells. In this study, we identified molecular events that occur during the anti-apoptotic effects of testosterone. At short times of exposure to H_2O_2, cells exhibit ERK2, Akt and Bad phosphorylation and an increase of HSP70 levels. At longer treatment intervals with the apoptotic agent, dephosphorylation of the proteins mentioned before, cytochrome c release and PARP cleavage occurs. Treatment with testosterone prior to H_2O_2 induced Bad inactivation, translocation of HSP90 to mitochondria and a decrease in Bax levels, showing a protective role of the hormone and a putative modulation of the apoptotic intrinsic pathway. Moreover, we observed an extra nuclear localization of the androgen receptor (AR) in mitochondria and microsomes. The fact that simultaneous treatments with testosterone, H_2O_2 and the AR antagonist, flutamide, reduce the effects of the hormone on Bax and PARP proteins, points to a possible participation of a non-classical AR in the anti-apoptotic effect of testosterone.

CB-P53

ESTRADIOL PROTECTS MUSCLE FROM APOPTOSIS INVOLVING MITOCHONDRIAL MEMBRANE POTENTIAL, Bax AND ERK

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We previously demonstrated that 17â-estradiol (E2), at physiological concentrations, abrogates apoptosis induced by oxidative stress (H_2O_2) in C2C12 skeletal muscle cells. We showed that the PI3K/Akt and MAPKs pathways are involved in these events. To further characterize the protective actions of E2, here we study the role of the proapoptotic protein Bax. By coimmunoprecipitation analysis, we show that Bax binds to the cytoplasmic protein 14-3-3 and ERK. When cells were treated with E2 increased Bax/14-3-3 interaction was observed. On the other hand, incubation of cultures with E2 diminished Bax/ERK complex levels, in accord with E2-dependent ERK translocation to mitochondria observed before. Apoptotic stimulation with H_2O_2 disrupts Bax/14-3-3 association and increases Bax/ERK interaction and these effects are reversed by E2 pretreatment. Moreover, we have investigated mitochondrial membrane depolarization by flow cytometry. Results show that H_2O_2 induces a decrease in mitochondrial membrane potential, which is prevented by E2. When the cells were preincubated with the ERK inhibitor U0126, the hormone was unable to avoid the effect of the oxidative stress suggesting a role for ERK in this event. These findings suggest that Bax, 14-3-3 and ERK are involved in negative regulation of muscle apoptosis by E2 affecting mitochondrial function.

CB-P54

COXSACKIEVIRUS B INFECTION INDUCES APOPTOSIS IN HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are self-renewing pluripotent cells that can differentiate to a wide range of specialized cells. Group B Coxsackie viruses (CVBs) produce acute myocarditis and chronic dilated cardiomyopathy. We have shown that hESCs and differentiated contractile embryoid bodies express CVB receptors and that CVBs infection of these cells causes a cytopathic effect compatible with cell death. The aim of the present work was to study if CVBs infection induces programmed cell death in hESCs. hESCs HUES-5 (H5) and WA-01 (H1) and CVBs (1 and 3) were used. Undifferentiated state of hESCs was validated by immunofluorescence and RT-PCR of pluripotent markers (TRA1-60, TRA1-81, SSEA-4, Oct-4 and Nanog). H1 and H5 cell viability (XTT assay) and apoptosis (DAPI staining, TUNEL and caspase-3-like activity assays) were measured at different time points post-infection (pi). Cell viability decreased moi dependently at 24hs CVB1 and CVB3 pi; DAPI and TUNEL positive apoptotic nuclei increased at 5 and 8 hours CVB3 pi and caspase-3-like activity peaks at 1 hour CVB3 pi. Quantification of anti-(Bcl-2, Bcl-XL) and pro-(Bax, Bad) apoptotic genes mRNA levels by RT-Real Time PCR showed a decrease in Bcl-XL mRNA levels for both H1 and H5 at 8hs CVB3 pi. Conclusions: cytopathic effect observed after hESCs infection with CVBs is compatible with an induction of apoptosis.

CB-P55

A PROTEOMIC APPROACH FOR THE IDENTIFICATION OF SERINE PROTEASE INHIBITORS IN SP AND MALE DUCT

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The presence of serine-protease inhibitors in seminal plasma (SP) and accessory sexual glands has been reported in several mammals. They play a role in balancing protease activities and are believed to be important in regulating the fertilization process, acting as decapacitating factors. An important application to improve the membrane alterations in cryopreserved sperm (resembling the capacitation process) might be the addition of these inhibitors to the sperm thawing medium. The aim of the present study was to investigate the presence of serine protease inhibitors in seminal vesicle (SV) and SP through proteomic analysis and to obtain recombinant inhibitors. Bidimensional electrophoresis was performed from mouse SV and bovine SP low-Mw protein extracts. A serine protease inhibitor, named SPINK3 was identified. This protein has a known inhibitory effect against Ca+2 uptake in mouse sperm. The corresponding cDNA was obtained by reverse transcription of mouse SV RNA. The cDNA encoding the mature protein was cloned into PGEX-4T-3 vector, expressed and the protein was purified. The biological activity of the fusion protein was confirmed by evaluating the acrosomal reaction and capacitation.
This is the first approach in obtaining this inhibitor with proteomic tools. Further studies are needed to analyze the ability of SPINK3 to protect against sperm cryoconservation damage.

CB-P56

PROTEIN PROFILING OF BOVINE OOCYTES *IN VITRO* MATURATED IN DEFINED MEDIA: A PROTEOMIC APPROACH

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Oocyte maturation and differentiation are accompanied by the expression of specific proteins. The maternal proteins are required for fertilization and the initiation of zygotic development. The identification of these proteins will provide insight into the regulation of early embryogenesis. The media currently used for *in vitro* oocyte maturation contains hormones and fetal bovine serum (FBS). The aim of this work was to evaluate the effect of a media with defined composition over bovine oocyte maturation by a proteomic approach. Oocytes were maturated in a media supplemented with r-hFSH and 10% FBS (control) or EGF, hyaluronan and cysteamine (EGF) until they reached the metaphase II stage. Denuded zona pelucida-free oocytes were analyzed by bidimensional electrophoresis and master gels were used to detect differentially expressed proteins. The results showed that 173 spots could be detected, where 68 were present in both treatments, 41 were exclusively found in the control condition and 64 were exclusive for the EGF condition. Among the spots shared by both treatments, 17 showed differential intensities, most of them increasing in the control condition. These results demonstrate an effect of the maturation media over oocyte proteomics. The identification of these proteins should serve as the molecular basis of maturation and a clue for the discovery of maturation markers.
(ANPCyT).

CB-P57

IDENTIFICATION OF A CALTRIN RECEPTOR PROTEIN IN RAT SPERMATOZOA

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Caltrin (calcium transport inhibitor) is a small and basic protein secreted by the seminal vesicles. During ejaculation, rat caltrin (6.2 kDa) specifically binds to the acrosomal region of the sperm head and inhibits sperm Ca^{2+} uptake. Thus, it prevents the spontaneous acrosomal exocytosis and protects the integrity required for sperm-egg interaction. The aim of this work was to identify the molecules responsible of the specific binding of caltrin to the sperm surface. The presence of receptor molecules was first examined by chemical cross-linking using NHS and EDC reagents to covalently bind caltrin to the receptors. Then, sperm extracts were subjected to SDS-PAGE and Western blotting with anti-caltrin antibodies. An immunoreactive band of 55 kDa was revealed suggesting the presence of a 49 kDa caltrin-receptor protein. This protein was isolated by affinity chromatography using recombinant caltrin bound to the fusion protein intein. Caltrin-intein complex expressed in *E. coli* was bound to chitin and then pour into a column. Sperm proteins extracted with 1% Triton X-100 were loaded in the column and those retained were eluted with 1 M NaCl and then analyzed by SDS-PAGE and MALDI TOF/MS. An enriched band of 48 kDa was identified as HongrES 1, an epididymal protein secreted by epithelial cells of the cauda. Like caltrin, HongrES 1 is synthesized under strict androgenic control.

CB-P58

IS RAT CALTRIN II PROTEIN A SECRETORY LEUKOCYTE PREOTEASE INHIBITOR?

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Rat caltrin (calcium transport inhibitor) is a small (6.2 kDa) protein from the seminal vesicle (SV) secretion that specifically binds to the sperm head during ejaculation and prevents the spontaneous acrosome reaction. It is also a potent inhibitor of sperm serine proteases. We have previously purified and partially sequenced a 15 kDa protein which inhibits sperm Ca^{2+} uptake. Although its partial amino acid sequence shows no identity with rat caltrin, it shares homology with some regions of that of guinea pig caltrin II, and SLPI (Secretory Leukocyte Protease Inhibitor) an inhibitor of different proteases including elastase, cathepsin G, chymotrypsin and trypsin. The aim of this work was to determine the complete sequence of this protein designated rat-caltrin II. Using a pool of SV cDNAs and specific and degenerated forward primers based on the sequences of SLPI and rat-caltrin II we amplified, by 3'RACE, a 600 bp fragment which sequence showed 98 % of identity with rat SLPI cDNA. Additionally, the predicted amino acid sequence was in agreement with that determined by MALDI TOF/MS of the purified rat-caltrin II protein. The remarkable homology allows to pointing out this cDNA as a product of rat SLPI gene suggesting a dual role of rat-caltrin II, as it was demonstrated with caltrin I.

CB-P59

EFFECTS OF EXOGENOUS SEXUAL HORMONES ON THE VAGINAL MICROBIOTA AND IMMUNE CELLS IN MICE

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The effect of progesterone (P) and estradiol (E) on several organs of an experimental animal model was evaluated. 2-months-old female BALB/c mice were inoculated with (P) (day -5) or E (day -2) to induce and maintain the different stages of the estrous cycle. The samples taken on days 0, 2, 6 and 8 post-injection of hormones were: blood, vaginal fluid (VF), vagina, spleen and bone marrow (BM). Most of the animals remained in diestrous-metaestrous stages until day 6 post-P-injection or in proestrous-estrous stages until day 8 post-E-injection. The numbers of enterobacteria, staphylococci and lactic acid bacteria in VF were higher in estrogenized animals. In VF, myeloid population ($Gr\text{-}1^+$) was dominant compared to T ($CD3^+$) and B ($B220^+$) lymphoid populations, determined by flow cytometry. On most of days, the $Gr\text{-}1^+$ cells of VF were higher in estrogenized mice. The hormonal treatments did not produce significant differences in the myeloid and T cell populations of BM or spleen. Only in spleen, a significant increase of mature B cells ($B220^{High}CD24^{Low}$) and a decrease of immature B cells ($B220^{Low}CD24^{High}$) was observed in P-inoculated mice compared with those E-inoculated. These results allow the selection of the most suitable experimental conditions to further evaluate the mechanisms of interaction between beneficial vaginal lactobacilli and the host in a murine experimental model.

CB-P60

MOLECULAR REGULATION OF N-CADHERIN PRECURSOR TRAFFICKING: ROLE OF PROTEIN TYROSINE PHOSPHATASE PTP1B

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N-cadherin, a cell-cell adhesion molecule, is synthesized as a precursor protein with a prodomain that is cleaved post-Golgi. Our previous data suggest that PTP1B promotes ER-Golgi trafficking of the precursor, likely by ensuring its binding to p120 catenin. To confirm the role of p120 in traffic, we analyzed endo H sensitive profiles of the precursor in cells knocked down for p120 expression. Preliminary results did not show differences with the control, suggesting that additional components bound to the precursor are involved in trafficking. In a search for relevant proteins, we analyzed the array of proteins associated with the wild type and a mutant precursor unable to bind p120.
Immunoprecipitation/Western blot analysis showed that N-ethylmaleimide sensitive factor, an ATPase that regulates vesicle fusion, associates with the WT precursor, but at lower levels with the mutant. Mass spectrometry analysis also revealed a decrease of spectrins á2/â1 and á-actinin 4 in the mutant complex, and in the complex of the WT precursor expressed in PTP1B KO cells. Spectrin interacts with the motor dynein, which mediates ER-Golgi transport, and with ankyrin G, a protein that binds to the precursor in a region overlapping with the p120 binding site. Thus, PTP1B and p120 may contribute to recruit components involved in transport and fusion to the N-cadherin precursor.
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CB-P61

Src ACTIVATION IN NASCENT ADHESIONS DEPENDS ON INTEGRINS, PTP1B AND PAXILLIN DEPHOSPHORYLATION

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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. Here, we examined the mechanisms underlying the localization of active Src in nascent cell-matrix adhesions. Immunofluorescence analysis of total Src showed a uniform punctate distribution in both PTP1B WT (WT) and PTP1B KO (KO) cells shortly plated on fibronectin. However, active Src accumulated in a peripheral ring of nascent adhesions only in WT cells. This distribution was lost when cells were plated on poly-L-lysine. Adhesion of WT cells to vitronectin, which specifically depends on beta3 integrin, showed accumulation of active Src in nascent adhesions. Conversely, inhibition of beta3 integrin on cells plated on fibronectin, which also adhere through beta1, abolished this distribution. Paxillin localization in nascent adhesions did not depend on Src expression, as revealed by analysis of SYF cells. However, active Src localization depended on paxillin, since null cells were unable to show its accumulation at the peripheral ring. Reconstitution with the double paxillin mutant Y31F/Y118F and with the single mutant Y118F, but not Y31F, rescued active Src localization to nascent adhesions. Thus, dephosphorylation of paxillin, likely by PTP1B, promotes Src activity at beta3 integrin-dependent nascent adhesion sites.
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CB-P62

CELL SIGNALING PATHWAYS INVOLVED IN p19INK4D ACTIVATION DURING SENESCENCE

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The cellular response to different stress stimuli is highly complex, involving sensor proteins that detect the damage and transmit signals to transducer proteins, which in turn activate numerous effectors implicated in specific cellular pathways.
Previous work in our laboratory showed that p19 is involved in cell cycle arrest, DNA repair and senescence. This suggests that p19 might be a part of a protein complex responsible for integrating information and deciding the cellular response to a particular type of stress. In this work we studied the signal transduction pathways involved in p19 induction by genotoxic and physiological senescence.Using reporter constructs we showed that physiological and genotoxic senescence activated the p19 promoter and that the effect of both conditions is additive. A senescence stimulus, low doses of a genotoxic drug, upregulated p19 for at least 10 days resulting in cell cycle arrest. In contrast, high doses of the same agent caused a transient induction and apoptosis. p53 decoy oligonucleotide prevented p19 induction following genotoxic senescence. Finally, for both types of senescence, p19 stimulation required ATM, Chk1 and p38 activities. These results suggest that the signaling pathways leading to p19 induction during senescence share components with those mediating the cellular DNA damage response.

CB-P63

OXIDATIVE STRESS STIMULATES p19INK4D RECRUITMENT TO CHROMATIN

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Exposure of mammalian cells to potassium bromate (KBrO3) generates oxidative DNA modifications, in particular 7, 8-dihydro-8-oxoguanine (8-oxoG), an oxidized form of guanine, which is highly mutagenic due to its capacity to pair with adenine during replication. Previous work in our laboratory has shown that p19 participates in the DNA damage response (DDR). Although its mechanism of action is still unknown, preliminary evidence suggests that p19 might interact with chromatin and facilitate the access of the repair machinery to sites of damaged DNA. Treatment of HEK293 and SH-SY5Y cells with the DNA damaging agent KBrO3 caused an increase in p19 expression. Moreover, upon DNA damage, p19, which is mostly cytoplasmic in normal cells, translocated to the nucleus and was found associated to chromatin. This interaction resisted detergent extraction indicating that p19 is tightly bound to chromatin after DNA damage. This chromatin fraction was enriched in acetylated histones and RNAPolII suggesting that p19 preferentially interacts with euchromatin. Finally, the access of the enzyme XbaI to a chromatinized plasmid was enhanced when it was incubated with an extract from cells overexpressing p19.
Taken together, these results suggest a role for p19 as a chromatin accessibility factor during the DNA damage response

CB-P64

CHARACTERIZATION OF SCLEROTOMAL GENES DURING *Xenopus laevis* EMBRYOGENESIS

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The axial skeleton develops from the sclerotome, a cell mass derived from the somites, segmentally repeated units located on either side of the neural tube. Those cells take up positions around the notochord and neural tube, when they leave the somites, for later differentiation to cartilage and bone. The anurans are characterized for having a highly specialized vertebral column with a reduced number of vertebral elements.
The amphibian sclerotomal cells have not been studied and knowledge of the molecular programs leading to morphogenesis of vertebral elements is relevant taking account the broad range of dysmorphic syndromes affecting axial skeleton formation.
In this work we characterize the expression pattern of the genes *Xpax1, Xpax9* and *Xuncx* by *in situ* hybridization and RT-PCR. We found that these transcription factors are expressed in the sclerotomal cells as well as in the pharyngeal arches from tail bud stage, which is consistent with other vertebrate. Comparison of the derived protein sequences of these genes with their orthologs reveals a striking conservation of their DNA-binding domain which has been maintained throughout of vertebrate evolution. We also carried out experiments of gain of function by microinjection of the mRNAs of these genes showing their potential role in the *Xenopus laevis* development.

CB-P65

CYCLIC AMP, CALMODULIN AND CALCIUM IMPLICATION IN *Xenopus laevis* VITELLOGENIN UPTAKE

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The transformation of oogonia into oocytes is commonly described as oogenesis. This is the differentiation of a precisely organized, fully mature and functioning oocyte, which is a result of a complex series of specific cellular and molecular events.
Vitellogenesis is one of the most important processes during the oogenesis phase in oviparous vertebrates. It is characterized by hepatic production of the glycoprotein vitellogenin, which is transported via the bloodstream to the ovary where the oocytes enter through receptor-mediated endocytosis.
In previous works we established that in the amphibian *Xenopus laevis*, cAMP is the diffusible signal molecule that is capable of passing through fully open heterologous gap junctions that trigger the vitellogenic process. In addition, we demonstrated a participation of the calcium binding protein Calmodulin (CaM) during *X. laevis* vitellogenin uptake.
In order to assess the physiological roles of cAMP, CaM and Calcium during *X. laevis* vitellogenesis, experiments with chemical antagonists/agonists were performed.
We showed that the cAMP molecule is upstream in the signaling pathway with regard to the Calmodulin/Calcium complex.

CB-P66

SCREENING FOR TARGET GENES OF A NUCLEIC ACID CHAPERONE ESSENTIAL FOR CRANIOFACIAL DEVELOPMENT

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Cellular nucleic acid binding protein (CNBP) is a highly conserved single-stranded nucleic acid binding protein required for craniofacial development. CNBP acts as nucleic acid chaperone rearranging the secondary structure of its targets and promoting the formation of G-quadruplexes (G4), stable nucleic acid structures described as novel elements for gene expression regulation. So far, the direct target genes regulated by CNBP remain unknown. Here, we performed a genome-wide screening for zebrafish CNBP binding sites using inverse one-hybrid strategy on genomic zebrafish and mouse libraries cloned in yeast. We obtained 76 and more than 170 clones from zebrafish and mouse libraries, respectively. Many of them were repeated, and 50% were chimeras consisting of unrelated genomic fragments cloned in tandem. Sequences were in silico mapped to the zebrafish and mouse genomes to define their location in or nearby annotated genes and putative promoter regions likely related to CNBP function. Forty % matched to annotated genes with variable functions, while 60% to intergenic regions. Several sequences were nearby clusters of small non-coding RNA genes (snRNA, rRNA, miRNA). Moreover, more than 80% of the sequences contained putative G4 formed by 2 tetrads. This is a 2-fold higher frequency than average in the analyzed genomes, meaning that CNBP prefers sequences with G4 formation potential.

CB-P67

ULTRAVIOLET B (UVB) INDUCED DNA DAMAGE AFFECTS ALTERNATIVE SPLICING IN SKIN CELLS

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The ultraviolet (UV) radiation from the Sun that reaches the Earth's surface is a combination of low (UVA, 320-400 nm) and high (UVB, 290-320 nm) energy light. UVB light causes two types of mutagenic DNA lesions: thymine dimers and (6-4) photo-products. UVB mutagenesis is a critical step in the generation of different forms of skin cancer, which develops almost exclusively in sun exposed areas. We have previously shown that RNA polymerase II (pol II) hyperphosphorylation induced by UVC (254 nm) irradiation of non-skin cells inhibits pol II elongation rates which in turn affects alternative splicing (AS) patterns, altering the synthesis of pro- and anti-apoptotic isoforms of key proteins like Bcl-x or Caspase 9 (C9). Since the UVC radiation is fully filtered by the ozone layer and AS regulation in skin pathologies has been poorly studied, we decided to extend our studies to human keratinocytes in culture treated with UVB (302 nm) light. We observed that pol II hyperphosphorylation is increased upon UVB irradiation, being this modification necessary for the observed change in AS of a model cassette exon. Moreover, UVB irradiation induces the pro-apoptotic mRNA isoforms of Bcl-x and C9 consistently with a key role of AS in skin response to DNA damage.

CB-P68

MODULATION OF ALTERNATIVE SPLICING BY CHANGES IN CHROMATIN STRUCTURE DURING NEURONAL DIFFERENTIATION

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The rate of RNA polymerase II elongation could influence splice site selection and the timing at which regulatory sequences are presented to the spliceosome. This mechanism can be modulated by intragenic chromatin structure that affects polymerase processivity or co-transcriptional recruitment of splicing factors to nascent RNA, highlighting a role for intragenic chromatin in the regulation of alternative splicing (AS). We have shown that neuronal differentiation triggers transcription-repressive intragenic histone modifications (such as H3K9me2 and H3K27me3) in the NCAM gene, correlating with an increase of its alternative exon 18 (E18) inclusion. Treatment of differentiated N2a cells with a DNA methylation inhibitor reverts the effect of differentiation on E18 inclusion, whereas treatment with a hyper-acetylating drug has the opposite effect. We study this process using DMSO-induced differentiation of N2a murine neuroblastoma cell line and are now working with P19 embryonal carcinoma cells, a stem cell-like line, which can be differentiated into neuronal precursors and neurons. To extend the analysis to other alternative splicing events, we used available ChIP-seq and AS microarray data to identify candidate genes with a correlated modulation of both histone marks and AS patterns during neuronal differentiation.

CB-P69

ORDER OF INTRON REMOVAL AROUND A MODEL ALTERNALTIVELY SPLICED EXON

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The mechanisms involved in alternative splicing regulation have not been deeply studied in *in vivo* models. We studied the relative order of removal of the introns flanking fibronectin EDI, a model alternative cassette exon. We transfected Hep3B cells with plasmids with a FN EDI minigene and we analyzed the relative order of removal of the introns flanking EDI by RT-PCR. We set up two qRT-PCR reactions which amplified the splicing intermediates resulting from either intron 1-first or intron 2-first pathways of intron removal. We show that there is a preferential removal of the intron downstream of the cassette exon before the upstream intron has been removed. Cis mutations and trans-acting factors that enhance EDI inclusion by different pathways change the pattern of intron removal in ways that are consistent with their mechanism of action. However, reduction of transcriptional elongation, also causing higher inclusion of the cassette exon, does not change the order of intron removal. We propose that instead of promoting excision of the upstream intron, slow Pol II elongation favors commitment to splicing of this intron before the downstream intron is synthesized. To verify this hypothesis we are assessing the recruitment of the splicing factor U2AF to the 3' splice site of the upstream intron under different conditions.

CB-P70

THE *Giardia lamblia* SUPER FAMILY 2 OF RNA HELICASES

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Antigenic variation in *Giardia lamblia* is posttranscriptional regulated by an RNAi mechanism that involves a bidentate RNAse III (Dicer). Strikingly, this does not present the RNA helicase domain, distinctive of other higher eukaryotes Dicer enzyme. We decided to study and characterize RNA helicases from the parasite that could potentially be involved in this mechanism. The RNA helicase DExD/H-box family involves the DEAD-box, DEAH-box, DExH-box and DExD-box subfamilies, all members of the Super Family 2 (SF2) of RNA helicases that present eight conserved motifs. We performed an extensive *in silico* analysis of the *Giardia* genome database and identified 31 RNA helicases from SF2. Phylogenetic and sequence analysis allowed us to separate them in 3 subfamilies, 21 DEAD-box, 6 DEAH-box and 4 Ski2p RNA helicases, some of them having high homology with well characterized RNA helicases from higher eukaryotes. In this work we present a comprehensive analysis of the *G. lamblia* SF2 RNA helicases, showing that none of them present extensive homology with the RNA helicase domain from other Dicers. Being Dicer an essential component of the RNAi machinery that controls the antigenic variation in this important human pathogen, the absence of an RNA helicase domain in this enzyme confirm the particular function of Dicer in regulating antigenic variation in *Giardia*.

CB-P71

EPIGENETIC MECHANISMS INVOLVED DURING ENCYSTATION AND ANTIGENIC VARIATION IN *Giardia lamblia*

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Giardia lamblia is one of the most common causes of intestinal disease in humans. To adapt to different environments both outside and inside of the host's small intestine, this early-branching eukaryote undergoes the adaptive processes of differentiation into cyst and antigenic variation, respectively. In this work, we studied if epigenetic factors, like histone modifications, are involved in both processes. *Giardia* lacks canonical histone H1 but possesses the core histones H2a, H2b, H3 and H4, which form the nucleosone where the DNA is wrapped. Using specific antibodies against histone modifications and chromatin inmunoprecipitation assays (CHIP), we determined the dynamics of different histone marks during encystation and antigenic variation. When *Giardia* clones were exposed to specific inhibitors of histone deacetylase enzymes, inhibition of encystation and a significant increase on the switching rate of surface antigens (VSPs) was observed. We also identified and characterized all *Giardia* histone deacetylase enzymes from the genome of the parasite, which can be separated in non-NAD+-dependent (HDACs) and NAD+-dependent. Our results indicated that several histone modifications, in particular acetylation of lysines in *Giardia* histone H3 and H4 are directly involved in controlling adaptation and differentiation of this important human parasite.

CB-P72

IDENTIFICATION, CLONING AND FUNCTIONAL CHARACTERIZATION OF A NOVEL *Giardia* LRP-LIKE PROTEIN

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As *Giardia lamblia* is unable to synthesize cholesterol de novo, this compound might be obtained from the intestinal milieu by endocytosis of lipoproteins. Here, we identified a putative *Giardia* LRP (GlLRP), a type-I membrane protein, which shares the substrate-N-terminal binding domain and a FXNPXY-type endocytic motif with the human Low-density lipoprotein Receptor related Proteins (LRPs). Expression of tagged-GlLRP showed that it was localized in the ER, lysosomal-like peripheral vacuoles, PM and nuclei. However, the FXNPXY-deleted GlLRP was retained at the PM suggesting that it is abnormally transported and processed. LDL and chylomicrons interacted with GlLRP, with this interaction being necessary for lipoprotein internalization. GlLRP was found to bind directly to the medium subunit of *Giardia* adaptor protein 2 (AP2), indicating that receptor mediated internalization occurs through an adaptin mechanism. Beside, by an antisense strategy we showed that GlLRP plays a pivotal role in parasite replication. Finally, we showed that the degradation of GlLRP was in part due to the action of a ã-secretase-like complex, which had a significant effect in its nuclear localization. We postulate that GlLRP is involved in the internalization of cholesterol from lipoproteins via a regulated AP2-dependent pathway and possesses a potential role in the intracellular signalling.

CB-P73

NOVEL FEATURES ON H2AX ROLE IN *Toxoplasma gondii*

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The histone variant H2AX possesses a C-terminal conserved SQ(E/D)Ö motif (Ö being an hydrophobic amino acid), which makes it different from the rest of the H2A histones. Phospho-H2AX at this SQ motif (ãH2AX) has become a DNA damage hallmark. In *Toxoplasma gondii*, H2AX is phosphorylated (S131) in the presence of oxidative damage. Interestingly, parasites grown under standard conditions show high levels of ãH2AX. In addition, H2AX is enriched at repressed promoters and silenced regions, and its expression increases during bradyzoite development, suggesting an extra role for H2AX in parasite biology. To study H2AX function and its phosphorylation in *T. gondii* we obtained two stable parasite lines expressing H2AX mutated at S131 for alanine, and two others expressing the wild type version of H2AX; both fused to c-myc tag at the N-terminus (c-mycH2AXS131A and c-mycH2AX respectively). The presence of c-mycH2AX and c-mycH2AXS131A were determined by Western blot assays. The recombinant proteins were detected with anti-c-myc in the four clones. Besides, c-mycH2AX was recognized by anti-ãH2AX while c-mycH2AXS131A was not. Their localization was analyzed by indirect immunofluorescence using the same antibodies. In both cases the fusion proteins were localized in the nucleus. With all these clones the replication and growth rates as well as conversion to bradyzoites will be analyzed.

CB-P74

FUNCTIONAL CHARACTERIZATION OF THE RNA BINDING PROTEIN TcSR62 FROM *T. cruzi*

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TcSR62 is a RNA binding protein from *Trypanosoma cruzi* belonging to the SR-related family. SR and SR-related proteins have multiple roles in mRNA metabolism, particularly as regulators of pre-mRNA processing. TcSR62 is present in all stages of the parasite and localized mainly to the nucleus. Previous results from our lab. showed that over-expression of TcSR62, or different mutant versions, in *T. brucei*, induced a significant decrease both in trans-splicing and in the mRNA levels of several analyzed genes. Therefore, it was of interest to perform a global microarray analysis on the *T. brucei* transcriptome to study the extent of such effect. These results will be presented in the poster.
As previous experiments suggested the involvement of TcSR62 in trans-splicing, we wanted to know whether this protein is an integral part of the spliceosome. As a first approach, we studied whether different U snRNAs and the Spliced Leader (SL) RNA co-immunoprecipitated (IP) with TcSR62. To do this, we performed IP of RNA–protein complexes from soluble extracts with a specific antiserum against TcSR62. After isolating the RNA fraction, the presence of SL and U snRNAs was determined by semi-quantitative RT-PCR. Interestingly, we identified all the U snRNAs analyzed and the SL RNA, suggesting that this protein may be a component of the trans-spliceosome.

CB-P75

LOCALIZATION AND ENZYMATIC ACTIVITY OF AURORA KINASES FROM *Trypanozoma cruzi*

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In all eukaryotes protein kinases are crucial players in the mechanism of cell cycle regulation. Among them are the Aurora kinases controlling important processes during mitosis. This family of enzymes was characterized in several organisms, from protozoan to mammals. One of the most interesting protozoan families are the trypanosomatids because of their complex life cycle, here are included *T. cruzi*, *T. brucei* and *Leishmania major*. Aurora kinases were identified in *L. major* and were deeply studied in *T. brucei* (TbAUK1). As in mammals, TbAUK1 is involved in mitotic events like spindle formation and dynamics, chromatin segregation and cytokinesis.
In *T. cruzi* we've already identified three genes that codify for aurora kinases (TcAUK1, 2 and 3) and have described their genomic organization and expression profile at mRNA level. We've also established TcAUK1 as a nuclear protein in epimastigotes that its expression seems to be regulated in a cell cycle-dependent way. In this work we extend the study of cellular localization to the others TcAUKs, expressing them as fusion proteins with GFP in epimastigotes of *T. cruzi*. As part of the biochemical characterization of these enzymes we performed kinase assays, using recombinant TcAUKs expressed in *E. coli*. With these assays we could established that TcAUKs show greater activity levels when Mn^{2+} is used as cofactor.

CB-P76

SELECTION OF REFERENCE GENES FOR QUANTITATIVE REAL TIME PCR STUDIES IN RAT MODELS OF HEPATOTOXICITY

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Drug-induced hepatotoxicity is an important cause of liver disease with significant medical, economic, legal, and regulatory implications. Experimental production of hepatic toxic injury has increased our understanding of this pathology. Application of reverse transcription quantitative real-time PCR is a powerful tool to reveal differences in gene expression in vivo as long as in vitro hepatotoxicity models. For a correct interpretation of the real-time PCR results, all data must be normalized using reference genes. The expression stability of 9 candidate reference genes (ALB, RL13A, SDHA, 18S, HPRT, B2M, CYCA, B-ACT, GAPDH) was determined in the liver of rats treated with classical hepatotoxicants. Histological studies were accomplished and serum transaminases levels were measured in order to corroborate the hepatoxic models with carbon tetrachloride (i.p.; 0, 0.1, 0.4, 0.7, 1 mL/kg) and thioacetamide (i.p.; 0, 10, 50, 150 mg/kg). Total hepatic RNA was isolated using Trizol reagent, and the expression of putative references genes was analyzed by the RT-qPCR technique. Using the geNorm application, B-ACT and GAPDH were found to be the most stable genes across the examined hepatotoxic models, and the use of both reference genes are recommended to generate an accurate normalization factor.

CB-P77

microRNAs AS POTENTIAL NEW BIOMARKERS FOR TUBERCULOSIS

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Tuberculosis (TB) may be regarded as a disease in which the immune response to *Mycobacterium tuberculosis*, its etiologic agent, is engaged both in protection and pathology. Current biomarkers cannot completely distinguish patients with mild or moderate forms of pulmonary TB from the ones that present a more severe disease. Both circulating and cell specific microRNAs (miRNAs) are promising biomarkers for several diseases, but their correlation with the progress of Tuberculosis has not been studied.
In this work we analyzed six miRNAs that could modulate different aspects of the immunendocrine response observed in TB patients: miR-18a, miR-223, miR-15b, miR-155, miR-142 and miR-146a. In a first approach, we standardized the protocols to determine the expression levels of the miRNAs in both plasma and peripheral blood mononuclear cell from healthy controls. Using the stem loop RT-PCR technique we were able to successfully detect them in blood samples. Then miRNA expression was investigated in a group of TB patients and compared to healthy controls to evaluate their potential prognostic value.
In sum, the results presented here demonstrate the feasibility of quantifying microRNA expression in patient tissues and suggest that they might serve as new biomarkers for the disease.

CB-P78

CORRELATION BETWEEN THE BLOOD RELEASE OF mir-122 AND TRANSAMINASES IN RAT MODELS OF HEPATOTOXICITY

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Drug-induced liver injury is a significant clinical problem. The plasma activities of alanine aminotransferase (ALTp) and aspartate aminotransferase (ASTp) are the main hepatotoxicity biomarkers in both animal models and human patients. Mir-122 is a small noncoding RNA that is expressed exclusively in the liver and it has been shown to increase its concentration in the blood of murine models of hepatotoxicity. We studied the liver histology and assessed the blood levels of mir-122 (mir122b), ALTp and ASTp in rats treated (n=18, i.p., 24 hs) with carbon tetrachloride (0, 0.1, 0.4, 0.7, 1 mL/kg), acetaminophen (0; 0.25; 0.5; 0.75; 1 g/kg) and thioacetamide (0, 10, 50, 150 mg/kg). Mir122b was determined in total blood RNA (Trizol isolation) by Stem Loop RT-qPCR. ALTp and ASTp were measured using commercial kits. Since the sample size for each dosage group was inadequate to test the assumption of normality, then both Pearson product-moment (Pe, parametric) and Spearman's rank (Sp, nonparametric) correlation coefficients were determined. The variables were expressed relative to control levels and plotted as log10. We observed a significant and very strong correlation between mir122b and ALTp (Pe: $r = 0.91$, $p<0.001$; Sp: $rs = 0.79$, $p<0.001$) and between mir122b and ASTp (Pe: $r = 0.93$, $p<0.001$; Sp: $rs = 0.92$, $p<0.001$) supporting the potential of mir-122 as blood biomarker of hepatotoxicity.

CB-P79

CADMIUM CHRONIC INTOXICATION: INDUCTION OF OXIDATIVE STRESS AND GENE EXPRESSION IN THE RAT HEART

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Cadmium-induced oxidative damage has been widely demonstrated by the increase of lipid peroxidation, and impairment of the enzymes required to prevent oxidative stress. Objective: The aim of this study was to assess the effect of Cd intoxication on non-proteic thiol grups contents, protein oxidation, and Nrf2 and Metallothionein (MT) expression in heart. Experimental model: Male Wistar rats were separated into four groups: 1(Controls, tap water), 2, 3 and 4(tap water with 15 ppm Cd ion, during 15, 30 and 60 days, respectively). Afterwards, animals were decapitated, under light ether anesthesia. Samples were kept at –20 ºC, until processed. Methods: Transcription factor Nrf2 and MT IIA expression levels were measured by RT-PCR. Total and reduced Glutathione, Catalase activity and protein carbonyls were assessed spectrofotometrically. Cd^{2+} levels in heart were measured using atomic absorption spectrometry. Results: Cd^{2+} levels were elevated in heart, in groups 2, 3 and 4 ($p<0,001$), but no changes were found in MT IIA expression levels. Total GSH and rGSH levels decreased and protein carbonyls increased in group 2 ($p<0.05$), Catalase activity augmented only in groups 3 and 4 ($p<0.001$). Nrf2 expression increased in groups 2 and 3 ($p<0,05$). Conclusion: Cd^{2+} intoxication entails oxidative stress and affects antioxidant enzymes activity and Nrf2 expression, in a time-of-exposure dependence.

CB-P80

TIME-CONCENTRATION-DEPENDENT BIOCHEMICAL RESPONSE TO TRANSIENT AGROCHEMICAL EXPOSURE IN TADPOLES

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We have studied the biochemical response of *Rhinella arenarum* larvae transiently exposed 4h to 1 mg/L azinfos methyl (AM). After 4h, tadpoles suffer a mild reduction in esterase activities, mainly carboxylesterase (CE) with a slight induction of acetylcholinesterase (AChE). Induction of the Phase-II detoxification pathway is observed, through an increase in glutathione-S-transferase (GST) activity, and GSH depletion after 48h of recovery.
In the present work, we deepen into time- and concentration-dependence of the biochemical response through high level and transient AM exposure (80 mg/l; 15 min). Both AChE and CE activities were deeply inhibited (20% and 80%) in transiently exposed larvae. On the contrary, neither GST nor GSH were affected by the exposure. Taken together, these results point to the varying response pattern in toad larvae, depending on the characteristic of agrochemical exposure. No Phase II detoxification metabolism would be induced by a sharp, sudden and fleeting exposure, and AM suicide sequestration by CE becomes not only the first, but the unique defense line.
These results show the importance of performing a deeper characterization of the biochemical alterations in a resistant species such as the toad under different intoxication patterns. This is necessary for developing a biomonitoring tool for the evaluation of ecological impact of agrochemicals.

CB-P81

THALLIUM ALTERS CYCLINS EXPRESSION IN RAT PHEOCHROMOCYTOMA (PC12) CELLS. MODULATORY EFFECTS OF EGF

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The mechanisms underlying the toxicity of the heavy metal thallium (Tl) are not elucidated yet. Previously, we demonstrated that Tl(I) and Tl(III) (5-100 ìM) altered cell cycle progression in PC12 cells, effect that was modulated by the epidermal growth factor (EGF). We next investigated in synchronized PC12 cells, the effects of Tl(I) and Tl(III) on the expression of the major cyclins that regulate cell cycle. All the experiments were performed after 24 h of reentering into cell cycle (when cells were mostly in S phase) with or without Tl and/or EGF. EGF did not prevent the decrease in cell viability due to Tl. In the absence of EGF, Tl(I) increased cyclin D1 content, without affecting cyclins A or B1. Tl(III) increased cyclin B1 expression, and did not affect cyclins A and D1. On the other hand, in the presence of EGF, Tl(I) slightly increased cyclins D1 and B1 contents, while Tl(III) markedly increased cyclins D1, decreased cyclin A, and did not affect cyclin B1. Interestingly, only in the presence of EGF both Tl(I) and Tl(III) increased H_2O_2 production that also could affect cell cycle progression. Together the experimental evidence suggest that both Tl(I) and Tl(III) affect cell cycle progression, effect that could partially account for the cytotoxic effects of these cations.

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CB-P82

HOMOPHILIC INTERACTIONS BETWEEN PROTOCADHERINS AND THEIR ROLE IN CELL ADHESION AND CELL SIGNALING

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Clustered protocadherins (Pcdh) are a group of neuronal surface glycoproteins that are expressed in specific regions of the central nervous system (CNS) of vertebrates (brain cortex, olfactory bulb, spinal chord, hippocampus), enriched at synapses. They belong to the Cadherin superfamily, involved in Ca2+ dependent cell adhesion, but their function remains unknown. They consist of over 50 genes arranged in three clusters (alpha, beta and gamma). The combinatorial expression of these variants could help establish, to some extent, neuronal identity. We know that single neurons express Pcdh isoforms monoallelically and stochastically, with the exception of the "c2 type" isoform.

We want to understand the nature of Pcdh interactions with other proteins in the CNS and whether these trigger cell adhesion and/or signaling processes. Our model consists of a mouse neuronal cell line stably expressing one Pcdh isoform at a time, fused to green fluorescent protein. These are challenged with the same or different isoforms. We have found that Pcdh engage in homophilic interactions and that different isoforms differ in their transport dynamics to and/or from the plasma membrane. Now we want to study their involvement in cell signaling.

CB-P83

ALTERATIONS OF F-ACTIN CYTOSKELETON DURING INCOMPATIBLE POLLEN REJECTION IN *Nicotania alata*

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The integrity of F-actin cytoskeleton is essential for polar growth in pollen tubes. So far, most studies of F-actin organization in pollen tubes were carried out on *in vitro* cultured pollen tubes. We studied the *in vivo* changes of F-actin during rejection of incompatible pollen tubes of *Nicotiana alata*. Compatible pollen tubes reached the ovary 72 h after pollination while incompatible ones were full stopped at fifth day after pollination, slightly below the middle style. Early after pollination about 70% of both compatible and incompatible pollen tubes showed an organized pattern of F-actin long cables along the main axis of the cell. The remaining tubes exhibited an altered pattern consisting of isolated fragments or punctate foci of F-actin. While these proportions were kept constant in compatible pollinations throughout the style, incompatible pollinations showed a progressive decrease of F-actin integrity of pollen tubes. Five days after pollination, 70% of incompatible pollen tubes showed a disorganized pattern of F-actin. We are studying if alteration of F-actin in incompatible pollen tubes preceeds the S-RNase releasing from vacuole to cytoplasm, the crucial step in *Nicotiana* pollen rejection. If so, incompatible pollen rejection would occur in two steps: a) F-actin disruption to stop growth and b) pollen RNA degradation and subsequent cell death.

CB-P84

POSSIBLE ROLE OF VAMP7 IN AUTOPHAGOSOME FORMATION

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Autophagy is a normal degradative pathway that involves the sequestration of cytoplasmic components and organelles in a vacuole called autophagosome which finally fuses with the lysosome to degrade the sequestered material. The protein LC3 is an autophagic marker present in eukaryotic cells as a soluble form (LC3-I) and a membrane-associated form (LC3-II). LC3-I is conjugated to a lipid molecule to generate LC3-II, which localizes to autophagosomes. We have analyzed by fluorescence microscopy and Western blot the participation of the fusion protein VAMP7 (V-SNARE) in autophagosome formation. Our results indicate that a population of LC3 positive structures is decorated with endogenous VAMP7. Furthermore, overexpression of the N-terminal domain of VAMP7, which inhibits the SNARE complex formation, alters the RFP-LC3 pattern from a punctate to a diffuse distribution in several cell types, indicating that LC3 is not associated to autophagosomal membranes. Moreover, silencing VAMP7 caused a marked decrease in the number of LC3 labeled structures. These results were confirmed by Western blot observing a decrease in the levels of the LC3-II when endogenous VAMP7 was knocked down. Taken together, our results suggest that VAMP7 is involved at the initial steps of autophagosome formation, probably allowing the delivery of ER membranes to the pre-autophagosomal structures.

EN-P01

STUDY OF AN UNUSUAL METHIONINE SULFOXIDE REDUCTASE FROM *Trypanosoma cruzi*

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Trypanosomatids parasitize a wide variety of invertebrate and vertebrate hosts. Many efforts has been made to understand the mechanisms by which these organisms neutralize reactive species (RS) but much less is know about the proteins responsible of repairing the damage created by RS.
Methionine sulfoxide reductases (Msrs) catalyze thiol-dependent reduction of oxidized methionine. MsrA and MsrB are the best know Msrs that repair methionine-S-sulfoxide and methionine-R-sulfoxide respectively. In addition, an *Escherichia coli* enzyme specific for free methionine-R-sulfoxide (fMsr) was recently discovered. This protein is present in some prokaryotes and unicellular eukaryotes.
We find two orthologs in the genome of *Trypanosoma cruzi*. These proteins contain an extra C-terminal domain with a possible function in the regulation of type 2A phosphatases.
In this work, we carried out the cloning, purification and characterization of the fMsr N-terminal domains of both alleles. They reduce methionine-R-sulfoxide utilizing tryparedoxin as electron donor. The catalytic efficiency of the two alleles differs in one order of magnitude. This discrepancy could be explained on basis to the quaternary structure as we can realize by gel filtration assays.
Efforts are being made to obtain the complete protein to understand the functional relationship between these domain fusions

EN-P02

CHARACTERIZATION OF A SOLVENT TOLERANT LIPASE FROM *Aspergillus niger* MYA 135

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Lipase catalyses the hydrolysis of triglycerides at the oil-water interfaces. This reaction is reversible and the enzyme also catalyses the synthesis of esters in microaqueous conditions. Previously, we reported the presence of three enzymatic bands with lipolytic activity from *Aspergillus niger* MYA 135 olive oil-induced supernatant. In the present work, we selected lipase 1 for its purification and characterization, especially for its stability in the presence of various organic solvents. This activity was purified by two methods, electro-elution and DEAE-Sepharose anion-exchange chromatography leading for each one to 8.4-fold and 47% and 16.6-fold and 53.4% of purification and recovery, respectively. Lipase 1 showed the following main characteristics: maximum activity at 37°C, pH 7.0; molecular mass, 68 KDa; pI 5.1; and a K_m value of 0.99 mM for C18 (p-NP stearate). The purified lipase was also digested with trypsin and analyzed by HPLC-MS/MS. The sequences of the peptides did not show similarity with other lipases. Concerning the reactivity of this enzyme in a solvent free medium, a transesterification activity value of 0.78 ± 0.01 U/L was obtained yielding ethyl stearate as a product. Thus, these results show a valuable solvent-tolerant lipase for biodiesel production.
This work was supported by grants PIP-CONICET 297 and CIUNT 26/D409.

EN-P03

EFFECT OF TUBULIN IN PMCA ACTIVITY DEPENDS ON LIPID COMPOSITION WHERE THE ENZYME IS IMMERSED

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In previous work we demonstrated that acetylated tubulin forms a complex with PMCA (Ca^{2+}-ATPase) and the formation of the complex *in vivo* inhibits the enzimatic activity. We recently demonstrated that the effect of tubulin on PMCA activity depends on the environment in which both proteins are immersed. The reconstitution of the PMCA in liposomes and the subsequent addition of tubulin caused the activation of the PMCA. In this work we study the effect of tubulin on the PMCA activity reconstituted in different lipids. For this proposes PMCA was purified from rat brain and reconstituted in liposomes with diacylglycerol (DAG), phosphatidic acid (PA) or phosphatidylcholine (PC). Results shown that tubulin is able to activate PMCA independently of the tubulin concentration when the enzyme was reconstituted in BE and PA. However, tubulin affect the enzyme activity in a dose dependent manner when the reconstitution was done in DAG or PC. Low tubulin concentrations (below 25 µg/ml) activates PMCA more than two times, while higher concentrations inhibited the enzyme. These results indicate that the effect of tubulin on the PMCA activity depends on the lipid composition in which the enzyme is immersed and the tubulin concentration.

EN-P04

LPX1P SERIN PROTEASE PARTICIPATES IN THE GLUCOSE-INDUCED *S. cerevisiae* H^+-ATPASE ACTIVATION

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In previous work we showed that acetylated tubulin 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 the tubulin dissociation is caused by degradation of the membrane tubulin by a serin like protease. In this work we show that the strain YOR084w, loosing a serin protease of 44 kDa and pI 8.4 is deficient in H^+-ATPase activation and tubulin degradation when stimulated with glucose, indicating that this protease could be involved in the H^+-ATPase activation mechanism. The complementation of the YOR084w strain with the wild type form of the gene LPX1 showed that glucose could activate the H^+-ATPase activity through the degradation of membrane tubulin. These results demonstrate that glucose activates the plasma membrane H^+-ATPase of *S. cerevisiae* in a mechanism which involves the hydrolysis of membrane tubulin by the action of Lpx1p which causes the dissociation of acetylated tubulin / H^+-ATPase complex.

EN-P05

MOUSE LIVER OXIDATIVE STRESS CAUSED BY CHRONIC PROTEIN MALNUTRITION AND DEN. PROTECTION BY MET

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Many liver diseases are linked to the occurrence of oxidative stress. Thus, cells have strategies to preserve their redox balance with enzymes such as superoxide dismutase (SOD) and catalase (CAT), and regular amino acid dietary supply. Conversely, diethylnitrosamine (DEN) form electrophilic species responsible for hepatic oxidative stress. This work studied the effect of chronic protein malnutrition, as well as supplementation with methionine, on the hepatic oxidative status of mice treated with DEN. BALB/c mice were fed during 5 days with a protein-free diet followed by 5 days of complete diet, repeated 3 times (3PFD-CD). In addition, both the effect of Met supplementation (3PFD+Met-CD) and DEN injection (i) were studied. The oxidative status of liver was evaluated analyzing the activities of SOD and CAT, and the level of protein carbonylation. 3PFD-CD decreased the activities of SOD (-32%) and CAT (-30%) while protein carbonylation increased (+74%). On the other hand, 3PFD-CDi decreased both, the activities of SOD (-70%) and the levels of protein carbonylation (-40%) but did not change that of CAT. Addition of Met to PFD preserved the normal values in all conditions. In conclusion, 3PFD-CD and DEN produce hepatic oxidative stress. Conversely, dietary Met proves to exert a main role on cellular protection by decreasing this stress.

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EN-P06

***IN VITRO* CHARACTERIZATION AND INHIBITION OF *Trypanosoma cruzi* AND *Trypanosoma brucei* PARP**

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In contrast to humans, *Trypanosoma cruzi* and *T. brucei*, causal agents of American and African trypanosomosis respectively, have only one PARP enzyme (TcPARP or TbPARP), which *in vivo* is activated by DNA damage. We have expressed both PARPs in bacterial systems and purified recombinant proteins for further activity analysis. According to size exclusion chromatography, the most active form of both recombinant TcPARP and TbPARP is a dimer. We optimized assay conditions for both enzymes, which share similar properties regarding their activity requirements. Despite the fact that none of them show characterized DNA binding domains, both enzymes are activated *in vitro* by nicked DNA. Notably, neither of them requires Mg^{2+} or other metal ions. Instead, as previously shown for TcPARP, trypanosomatid PARPs are inhibited by many divalent cations. A panel of compounds known as PARP inhibitors was tested for inhibitory capacity on TcPARP and TbPARP. While some compounds did not inhibit either significantly at 10 µM concentration, most showed very similar inhibition of both enzymes. The most potent inhibitors tested were Olaparib and EB-47, the latter showing 10-fold selectivity for trypanosome enzymes over hPARP-1. Inhibitors will be tested for their ability to affect parasites PARP activity *in vivo*, as well as its effects on DNA damage response, post-stress survival and cell cycle.

EN-P07

EXPRESSION OF GPDH ISOFORMS IN FLIGHT MUSCLE FROM WILD AND LABORATORY *Triatoma infestans* ADULTS

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Triatoma infestans (*T. infestans*), vector of Chagas disease, acquires wings and develops flight ability after shedding the last nymphal stage to adult. The flight is important in the recolonization of the housing post fumigation. The enzyme glycerol-3-phosphate dehydrogenase (GPDH) is involved in providing energy for flight. In *T. infestans*, we characterized two isoforms of GPDH transcripts (GPDH-1 and GPDH-2). Previous studies showed that its expression in flight muscle varies during the development of flight ability, according to sex and to changes in temperature and time of intake. The aim of this study was to analyze the expression of the isoforms of GPDH in flight muscle of *T. infestans* adult wild and laboratory colonies. We were performed semiquantitative RT-PCR, non-denaturing PAGE electrophoresis revealed with specific enzyme activity and determination of enzymatic total activity. We found that GPDH-1 is the predominant isoform in flight muscle *T. infestans* adult wild and laboratory colonies. In laboratory colonies, comparing the expression in the first with the second generation, there are an increase in the expression of GPDH-2 and a decrease in the expression of GPDH-1. This is consistent with the metabolic functions described for isoforms of GPDH and the adjustment to a regular supply of food.

EN-P08

CARBOHYDRATE METABOLISM IN *Nitrosomas europaea*: CHARACTERIZATION OF ADP-GLUCOSE PYROPHOSPHORYLASE

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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 polysaccharide biosynthetic pathway. Transcripts of the *glgC* and *glgA* (glycogen synthase) genes in *N. europaea* were evidenced. The recombinant ADPGlc PPase (*Neu*ADPGlc PPase) enzyme was previously expressed in our laboratory in an active form. In the present work, the enzyme was characterized regards the influence of physicochemical variables (temperature, pH), stability as well as its ability to use different divalent metals as cofactors. Also, we determined that the enzyme can use UTP as an alternative substrate. *Neu*ADPGlc PPase remained stable after 2 hours incubation between 0-45ºC, and more than 24 hours at room temperature. Optimum pH range was between 9.5-10.0, showing high stability at alkaline pH (~9). The enzyme utilized different divalent cations with a preference order of $Co^{2+} > Mn^{2+} > Mg^{2+}$; respectively displaying $S_{0.5}$ values of 0.5 mM, 1 mM, and 29 mM, with similar V_{max}. The apparent affinity of the enzyme for the metal increased significantly at alkaline pH or in the presence of pyruvate (activator). The distinctive kinetic and regulatory properties of *Neu*ADPGlc PPase are relevant to understand metabolism of chemolitotrophes.

EN-P09
STUDY OF ENZYMES INVOLVED IN GLYCOGEN METABOLISM IN *Giardia lamblia*
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Giardia lamblia is the etiological agent of giardiasis, an intestinal infection most prevalent in children. Research on this protozoon is driven both by its impact on public health and its relevance for understanding evolution of eukaryotes, since it is a member of one of the earliest diverging eukaryotic lineages. It is known that glucose is a key source of metabolic energy for the organism, being the monosaccharide accumulated as glycogen in the trophozoite stage. We cloned the genes coding for glycogen synthase (EC 2.4.1.11) and UDP-glucose pyrophosphorylase (EC 2.7.7.9; UDPGlcPPase) from genomic DNA of *G. lamblia*. The latter gene was expressed in heterologous cells (*Escherichia coli*), and the recombinant enzyme was purified and characterized in its kinetic properties. *Gla*UDPGlcPPase exhibited typical hyperbolic saturation kinetics for substrates, with K_m values of 0.13 mM (UTP), 0.14 mM (Glc1P) and 0.55 mM (Mg^{2+}). The enzyme was inactivated by oxidants as diamide, hydrogen peroxide and sodium nitroprusside. The inactivation was reversed by reducing agents: DTT and thioredoxin (from *Entamoeba histolytica* and *Trypanosoma brucei*). Results support the occurrence of a physiological redox mechanism for modulation of UDPGlcPPase activity in protozoa (and other eukaryotes). Such a mechanism would be critical to regulate carbohydrates metabolism and to determine virulence in *G. Lamblia*.

EN-P10
ENZYMATIC ACETYLATION OF ppGalNAc-T2 CONTROLS ITS GLYCOSYLTRANSFERASE ACTIVITY
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Mucin-type O-glycosylation is initiated by UDP-Nacetyl galactosamine:polypeptide N-acetylgalactosaminyltransferases (ppGalN Ac-Ts). ppGalNAc-Ts are type II transmembrane proteins with a Golgi luminal region containing a catalytic domain and a C-terminal R-type lectin domain. Here we study the enzymatic acetylation effect on the GalNAc-transferase activity of ppGalNAc-T2. Recombinant human ppGalNAc-T2 was expressed in insect cells and purified to homogeneity by affinity chromatography. Purified ppGalNAc-T2 was acetylated in presence of p300 acetyltransferase or without acetyltransferase. ppGalNAc-T2 acetylation degree was demonstrated by western blots using anti-acetylated lysine antibody. The mutant ppGalNAc-T2 K521Q, mimicking an acetylated amino acid in lectin domain, was also analyzed. Glycosyltransferase activity was studied using several mucin peptide acceptors. ppGalNAc-T2 and ppGalNAc-T2 K521Q showed similar glycosyltransferase activity with MUC1, MUC2 and MUC5B peptide acceptors. ppGalNAc-T2 acetylated with p300 or auto-acetylated showed lower enzymatic activity using MUC1 and MUC5B peptide acceptor, and no difference on MUC2 acceptor. Thus, the presence of acetyl residues on defined positions of ppGalNAc-T2 controls its glycosyltransferase activity depending on the mucin peptide acceptor.

EN-P11
PHOSPHOTRANSFERASE ACTIVITY OF *Pseudomonas aeruginosa* PAO1 POLYPHOSPHATE PHOSPHATASE
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In *P. aeruginosa* PAO1 the gene PA5241 encodes an exopolyphosphatase (PPX), which catalyzes the hydrolysis of PP_n to PP_{n-1} + Pi. PPX shares structural homology with ATP-binding proteins. Therefore, we propose that PPX can transfer the Pi released from PP_n to ADP to form ATP. The DNA fragment corresponding to PA5241 was cloned in pCR2.1-TOPO, expressed as N-terminal fusion to 6xHis-tag in pET-15b (Novagen), and protein purified on Ni-agarose columns (Qiagen). The 6xHis-tag was removed by a thrombin cleavage capture kit (Novagen). ATP was determined with the kit "Luciferin Luciferase Reaction" (Molecular Probes) and PPX by Pi released. In conditions used to test PPX activity (8 µM PP_{65}, 5 mM Mg^{2+}, 80 mM K^+), PPX was capable of transfer Pi to ADP. The transferase activity was dependent on Mg^{2+} and was not activated by K^+, unlike previously described for PPX activity. The kinetic behavior of both enzymes was compared. PPX showed a Km_{app} of 3.7 µM for PP_{65}, and a $K_{0.5}$ of 0.32 mM and 0.29 for Mg^{2+} in the absence and presence of 80 mM K^+, respectively. The transferase activity showed a Km_{app} of 1.6 µM for PP_{65}, and a Ka of 0.16 mM for Mg^{2+}. These results show that PPX has a second enzymatic activity capable of catalyzing the reaction PP_{65}+ADP - PP_{64}+ATP. These two functions can release Pi or produce ATP to meet the nutritional needs to generate energy or initiate metabolic processes

EN-P12
Streptomyces omiyaensis SSM 5670, POLYHYDROXYALKANOATES DEPOLIMERASE ACTIVITY IN MATURE COMPOST
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During polyhydroxyalkanates (PHAs) composting process, these bioplastics are biodegraded by the action of microbial depolymerases.
The homopolymer of butyric acid (PHB) posses the highest biodegradation average in mature compost. The biodegradation of the PHB–co–PHV12% was 1.1% smaller than the biodegradation of PHB. When polycaprolactone was incorporated to PHB the biodegradation diminished 50%. When polyethylene of low density was incorporated the biodegradation diminished 23%. To these biodegradation an increased fungi growth was observed in the first cultivation phase. After 30 days UFC fungi decreased. In this last phase, an increase of mesophilic bacteria increased drastically. Previously we isolated and identified the bacterium *Streptomyces omiyaensis* SSM5670 as capable to biodegrade PHAs. The activity of the purified enzyme was evaluated measuring those derived of methyl ester monomers by gas chromatography and the weight decreased of prepared bioplastics by casting, pressed and by pneumatic injection in mature compost. Samples were incubated to 37°C, 15 days. Samples obtained by casting were degraded 70% faster than those by pneumatic injection or hot pressed samples, probably because these samples were exposed to 160°C previous to molded and the hydrophobic properties of the biopolymer increased diminishing the enzyme contact.

EN-P13
UNUSUAL *T. cruzi* ADENYLATE KINASE ISOFORM LOCATED IN THE CELL NUCLEUS
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Cellular energetic homeostasis is partially maintained by the enzymes adenylate kinases (Adks) and nucleoside diphosphate kinases (NDPKs), which are involved in nucleotide interconvertion. In typical eukaryotic cells, there are very few isoforms of Adks and NDPKs, however, in trypanosomatids there have been characterized a large number of isoforms. This unusual number could be explained by the compartmentalized metabolism, which characterizes these organisms. In this work we studied a nuclear Adk isoform (Adkn) of *T. cruzi.* We measured the biochemical activity of this enzyme and generated transgenic parasites which express the protein fused to GFP to determine its subcellular localization. We also studied the Adkn trafficking between cytoplasm and nucleus under different drugs or media treatments, and also along the parasites growth curve. Furthermore, we mapped the non-canonical nuclear localization signal, by segmenting the protein and making fusions to GFP. 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.

EN-P14
OXIDATIVE STRESS IN A SEMELPAROUS LIFE HISTORY: THE CASE OF THE OCTOPUS *Octopus tehuelchus*
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Oxidative stress (OS) has been postulated as a physiological cost of reproduction and a contributing factor to the ageing process. *O. tehuelchus* has a short life cycle with a single reproductive event (i.e. semelparity) before its death. The aim of this work is to analyze OS parameters in the digestive gland, gills, and oviducal glands (OG) of female *O. tehuelchus* collected from natural populations at spawning (SW) and advance spent stages (SP). Results showed that glutathione (GSH) concentration increased in gills and decreased significantly in OG at SP (451% and 83% respectively). Superoxide dismutase proved a low activity and only a tendency to decrease in gills from SW to SP. Malondialdehyde concentration in OG showed a trend to increase at SP (36%). These results could indicate that antioxidant response at these stages is principally held by non-enzymatic scavengers. On the other hand, SP females increased their activity by the active ventilation of the eggs, while their OG (in which fecundation takes place) become nonfunctional. Thus, the stage-related response observed for the GSH in gills and OG seems to be linked to the functional state of the organ. Although reactive oxygen species remain to be quantified, these results suggest that the low level of antioxidant protection compared with those of other taxa would be linked to the short lifespan (~2 years) of this specie.

EN-P15
KINETIC STUDY OF THE EFFECT OF SULFITE ON THE ACTIVITY OF THE MITOCHONDRIAL F_1-ATPASE
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The ATPase activity of F_1 is affected by various anions. It is stimulated by bicarbonate, likely by binding to a non-catalytic nucleotide binding site, whereas sulfate, that binds to catalytic sites of the enzyme, behaves as a general modifier (it inhibits at low and stimulates at high [ATP]).
The time course of the ATPase activity of F_1 shows a complex behavior. Even in the presence of an ATP regenerating-system (constant [ATP] and [ADP] ˜ 0), a progressive modification of the reaction velocity can be observed till a steady-state is attained. Values for initial (v_i) and final (v_f) velocities can be estimated. It has been postulated that a non-catalytic site would be involved in such a behavior.
Sulfite stimulated the ATPase activity of F_1 at concentrations smaller than 5 mM and inhibited it at higher concentrations. The stimulatory effect was observed not only on v_i but on v_f as well. However the effect was significantly bigger on the former than on the latter. The apparent $K_{A(sulfite)}$ did not significantly depend on the [ATP]. The inhibition observed at higher [sulfite] was complete with K_i values that slightly depended on [ATP]. These results support the hypothesis that sulfite binds with high affinity to the non-catalytic nucleotide binding sites stimulating ATP hydrolysis and with lower affinity to catalytic sites inhibiting the hydrolytic reaction.

EN-P16
STRUCTURE AND KINETICS OF PLASTIDIC NADP-MALIC ENZYMES FROM C_4 MONOCOT SPECIES
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The origin of C_4 photosynthesis involved several morphological and biochemical adaptations. It is generally assumed that C_4 specific enzymes arose by the acquisition of novel regulatory properties of duplicated copies of enzymes already present in the C_3 ancestors. Maize and sorghum are C_4 grasses in which a chloroplastic isoform of NADP-Malic enzyme (NADP-ME) decarboxylates malate to increase the concentration of CO_2 in the surroundings of RuBisCO. These monocot plants along with rice, a C_3 monocot, constitute a good model for the study of C_4 evolution. Besides, maize and sorghum have two plastidic isoforms of NADP-ME, one involved in photosynthesis which is defined here as C_4 type, whereas rice has only one isoform in this organelle. The aim of this work was to asses whether the previously reported kinetical and structural features that distinguish $C_4$from non-C_4 isoforms in maize, can be found in sorghum and rice enzymes. First, the proteins were expressed in *E. coli* and purified by affinity chromatography. Kinetical parameters such as kcat, K substrates and substrate inhibition were assayed at pH7 and pH8. Additionally, the quaternary structure was determined by non denaturing electrophoresis. All the data obtained shows that the sequence-based classification of NADP-ME isoforms as C_4 or non-C_4 is in accordance with the kinetical and structural differences here presented.

EN-P17

SUCROSE CATABOLISM IN FILAMENTOUS AND UNICELULLAR CYANOBACTERIA UNDER STRESS CONDITIONS

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In cyanobacteria Suc is synthesized through a two-step pathway involving Suc-phosphate synthase and Suc-phosphate phosphatase and degraded by Suc Synthase (SuS) or Alkaline-Neutral Invertases (A/N-Inv). Suc cleavage by SuS had only been reported in filamentous heterocyst-forming cyanobacteria. *Microcystis aeruginosa* is a unicellular non-N_2 fixing strain, well-known as one of the most common bloom-forming strain in fresh water environments. By sequence analysis we retrieved an open reading frame homologous to SuS encoding gene in the *M. aeruginosa* PCC7806 genome. The sequence was cloned and functionally characterized by expression in *E. coli* cells. In addition, the SuS activity was measured in cell free extracts. The expression of SuS and A/N-Invs was also studied in *Nostoc* sp. PCC7120, a filamentous strain and in *M. aeruginosa*. Cultures of both cyanobacteria were subjected to different conditions of light, nitrogen and salt stress. Expression analysis showed that while the response to salt stress was similar in both strains, a different expression pattern was obtained for SuS and A/N-Inv encoding genes under dark and light conditions. Also SuS and A-N/Inv expression was activated in diazotrophic growth. Our results indicate a distinct role for the Suc catabolism proteins in unicellular and filamentous nitrogen fixing strains.

PICT 21227, PIP0134, UNMDP and FIBA.

EN-P18

CHARACTERIZATION OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM *Streptococcus mutans*

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Streptococcus mutans is the leading cause of dental caries worldwide. When growing in excess of sugars, the microorganism accumulates a glycogen-like polysaccharide that mainly contributes to cariogenesis. We cloned *glgC* and *glgD* genes, coding for ADP-glucose pyrophosphorylase (ADPGlcPPase), a key enzyme for glycogen synthesis in most bacteria. Vectors were constructed for the expression of each gene separately or together. The GlgC protein showed ADPGlcPPase activity, while GlgD was inactive. Interestingly, GlgCD was 10-fold more active than GlgC. Both proteins, GlgC and GlgCD were purified and kinetically characterized, with remarkable differences between them. Fru-1,6-bisP activated GlgC by 2-fold, having no effect on GlgCD. Conversely, PEP inhibited GlgCD but not GlgC. GlgCD was also inhibited by inorganic salts. Results suggest that regulation of *S. mutans* ADPGlcPPase activity could be exerted at two levels: by changes in the protein oligomeric state and by allosteric regulation. The former regulation would comprise dissimilar expression of *glgC*/*glgD* genes and/or specific arrangement of protein subunits. Fru-1,6-bisP and PEP would be critical metabolites for allosteric regulation. Since glycogen synthesis occurs by different pathways in mammals and bacteria, it is worth of highlight that ADPGlcPPase can be visualized as a particular target for controlling *S. mutans* virulence.

LI-P01

CHOLESTEROL DEPLETION AFFECTS MEMBRANE LIPID ORDER AND GM1 LOCALIZATION IN AMPHIBIAN OOCYTE RAFTS

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The fluorescent probe Laurdan was used to study the effect of progesterone-induced maturation and cholesterol depletion mediated by methyl-ß-cyclodextrin (MßCD) on biophysical properties of membranes from *Rh. arenarum* ovarian oocytes. MßCD treatment was performed to determine changes in the localization of GM1 which could be indicative of membrane raft disorganization. The effect of maturation, induced either by progesterone or by ceramide, on the localization of GM1 was also analyzed. Functional studies regarding tyrosine phosphorylation were performed in light membranes (L) evidencing that multiple substrates and active tyrosine kinases are already organized in immature oocytes. Plasma membrane fractions showed two different thermotropic profiles of generalized polarization (GP) analyzed by direct excitation and by FRET. MßCD treatment decreased the GP value of L, indicating a greater lipid disorder whereas heavy membranes (H) evidenced an increase in membrane lipid order. Levels of ganglioside GM1 showed that after drug treatment the signal decreases in L. As a result, both fractions become more similar and this is reflected in their biophysical states. Progesterone modified plasma membrane fluidity increasing lipid order of both fractions. Ceramide affected the distribution of GM1 among membrane fractions while progesterone seems not to affectmembrane micro microdomain integriyt

LI-P02

STUDY OF TWO DGAT GENES INVOLVED IN TRIACYLGLYCEROLS BIOSYNTHESIS IN *Rhodococcus opacus* Pd630

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Rhodococcus opacus PD630 is an oleaginous bacterium able to accumulate significant amounts of triacylglycerols (TAG) (up to 60% of CDW) after growth on different carbon sources. The last step of TAG biosynthesis is catalyzed by diacylglycerol acyltransferases enzymes (DGAT). Strain PD630 possesses 10 atf genes encoding for DGAT isoenzymes. We studied two DGAT genes (*atf1* and *atf2*), which exhibited higher acyltransferase activity when expressed in *E.coli*, by means of gene disruption and over-expression procedures. The disruption of both genes caused a decrease of cellular TAG content in comparison with the wild type (WT) strain. Whereas the WT strain accumulated 64,7% (CD) of TAG after cultivation in MSM0.1 medium with gluconate during 48 h at 28ºC, *atf1* and *atf2* single mutants produced 43,1% and 41,2 % of TAG, respectively. The fatty acid profiles of the mutants obtained after preparative TLC of TAG fraction was similar to that of the WT strain. On the other hand, over-expression of the genes cloned under a strong acetamide promoter of pJAM2 vector in the strain PD630 caused an increase of TAG content in comparison with the WT after cultivation on glucose as sole carbon source. The results indicate that *atf1* and *atf2* genes are involved in TAG biosynthesis in strain PD630 and their overproduction increase TAG contents in cells.

LI-P03

EXTRACELLULAR LIPIDS PRODUCED BY DIFFERENT *Rhodococcus* SPECIES

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Rhodococcus bacteria accumulate variable amounts of triglycerides (TAG) during cultivation on diverse substrates. The intracellular TAG accumulation by rhodococci is a well established feature, but the extracellular export of TAG remains to be investigated. In this study we analyzed the ability of *Rhodococcus* species, such as *R.opacus, R.jostii, R.wratislaviensis, R.ruber, R.erythropolis* and *R.fascians*, to produce extracellular TAG. All studied strains were able to export TAG into the culture medium, and in proportion, those species with lower content of intracellular TAG, produced higher amounts of extracellular TAG. Interestingly, intracellular and extracellular TAG produced by all studied species exhibited different fatty acid (FA) composition as revealed by preparative thin layer and gas chromatography analyses. Monounsaturated FA occurred predominantly in intracellular TAG, whereas extracellular TAG contained almost exclusively saturated FA (86.5-100% of total FA). Extracellular TAG from all rhodococcal species were composed predominantly by C16:0 and C18:0 (76-93%), while C18:1 was one of the major FA in intracellular TAG. The presence of TAG with different FA composition inside and outside the cells suggests the occurrence of an export mechanism for lipids in rhodococci which probably involve export proteins.

LI-P04

SELECTIVE INHIBITION OF CK OR CCTα IMPAIRS NEURONAL DIFFERENTIATION INDUCED BY RETINOIC ACID

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Neuronal differentiation implies an increase in cell membrane phospholipids in order to face the demand for neurite outgrowth.
In Neuro2a cell line, retinoic acid (RA) induced differentiation is driven by MAP kinase signalling and promotes an increase in phosphatidylcholine (PtdCho) synthesis. In this way, coordinated transcriptional and post-translational mechanisms are involved in the sequential activation of two of the key enzymes of the Kennedy pathway: CTP:phosphocholine cytidylyltransferase alpha (CCTα) biochemical activation, choline kinase (CK) mRNA stabilization, and an enhanced expression of both of these genes.
The aim of this study is to further characterize the role of CK and CCTα in the neuronal differentiation process. Selective inhibition of these enzimes using siRNA approaches, significantly abrogates the extension of neurites. Moreover, the use of pharmacological inhibitors of CK or CCTα produces a reduction in the percentage of differentiated neuronal cells after RA stimulation.
According to these results, the decrease in phosphatydilcholine or its derivatives may alter the production of lipids second messengers which could modify signalling cascades involved in neuronal differentiation.

LI-P05

CTP:PHOSPHOCHOLINE CYTIDYLYL TRANSFERASE EXPRESSION THROUGHOUT *Bufo arenarum* (AMPHIBIA) EMBRYOGENESIS

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Phosphatidylcholine plays fundamental structural and functional roles. In nucleated cells it is synthesized through the Kennedy pathway where the regulatory step isbcatalyzed by CTP: phosphocholine cytidylyl transferase (CT). The CTα isoform expresses in every animal tissue and presents two differential states: a cytosolic-inactive state and a membrane bound-active state. *Pcyt1a* knock-out mouse fail to form blastocysts demonstrating the essential role of CTα early during embryonic development. Our goals were to:1) detect CTα mRNA and protein in *B. arenarum* oocytes, 2) study CTα protein expression and distribution between cytosolic and membrane fractions during development. mRNA encoding Cta was analyzed by RT-PCR using mouse-specific primers in B arenarum total ovary RNA. Immunoblotting with anti-CT policlonal antibody confirmed the presence of Cta in oocytes. Total protein samples (ET) from embryos of different stages showed constant levels of CTa until morula. In blastocyst it starts decreasing reaching a minimum at neural plate after which it begins to recover. Immunoblot analysis demonstrated that cytosolic/total CTα protein ratio is constant during embryogenesis.
Our results suggest that CTα is present in *B. arenarum* oocytes and shows sequence similarity to mammalian CTα. Maternal CTα protein seems to fulfil the metabolic requirements during early

LI-P06

PIP5KI GAMMA AND ARF6 ARE REQUIRED DURING ACROSOMAL EXOCYTOSIS IN HUMAN SPERMATOZOA

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During fertilization, the spermatozoon must penetrate the zona pellucida to reach the oolema. Only sperm that have completed the acrosome reaction (AR) can successfully accomplish this task. The AR is a calcium-regulated exocytosis where the membrane of the single secretory vesicle, the acrosome, fuses to the plasma membrane. We have recently demonstrated that the small GTPase Arf6 is not only present in sperm cells but when activated induces AR in the complete absence of calcium. On the other hand, we demonstrated by TLC that active Arf6 increases spermatozoa's phosphatidylinositol 4,5-bisphosphate (PIP2) synthesis. It has been described in other cell types that Arf6 regulates the activity of phosphatidylinositol 4-phosphate 5-kinase (PI4P5K) that catalyzes the conversion of phosphatidylinositol 4-phosphate (PI4P) in PIP2. So, we moved on to analyze PI4P5K function in sperm cells. By Western blot and IFI we demonstrated that PI4P5K is present in human sperm cells. We observed that Arf6 directly activate a recombinant PI4P5K by using purified proteins in the presence of PI4P and 32Pγ ATP. The recombinant active kinase did not affect calcium-induced acrosomal exocytosis in functional assays but the antibody against the PI4P5K inhibited calcium-triggered AR suggesting that this enzyme is required for exocytosis.

LI-P07

MANIPULATION OF THE TOPOLOGY OF A MEMBRANE SUPPORTED ON SOLID: DEBUGGING AND CHARACTERIZATION

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The construction of functionalized surfaces for use in nanotechnology and biotechnology has opened a new area in materials science. Advances in microfabrication and nanofabrication processes are also opening new opportunities to investigate complex questions of cell biology in a way not previously possible. In particular, the spatial regulation of cellular processes can be studied through the design of physical and chemical conditions to which the cell is exposed. Lithographic methods and selective chemical modification schemes can provide biocompatible surfaces that control cellular interactions on the micron and submicron scales on which cells are organized. Combined with fluorescence microscopy and other techniques of cell biology, a widely expanded toolbox is becoming available.
In this work we present our progress in the development of the technique of Micro Contact Printing and results obtained on the modification of the topology of supported membranes, the mobility of membrane components, and the ability of the membrane to bind ligands of interest.

LI-P08

EFFECT OF FATTY ACIDS ON THE SECRETION OF PROINFLAMMATORY CYTOKINES IN INTESTINAL EPITHELIAL CELLS

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Intestinal epithelial cells (IEC) form the first line of defense against microorganisms and food derived antigens contained in the intestinal lumen. As such, IEC also participates in the assimilation and processing of a large quantity of lipids incorporated with the diet. These cells express Intestinal Fatty Acid Binding Protein (IFABP) and Liver-FABP. The classical functions attributed to these proteins are as cytosolic buffers and transporters of hydrophobic ligands. Nevertheless, new insigths have been suggested, FABP could have a role in the regulation of lipid metabolism and other cellular processes. In this work we planned to study the effect of principal dietary fatty acids on the secretion of proinflammatory cytokines in the Caco-2 cell intestinal epithelial model. The role of l- and LFABP will be also assessed using this model. We analyzed this effect by evaluation of mRNA expression level of cytokines such as IL6, IL8, CCL2 and CXCL10 by Real Time-PCR. Preliminary results suggest that oleic and palmitic acid would be increasing mRNA cytokines levels in a time dependent manner. What is more, the analysis of the effect of these fatty acids in the secretion of proinflammatory cytokines using the Caco-2 LFABP knock down model previously obtained in our laboratory will contribute to the knowledge of new functions for this proteins involved in the immunity of IEC

LI-P09

IDENTIFICATION, CLONING AND EXPRESSION OF A NOVEL PHOSPHOLIPASE A FROM *Leishmania braziliensis*

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We have reported in *Trypanosoma cruzi* infective stages the presence of Phospholipase A1 (PLA1), a membrane and secreted activity that modified Vero cells lipid profile and activated PKC, suggesting its involvement in the signaling events of parasite invasion (Belaunzarán *et al.*, 2007). To extend these studies to *Leishmania braziliensis*, we here describe the cloning and expression of PLA from this parasite. We identify in the genome database TriTrypDb (http://tritrypdb.org/tritrypdb/) 2 putative genes with lipase motif GXSXG: LbrM33_V2.1730 and LbrM31_V2.2750. As the first codifies for a putative precursor, LbrM31_V2.2750 was chosen. Primers were designed and PCR was done using genomic DNA of the reference clone MHOM/BR/75M2904. A unique band (1129 bp) was obtained and cloned into pGEMT vector, sequence was corroborated and then cloned into pET30a and pGEX-6p1 plasmids. Expression was assayed at 21-37ºC and 0.05-1mM IPTG. The best yields were obtained with pET30a in BL21C43(DE3)pLysS cells at 1mM IPTG, 37°C, 3 hs. Protein identity was confirmed by Western blot with anti-*T. cruzi* and anti-*T. brucei* PLA1 antibodies. Enzyme activity was assay with 14C Phosphatidylcholine confirming that the expressed gene has PLA activity. Further studies will allow us to determine the role of PLA in L. braziliensis biology as well as its potential pathogenic effects.

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LI-P10

HYPERTONICITY INDUCES OPPOSITE REGULATION OF PPARγ AND CYCLOOXYGENASE 2 EXPRESSION IN RENAL CELLS

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Peroxisome proliferator-activated receptors (PPARs) regulates the transcription of lipid metabolism related-genes including cyclooxygenase (COX2) which is considered an osmoprotective molecule in renal cells. Although PPARγ was demonstrated to be present in renal cells, the relationship between both proteins has not been established. The present work explores whether PPARγ regulates COX2 expression in renal epithelial cells in isotonic and hypertonic conditions. MDCK cell cultures were grown in isotonic (298 mOsm/Kg H2O) and NaCl-hypertonic (500 mOsm/Kg H2O) media in the absence or presence of PPARγ agonists, rosiglitazone (Rosi) and 15-deoxy-Δ12,14-PGJ2 (PGJ2), or antagonist, GW9662 (GW). After 24 h, treated cells were collected and submitted to westernblot analysis for PPARγ and COX2 In parallel experiments, immunofluorescence microscopy was performed. In isotonicity, Rosi and PGJ2 decreased COX2 expression, but they did not affect PPARγ protein. Therefore, the activation of PPARγ represses COX2 expression. In hypertonicity, the NaCl-induced increase of COX2 level was not prevented by GW, suggesting that PPARγ is not involved. But, when PPARγ was evaluated, the active form of PPARγ 2 was withdrawn. Taken together these results suggest that the up-regulation of the osmoprotective gene COX2 in cells submitted to high-NaCl medium only occurs after

LI-P11

THERMAL BEHAVIOR OF SPHINGOMYELINS WITH VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA)

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Rat germ cells and spermatozoa contain species of SM and Cer with 28:4n-6 and 30:5n-6, followed by 32:5n-6, as main nonhydroxy (n) and 2-hydroxy (2-OH) VLCPUFA. The aim of this study is to gain some insight into the properties of these unique sphingolipids in membranes. Total SM from rat testis was partially separated by TLC into two main fractions: SM1, containing n-VLCPUFA, and SM2, containing similar proportions of 16:0 and 18:0 as well as 2-OH VLCPUFA. Each was then separated into molecular species by HPLC. Liposomes formed with these fractions or species were prepared and the generalized polarization of the fluorescent probe Laurdan as a function of temperature was followed. The effects of adding various % of these SM upon the transition temperature (Tt) of dipalmitoyl and dimiristoyl phosphatidylcholine (DPPC, DMPC) were also determined. The Tt of SM1 was significantly lower than that of SM2. In turn, the Tt of SM2 was lower than that of 16:0-SM, 18:0-SM, or a 50:50 mixture of 16:0-SM/18:0-SM, indicating that the presence of SM with 2-OH VLCPUFA in SM2 accounts for this decrease. The Tt of DPPC and DMPC decreased and increased with SM2, respectively, and decreased when SM1 or species with n-VLCPUFA were added. The potential increase in Tt that could have resulted from their unusually long carbon chains is thus overriden by the effect the double bonds have in decreasing it

LI-P12

LIPIDS WITH LONG CHAIN POLYENOIC FATTY ACIDS (PUFA) OF THE n-9 SERIES IN RAT EPIDIDYMAL CELLS

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In rats, epididymal sperm maturation is associated with the acquisition of glycerophospholipids (GPL) with uncommon C20-C24 carbon PUFA of the n-9 series, particularly 22:4n-9-rich plasmenylcholine. Little is known about how the epididymal epithelium participates in this accretion. In this study, three epididymal regions (caput, corpus, cauda) were isolated for lipid and fatty acid analysis. Unexpectedly, the main n-9 PUFA-rich epididymal GPL including plasmalogens were those of ethanolamine rather than those of choline. Epididymal triacylglycerols (TAG) were highly enriched in 18:1n-9, a precursor of 22:4n-9, whereas the ether-linked triglycerides (ADG), as the GPL, were rich in C20-C24 n-9 PUFA (mainly 22:4n-9). The epididymal n-9 PUFA-rich ADG were made up by important proportions of 1-O-alkyl and 1-alk-1´enyl diacylglycerols. This contrasts with the ADG of testis, where the former subclass predominates over the latter and both are rich in 22:5n-6. Although 22:4n-9-rich GPL and ADG were present in the three epididymal segments, they were significantly more concentrated in the corpus, the metabolically most active region of the epididymis. Our results suggest an active involvement of epididymal cells in the lipid remodeling undergone by spermatozoa during their maturation, most of which takes place before the mature gametes are stored in the cauda region.

LI-P13
L-FABP COLOCALIZES WITH NUCLEAR LIPID DROPLETS
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We have already shown that: 1) L-FABP promotes endonuclear FA mobilization and release to different nuclear and cellular compartments and 2) neutral lipids are organized in discrete non polar domains inside the nuclei (N), Nuclear Lipid Droplets (NLD), which consist of a hydrophobic core of neutral lipids (NL) covered by a monolayer of PL and associated proteins. So, the aim of the present work was to elucidate if L-FABP can mobilize FA to NLD. With this purpose N were incubated *in vitro* with L-FABP, 20:4n-6 (AA), ATP and / or CoA in different experimental conditions. L-FABP distribution was analyzed by immunofluorescence by fluorescense confocal microscopy. N was stained with DAPI and neutral lipids with BODIPY493/503. L-FABP was internalized in a diffuse pattern of distribution with several intranuclear foci. These foci presented a contact zone (colocalization) with NLD. In particular when N were incubated with L-FABP-AA, NLD were surrounded by L-FABP. In conclusion, these results suggest that L-FABP targets AA directly to NLD. L-FABP foci could represent either alternative soluble AA pools or a nuclear domain that keeps a close relationship with NLD.

LI-P14
SYNERGISTIC EFFECTS OF MONOTERPENES ON Hep G2 AND A549 TUMOR CELLS
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High intake of fruits and vegetables correlates epidemiologically with a reduction in cancer incidence. Among their components, the isoprenoids are phytochemicals that could have antitumor activity because of their multiple effects on mevalonate pathway. The aim of this work was to study the effect of linalool (L), cineole (C) or their combination on cell proliferation (CP) and lipid content in HepG2 and A549 human tumor cells. Cells were treated with individual monoterpenes (0-10000 µM) for 48 h and their IC50 (dose resulting in 50% cell growth inhibition) were determined. Synergism between both monoterpenes was evaluated using half of their IC50 values. Cellular proliferation, cholesterol (Cho), triglyceride (TAG) and phosphorus (P) content were determined. In HepG2 cells, L and C individually produced 11 and 9% inhibition of CP respectively, without altering the lipid content, while their combination decreased CP (40%), content of Cho (20%) and TAG (36%). In A549 cells, L and C inhibited CP by 10 and 15% respectively, resulting their combination in a decrease in CP (59%). In conclusion, according to our results, L and C have synergistic effects on the inhibition of CP and diminution of Cho and TAG in human tumor cells.

LI-P15
CELL CHOLESTEROL REMOVAL BY DISCOIDAL HDL: INFLUENCE OF APOLIPOPROTEIN A-I HELIX REGISTRY
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Discoidal high-density lipoproteins (dHDL) are key intermediates in apolipoprotein A-I (apoAI) mediated cell lipid efflux. Due to the difficulty of dHDL isolation, the current knowledge about these complexes comes from studies using dHDL reconstituted by detergent dialysis, although they can also be generated by the spontaneous reaction of apoAI with phospholipid vesicles at their phase transition temperature, a procedure that may be similar to the "*in vivo*" apoAI lipidation.
We show evidence that spontaneously generated dHDL are more active than those obtained by cholate-dialysis in promoting cholesterol efflux from murine macrophagues. Moreover, through FRET measurements with single tryptophan or fluorescent-labeled cysteine mutants of apoAI, it is detected that spontaneously generated dHDL consist predominantly of a unique apoAI configuration with a fix helix registry (LL5/2) in opposition to cholate-dialysis generated dHDL which consist of at least two configurations of variable helix registry (LL5/5 and LL5/2).
Thus, our data indicate that only dHDL containig the LL5/2 configuration promote cell cholesterol efflux. As we previously proposed, the central 3-4 helix pairs form an intermolecular membrane-inserting bundle, which is possible in LL5/2 but not in LL5/5 configuration. Therefore, the formation of this intermolecular bundle should be essential for the dHDL activity.

LI-P16
IMPROVEMENT OF POLYUNSATURATED FATTY ACID (PUFA) PRODUCTION IN *S. cerevisiae*
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Fatty acids (FA) must be activated to their coenzyme A derivatives, prior to further metabolism. This process is catalyzed by acyl-CoA synthetases (ACS).
In previous works, four *Trypanosoma brucei* ACS genes (Tb-ACS 1, 2, 3 and 4) have been characterized by their expression in *E. coli*. It was found that although ACS1 prefers saturated FA, it is the only one that performs well with PUFAs, like araquidonic and docosahexanoic acids.
We achieved expression of trypanosomatid enzymes of PUFA biosynthesis in *S. cerevisiae*. Fatty acyl CoAs, formed by endogenous ACSs or exogenously supplied FAs are substrates of heterologous desaturases (DES) and elongases (ELO). Since ACSs from yeast have low affinity by PUFAs we hypothesized that co-expression of Tb-ACS1 will increase levels of activated FAs and help to improve recovered activities of ELO and DES, with potential application in biotechnology.
We cloned Tb-ACS1 in the yeast expression vector p425 and determined FA profiles of S. cerevisiae harbouring the empty vector or p425-Tb-ACS1, after supplementation with different substrates. Results show that yeast expressing Tb-ACS1 carried out a better activation of exogenous long chain and unsaturated FAs.
We also co-expressed the ACS1-Tb with the *L. major* ELO5. Surprisingly, the elongase was inactive in this background. A discussion about possible explanations for this result is presented.

LI-P17
GLYCEROL-3-P ACYLTRANSFERASE 2 STIMULATES THE SYNTHESIS OF ARACHIDONIC-ACID-RICH TRIACYLGLYCEROLS

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Glycerol-3-phosphate acyltransferase 2 (GPAT2) is a mitochondrial GPAT isoform highly expressed in testis. GPAT catalyzes the first and committed step in *de novo* glycerolipid synthesis. In particular GPAT2 has no preference for saturated acyl-CoA substrates, so we hypothesized that it would direct the synthesis of triacylglycerols (TAG) rich in polyunsaturated fatty acids (PUFA) involved in the traffic and metabolism of PUFA in testis. We have previously shown that GPAT2 overexpression in CHOK1 cells increased TAG content. To determine the specific role of GPAT2 we used GPAT2-overexpressing CHOK1 cells to measure GPAT and AGPAT activities using different acyl-CoAs and determine the fatty acid (FA) composition of TAG extracted from cells incubated with 10% FBS DMEM (control media) or with media supplemented with different FA. GPAT2 overexpression increased the incorporation of 18:1n9 and 18:2n9, respecting to control cells, when cells were incubated in media supplemented with 50 µM of the corresponding exogenous FA. When cells were incubated in control media GPAT2-overexpressing cells increased the incorporation of arachidonic acid into TAG. Consistently, in GPAT2 overexpressing cells both GPAT and AGPAT activities increased 2 fold only when arachidonoyl-CoA was used as a substrate. Our results suggest that GPAT2 is involved in arachidonic acid enriched TAG synthesis.

LI-P18
TEMPORAL REGULATION OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN PERIPHERAL OSCILLATORS

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3–10% of transcriptome mostly involved in essential metabolic pathways is under circadian regulation. Circadian oscillators are present in different peripheral organs and tissues and even in immortalized cell lines. We reported that the phospholipid synthesis oscillates in synchronized fibroblast cultures under an intrinsic clock control. However, it was unknown whether the phosphatidylcholine (PC) metabolism was temporally regulated. To this end, we studied the temporal expression and activity of CTP:phosphocholine cytidylyltranferase (CT), the key PC synthesising enzyme. We found that higher labeled PC levels observed at 6.5 h after serum shock (SS) correlates with an increase in CT activity at this time. CT activity remains low and peaks again by 33 h after SS. We found detectable mRNA levels for all CT isoforms and a significant increase of CTα1 mRNA at 3 h of SS by qPCR, returning to basal levels later on. In contrast the CTβ2 mRNA was found elevated between 6-18 h post SS. The contribution of CTα1 to CTβ2 was higher at all times (0-36h). Preliminary results show that CTα protein remains constant during 36 h while CTb isoforms exhibit higher levels after 9 h SS. Results suggest that the PC biosynthesis is driven by a circadian clock operating at different levels (enzyme activity/transcription, etc), and that CT isoforms are differentially regulated in synchronized cells.

LI-P19
A PROTEOMIC APPROACH TO THE STUDY OF AQUATIC POLLUTION IN A SHRIMP EXPOSED TO CRUDE OIL HYDROCARBONS

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In order to mimic the biological effects of a freshwater oil spill, we examined the differential protein expression as potential biomarker in the freshwater shrimp *Macrobrachium borellii* experimentally exposed to the water soluble fraction (WSF) of crude oil (mostly a mixture of low-boiling aromatics which closely resembles the aromatic hydrocarbon of diesel oil fuel). To this aim, midgut gland proteome was studied by proteomic analysis. Protein mixtures were extracted from shrimps exposed for 7 days to a subletal concentration of WSF (0.34 ppm). Midgut gland from non exposed shrimp was used as control. Protein extracts were subsequently subject to 2D gel electrophoresis and spots from sample and control gels compared to identify differentially-expressed proteins. Spots were punched out of the gel and analyzed by ESI-TOF/TOF mass spectrometry. Identity assigned in a MASCOT search mined against all known proteins. Exposure to pollutants revealed the presence of several proteins over- and underexpressed. The transcription levels of genes from a subset of the identified proteins were then analyzed by real-time PCR.
The analysis of differentially expressed proteins in shrimp exposed to hydrocarbon provided a new insight in the identification of potential biomarkers highlighting the usefulness of employing 2DE-based proteomic approach to aquatic pollution studies.

LI-P20
PROTECTIVE EFFECT OF LIPOIC ACID TO PESTICIDE-INDUCED BRAIN DAMAGE

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We have previously demonstrated that the administration of low doses of dimethoate, glyphosate and zineb to rats (i.p. 1/250 LD50, 3 times a week/ 5 weeks), provokes severe oxidative damage to macromolecules in liver and in certain brain regions (substantia nigra, SN and cortex, CC). Lipoic acid (LA) is considered an ideal antioxidant due to its ability to scavenge reactive species and reset the levels of endogenous and exogenous antioxidants. To investigate the protective effect we administered LA (i.p. 50 and 100 mg/Kg body weight) simultaneously with the pesticide mixture for 5 weeks. Both doses prevent the formation of protein carbonyls (PCOs) and lipid peroxides (MDA) reverting the increases induced by pesticide treatment to almost basal values. LA demonstrates important antioxidant properties especially in SN, since pesticide treatment increases the MDA levels in 136% in SN and in 80% in CC and the LA treatment was able to completely normalize the values in both regions. LA is also able to restore the lower levels of α-tocopherol in the pesticide-treated group in all the tissues studied. The content of total glutathione in pesticide–treated rats doubles control ones and LA administration normalizes it. LA seems to be a protective agent against pesticide-induced damages and would be a promising therapeutic strategy in neurodegenerative disorders such as Parkinson's disease.

LI-P21

CU OVERLOAD INDUCED-DAMAGES IN CULTURED CELLS EFFECT OF CARNOSINE AND NEOCUPROINE

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Cu overload is dangerous mainly because it can participate in the Häber-Weiss reaction producing ROS. As a consequence of pollution people are exposed to Cu overload under a sub-clinical and sub-symptomatological condition which obviously is very difficult to detect. So, we investigated (i) the possible use of chelator molecules, carnosine (CR) and neocuproine (NC) to prevent Cu overload-induced damages on cellular lipids and proteins tested in HepG2 and A-549 human cells; and (ii) the differential response of these chelators in relation to the protective action and the type of Cu ion involved. Cu treatment enhanced the formation of protein carbonyls, TBARs and nitrate plus nitrites with a concomitant decrease in cell survival estimated by trypan dye exclusion and LDH leakage. Simultaneous treatment with the chelators demonstrated that CR is more efficient than NC in protecting both types of cells from the effect of Cu on both the cell-associated damages and the decrease of cellular viability. This observation is supported by the fact that CR is not only a complexing agent for Cu(II) but also an effective antioxidant that can dismutate $O_2^{\cdot}$, scavenge OH. and neutralize TBARs formation. CR should be investigated in order to establish its putative utility as a supplement to prevent Cu-associated damages in humans.

LI-P22

BIOSYNTHESIS OF GLYCEROPHOSPHOLIPIDS AND SPHINGOLIPIDS IN ISOLATED SPERMATOGENIC CELLS

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In addition to glycerophospholipids (GPL) with 16:0 and 22:5n-6, pachytene spermatocytes and round spermatids were recently shown to be rich in sphingomyelins (SM) and ceramides (Cer) with almost exclusively very long chain polyenes (VLCPUFA). How germ cells biosynthesize these highly unsaturated lipids remains to be established. In this study, the incorporation of [3H]-palmitic acid and [3H]-serine into lipids of each of these cells was compared. Most of the label from [3H]-palmitic acid and serine was incorporated into choline and ethanolamine GPL, respectively. Although in lower proportion than GPL, sphingolipids were also labeled with both precursors. Cer was more actively labeled in spermatocytes than in spermatids, the opposite occurred with SM, and glucosylCer (GlcCer) was the most actively labeled sphingolipid in both cell types. This was confirmed with the fluorescent marker NBD C6-Cer, which was incorporated in both cell types to produce fluorescent SM and GlcCer, in proportions that were consistent with those observed with the radiolabeled precursors: a higher labeling of SM in spermatids than in spermatocytes and an active labeling of GlcCer in both. Taken together, the present results indicate that germ cells are able to perform the *de novo* synthesis of their own phospholipids, including and GPL and sphingolipids, independently of Sertoli cells.

LI-P23

CHARACTERIZATION AND REGULATION OF DIACYLGLYCEROL LIPASE IN ISOLATED NUCLEI FROM RAT CEREBELLUM

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Using [^{3}H]PtdOH as substrate, we demonstrated the existence of several metabolic pathways which involve phosphatidate phosphohydrolase (LPP), diacylglyceride lipase (DAGL) and monoacylglycerol lipase (MAGL) activities in the nucleus. These activities modulate changes in its lipid composition, generating second messengers implicated in intranuclear signaling. The aim of the present work was to evaluate and characterize DAGL activity using exogenously added [^{3}H] diacylglycerol ([^{3}H]DAG) as substrate. We also studied its regulation by retinoic acid. 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 indicate that MAG formation increased up to two minutes, 700 µM of substrate and about 50 µg of proteins. Interestingly, MAG production occurred at a higher DAG concentration (150 µM) than that used (60 µM) when DAG was generated from PtdOH. In addition, the nuclear membranes pre-incubated with a nuclear receptor RAR/RXR agonist, all-trans retinoic acid (10 µM), showed a significant DAGL inhibition of about 70%. Taken together, our results demonstrate the presence of an important regulated-enzyme related to signaling pathways in rat cerebellum nuclei.

LI-P24

EFFECTS OF MANDARIN ESSENTIAL OIL ON CELLULAR GROWTH AND LIPID METABOLISM

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Essential oil is a mixture of fragrant volatile compounds, named after the aromatic characteristics of plant materials from which they can be isolated. They basically consist of two classes of compounds: terpenes and phenylpropenes. Many terpenes have multiple effects on mevalonate metabolism and antiproliferative effect on various cell lines. The aim of this work was to study the effect of essential oil of mandarin (Citrus reticulata Blanco) (AEM) and its major component, limonene (LM) on cell growth and lipid content in A549 and HEPG2 human tumor cells. The AEM was obtained by cold pressing the fruit peel. The effect on cellular proliferation and viability was performed using MTT test. Cholesterol, triglyceride and phosphorus were determined for the lipid analysis. Results indicate that AEM and LM inhibit cell proliferation of both tumor lines. Preliminary data showed that HEPG2 and A549 treated with AEM had lower IC50 values (dose resulting in 50% cell growth inhibition) than cells treated with LM. A549 cells incubated with the AEM IC50 value diminished the content of total cholesterol and triglyceride. We concluded that C. reticulata AEM has an antiproliferative effect on human tumor cells and that LM, the major terpene component, is partially responsible for the inhibition of cell proliferation

LI-P25

QUANTIFICATION OF AGEING-ASSOCIATED CHANGES USING LIPID PARAMETERS IN THE MEDFLY *Ceratitis capitata*

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Aging is defined as the pattern of change in mortality over age. To study the combinatorial effects of stress and age in Medfly adults, reference biochemical and physiological parameters were studied. The age- sex- and tagma-dependent changes in storage molecules (lipids, glycogen and proteins), and in lipid peroxidation and protein carbonylation were quantified. The decay in male locomotor activity started very early after emergence but no deaths were registered until the fourth week. Then 50% of the males died during the next two weeks. The maximum level of total lipids was attained at day 15 in thorax/muscle and in head/brain and then diminished with age. However free and bound body sterols increased with age with the exception of head/brain free sterols that decreased at day 15 to later return to previous level. Significantly lipid peroxidation in head and thorax was high until day 15, and then decreased. Our results demonstrate that significant changes occur in male brain and muscle lipid profiles from days 15 to 20, just preceeding the initiation of deaths

LI-P26

ROLE OF PLASMA MEMBRANE LIPID COMPOSITION ON CELLULAR HOMEOSTASIS

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Evidence suggests that membrane-associated signaling and hence cell metabolism and viability, depend on membrane lipid composition. Our aim was to analyze the influence of membrane properties on cell homeostasis. For this purpose we constructed two permanent cell lines over expressing fatty acid desaturases: to begin with, CHO KI cells were transfected with rat Stearoyl CoA desaturase resulting in an increase of monounsaturated fatty acids at the plasma membrane (PM) (SAIB 2007 and 2008). Now we show results concerning a double transfectant over expressing both Δ5 and Δ6 desaturases (Δ5/Δ6-cells), which turned into a rise of polyunsaturated fatty acids. Furthermore, this cell-line showed lower contents of sphingomyelin and free cholesterol relative to phospholipids at the PM, consistent with a lower cholesterol removal by apoA-I, as well as an increase in the sterified-Cholesterol/free-cholesterol ratio. In addition, we analyzed PM properties through Laurdan Generalized Polarization (GP) by two-photon fluorescence microscopy. Preliminary data suggest higher membrane fluidity in Δ5/Δ6-cells PM when compared to control cells. Moreover, Δ5/Δ6-cells also showed differences in mitochondrial reduction activity. Together, our results indicate that membrane composition plays a role in lateral domain organization and cholesterol homeostasis and thus, in cell signaling and metabolism.

LI-P27

INTERACTION OF FCCP WITH LIPOSOMES. DEPENDENCE ON TEMPERATURE AND ON THE PHASE STATE

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FCCP (carbony lcyanide p-trifluoromethoxyphenyl hydrazone) is a protonophoric uncoupler. However at higher concentrations it exerts additional effects on the mitochondrial energy metabolism. We have previously shown that the binding of aniline-naphtalene sulphonate (ANS) to liposomes is also affected by FCCP.
We decided to study the effect of FCCP on the binding of ANS to DOPC liposomes at different temperatures (in the lamellar liquid crystalline mesophase) and to DPPC liposomes at 25ºC (gel phase) and 50ºC (liquid crystalline phase).
When DOPC was used, IC50(FCCP) values increases with temperature. Hence, the binding of FCCP to the bilayer is an exotermic process. The apparent Kd(ANS) also increases with temperature in the absence of FCCP, whereas it becomes independent of the temperature in its presence.
Conversely, the apparent Kd(ANS) for the binding of ANS to DPPC liposomes at 50ºC is significantly lower than that obtained at 25Cº either in the absence or in the presence of FCCP. Hence the membrane phase influences the binding of ANS differently than temperature. However, IC50(FCCP) values obtained with DPPC do not significantly differ from those obtained with DOPC liposomes.
We can conclude that binding of FCCP to the bilayer is not dependent on the mesophase, and suggest that penetration of FCCP in the bilayer is not required for the inhibition of ANS binding.

LI-P28

AGE-RELATED REGULATION AND COMPARTAMENTALIZATION OF DIACYLGLYCEROL SIGNALING DURING OXIDATIVE STRESS

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We have previously demonstrated that iron-induced oxidative stress stimulates diacylglycerol (DAG) generation from phosphatidylcholine (PC) in isolated cerebral cortex synaptic endings (Syn) obtained from adult and aged rats (Mateos et al., 2008). This lipid messenger can be generated either by a PC-specific phospholipase C (PC-PLC) or through the action of phospholipase D (PLD). In this work we study the mechanisms that govern DAG signaling during oxidative injury. DAG formation was differentially affected by the use of PIP2-PLC, PKC and ERK kinases inhibitors (U73122, BIM and U0126, respectively). U73122 partially inhibited DAG generation in adult rats. In aged animals, PKC and ERK inhibition showed to decrease (20 %) DAG generation induced by oxidative injury. The co-localization of PLD1 and PC-PLC with flotillin, and the DAG generation were not affected neither by aging nor by oxidative stress in isolated membrane rafts (MR). In addition, the iron-induced increase in lipid peroxidation levels observed in Syn was absent in MR. The reduced lipid peroxidation levels in MR, were in accordance with a decrease (68%) in polyunsaturated fatty acid composition with respect to Syn. Our results show that during oxidative injury: i) PC hydrolysis is differentially regulated by aging and ii) PC-PLC and PLD compartmentalization could be a mechanism for preventing DAG rise.

LI-P29
THE ROLE OF MAPKS AND PLA2 IN A RETINAL MODEL OF NEURODEGENERATION
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Free iron is considered as a neurotoxic agent that generates an imbalance in cellular redox mechanisms, producing lipid peroxidation, mitochondrial damage, and cell death. Iron exposure of bovine retinas constitutes a suitable experimental model of age-related macular degeneration. In this model, we have previously reported the activation of different phospholipase A2 (PLA2) isoforms (SAIB 2009). The aim of this work, was to characterize the role of cPLA2, iPLA2 and MAPKs in retinas exposed to increasing Fe2+ concentrations (25, 200 and 800 µM). Mitochondrial function and lipid peroxidation levels (measured as MTT reduction and TBARS, respectively) were determined as markers of oxidative damage. TBARS generation increased in an iron concentration dependent manner, whereas mitochondrial function was only affected at 800 µM. This was associated with the activation of ERK1/2 and p38 MAPKs. TBARS generation was significantly decreased in the presence of ATK (iPLA2 and cPLA2 inhibitor) and BEL (iPLA2 inhibitor). ATK and BEL were able to delay ERK activation at Fe 25 µM. Results suggest that both PLA2 isoforms participate in the generation of retinal oxidative damage and also modulate MAPK activation.
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LI-P30
LIPID DYNAMICS OF ARACHNID LIPOPROTEINS
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It has been already reported that hemolymph lipids in the spider P. pythagoricus are transported by HDL1 and VHDL lipoproteins. In the present work we studied *in vitro* the lipid transfer among intestinal diverticula (ID), principal organ involved in lipid biosynthesis, and either hemolymph (HL) or purified lipoproteins as well as between HL lipoproteins. ID and HL were labeled *in* vivo with 14C-palmitic acid. *In vitro* incubations were between ID and HL, as well as between isolated lipoproteins and ID were performed, being lipid transfer quantified by radio-TLC.
Lipoproteins (VHDL and in a minor extent by HDL1) released 76% of its lipids to ID. Isolated lipoproteins showed the same behavior to that observed with whole hemolymph.
Lipid uptake in hemolymph was greater in HDL1 (67%), though it was similar when the isolated lipoproteins (50% each) were assayed.
Lipid uptake and release in these systems were found to be constant within a wide range of starting lipid label.
Intestinal diverticula were found to retain free fatty acids and tryacilglicerides as only 28% of the label were transferred to lipoproteins while 76% was taken up from HL. A similar trend was observed regarding lipoprotein PL

LI-P31
PHASE TRANSITION BEHAVIOUR IN GIANT UNILAMELLAR VESICLES CONTAINING CERAMIDES
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It is generally plasma membrane lipids contribute to cellular processes, although very little is known regarding the underlying physical basis by which lipids accomplish this function, one hypothesis is that the cell membrane contain dynamic submicrometer domains that are enriched in certain lipids , and these can change in size, composition and stability in response to cues. There is evidence showing that also in complex cell membranes, the tendency of certain lipid mixtures to phase separate is maintained, especially those containing cholesterol and a mixture of saturated and unsaturated lipids. We are particularly interested in the dynamics of the coexisting domains near the phase transition temperature as a function of composition, since previous data of ours suggests the possibility of these mixtures showing a critical point.When a multicomponent system aproaches a critical point the two coexisting phases become identical and the system needs very litle energy to maintain inhomogeneous composition.This critical point depends mainly on de temperature and the composition.Our model system consists of Giant Unilamellar Vesicles (GUVs) composed of dioleoylPC (DOPC)-Brain sphingomyelin (BSM)-Brain ceramide (BCer)-Cholesterol (CHOL), at different proportions BSM:BCer. We have characterized this system by means of confocal fluorescence microscopy at different temperatures.

LI-P32
EFFECT OF RECONSTITUTED DISCOIDAL LIPOPROTEINS ON LIPID MOBILIZATION IN TWO CELL LINES
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Discoidal high density lipoproteins (dHDL) play a key role in apolipoprotein A-I (apoAI) mediated cell lipid efflux. We have used reconstituted dHDL of different size either containing or not cholesterol (C) with the aim of comparing their ability to modify the cellular distribution between C and cholesteryl esters (CE) as well as to promote cellular efflux of choline-containing phospholipid as phosphatidylcholine (PC) or sphingomyelin (SM). Two cell lines were used, one of them (CHOK1) with limited capacity to store CE, and the second one (Raw 264.7 macrophages) capable to accumulate large amounts of CE until they are transformed in foam cells responsible for the atherosclerotic plaque formation. In RAW cells, dHDL tend to increase the cellular CE/C ratio although it is statistically significant only in the case of C-containing 96 Å discs. On the other hand, in CHO cells lipid-free apoAI and large (120 Å) C-free dHDL decrease the cellular CE/C ratio indicating a lower availability of C to be esterified. With both cell lines, only PC (but not SM) efflux is evoked by apoAI or dHDL although some dependence on the cell type is observed. In RAW cells, apoAI and all dHDL types with the only exception of C-free 96 Å discs promote PC efflux and decrease the cellular PC/SM ratio, but only apoAI and C-free 78 Å discs are active in this process concerning CHO cells.

LI-P33

EFFECTS OF DIET SUPPLEMENTATION WITH GERANIOL ON LIPID METABOLISM AND TUMOR GROWTH IN NUDE MICE

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Products of plant mevalonate metabolism, like geraniol (GOH), have multiple pharmacologic effects on animal mevalonate metabolism; some of them may account for their effects on some tumor cells. To investigate the impact of dietary geraniol on lipid metabolism and growth of tumor cells "*in-vivo*", we implanted A549 cell line in nude mice. Animals were fed a control or experimental (25; 50 or 75 mmol.GOH/kg diet) diet for 25 days after tumor implant. Tumors were measured and animals were weighed twice a week. On day 25 mice were killed and livers, blood and tumors were removed. Three hours before sacrifice, animals were injected with 25 µCi de 14C-acetate. Cholesterolemia, incorporation of radioactivity into liver and tumor lipids, HMGCoA reductase and SREBP2 were determined. Higher doses of GOH significantly reduced tumor growth but only the highest dose significantly reduced mice body weight. Serum cholesterol diminished in GOH treated host mice (12±1.5%) and non-host mice (7±1.5%). GOH decreased 14C-acetate incorporated into total and nonsaponifiable lipids in liver and tumor of treated mice, and reduced HMGCoA reductase levels (23.9±1.9%) and SREBP2 levels (24.1±2%) in non-host mice liver. Host mice SREBP2 level was only reduced with the highest GOH dose. We concluded that geraniol inhibited tumor growth, diminished serum cholesterol and modified tumor and liver lipid metabolism.

LI-P34

INTRANUCLEAR SPECKLE LOCALIZATION OF CYTIDYLYLTRANSFERASE ALPHA SERVES AS A REGULATORY MECHANISM

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Cytidylyltransferase alpha (CCTα) is an amphitropic enzyme, but in most cell lines it has been localized predominantly in the nucleus. We have demonstrated that hypertonicity (stimulus that evokes differentiation) induces lamin A/C distribution to nucleoplasmic speckles and CCTα colocalizes with these intranuclear structures. In the present work we evaluated the importance of CCTα intranuclear redistribution and its relationship with enzyme activity and PtdCho synthesis. Silencing of Lamin A/C caused decrease in CCTα speckles number and an increase in CCTa activity. To evaluate CCTα speckle dynamics, cells were treated with oleic acid. Treatment was able to mobilize CCTα outside of speckles and was redistributed to nuclear envelope. Interestedly CCTα relocalization occurred only in MDCK cells subjected to hypertonicity but not in control cells. Oleic acid stimulus induced higher increase in PtdCho synthesis in control cells than hypertonic treated cells. No changes were observed in CCTa activity. Results herein demonstrate that oleic acid induces CCTα redistribution which occurs only in hypertonicity subjected MDCK cell, suggesting that in differentiated cells, CCTα-speckles are necessary for its re-localization. Under hypertonic condition intranuclear CCTα distribution in nuclear speckles could serve as a localized platform that facilitates CCTα mobilization.

SB-P01

STRUCTURAL EFFECTS OF A MUTATION RESPONSIBLE FOR A GLYCOGENIN RELATED NOVEL GLYCOGEN STORAGE DISEASE

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Glycogen Storage Diseases (GSD) are inherited metabolic disorders of glycogen metabolism. They are classified based on the enzyme deficiency and the affected tissue. The most recently described GSD is due to the deficiency of glycogenin-1, caused by a Thr82Met mutation. Glycogenin is a self-glucosylating protein involved in the initiation of glycogen synthesis. In the presence of UDP-glucose and Mn^{+2} it catalyzes the formation of a short glucose polymer covalently attached to its Tyr194 hydroxyl group. Glycogenin-1 is one of the two human glycogenin isoforms and is mainly expressed in muscle. The second isoform, glycogenin-2, is the liver protein. Human glycogenin-1 displays 93% identity with rabbit skeletal muscle glycogenin, the best studied member of this protein family and the only one whose three dimensional structure has been solved. Thr82 is not only present in human and rabbit glycogenins, it is also conserved in many members of the family. According to the reaction mechanism proposed for the protein, this residue would not be directly involved neither in catalysis nor in substrate binding. To explore the reasons for Thr82Met mutation induced loss of function, we have prepared this mutant form of rabbit glycogenin. We report here the crystal structure of apo- and UDP-glucose/Mn^{+2} bound Thr82Met mutant and the comparison of these results with those of the wild type enzyme.

SB-P02

CONTRIBUTION TO THE KNOWLEDGE OF THE SALIVA PROTEOME AND ITS IMPORTANCE AS A DIAGNOSIS TOOL

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The saliva proteome includes a number of proteins involved in the homeostasis of the oral cavity and there is currently great interest in its utilization as a diagnosis tool. In this work we studied parotid saliva samples from normal children as well as those from children with JIA (Juvenile Idiopathic Arthritis). The 2D-polyacrylamide gel electrophoresis denoted their high complexity degree. Then, two methodologies were used: a) samples were resolved by molecular filtration and fractions analyzed by HPLC-ESI-MS; b) samples were digested by trypsin and then analyzed by nano HPLC-ESI-MS/MS. Results indicate sample degradation prior to saliva analysis and many peptides from parotid saliva proteins, such as alpha amylase and proline rich proteins were found. On the other hand, several proteins were described in normal human saliva for the first time (an actin-binding protein, an alpha enolase, fragments of the immunoglobulin kappa chain, a thioltransferase I, a leucocyte elastase inhibitor, apoliprotein A1). In addition, we found other two proteins only present in JIA samples and never described in normal human saliva. We also reported the finding of a 18460 Da molecular mass present in all JIA samples but absent in normal ones which could be used as a molecular marker for early detection of the disease.

SB-P03

ACTIVATION OF LIMULUS COAGULATION FACTOR G BY SCLEROGLUCAN AS A BIOLOGICAL ACTIVITY MEASUREMENT

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The ability of scleroglucan (ß-1,3-ß-1,6 glucan) conformers to activate Limulus coagulation factor G was evaluated. Studies pursued to get a further insight into the scleroglucan structure-function relationship. Lab-scale produced (EPS I, EPS II, EPSi) and commercial (LSCL) scleroglucans were tested on their ability to activate factor G of the Limulus coagulation cascade (Glucatell Test, Pyrolab). Native triplex conformation was obtained by dissolving scleroglucan (2 pg/mL) in distilled water. Thermally- (150°C, 30 min) and alkali-treated (0•2 N NaOH, 10 min) scleroglucans were also prepared. Single helices were further evaluated at 2 ng/mL and 2 µg/mL. All scleroglucans were endotoxin-free (LAL-Test, Pyrolab). Triplex conformation had stronger ability to activate factor G than single helix, with EPS I and EPS II as the most active polymers. Thermal and alkaline denaturation significantly reduced scleroglucan reactivity, being EPS II the most stable glucan, whilst EPSi and LSCL were the most affected ones (91-99% reduction). Single helix activation ability was significantly dependent on polysaccharide concentration. Scleroglucans from *S. rolfsii* ATCC 201126 (EPS I and EPS II) exhibited marked clotting activity against factor G and were more effective than commercial scleroglucan. Denaturation would lead to lower biological activity and it would be dependent on EPS concentration.

SB-P04

FRET-ASSISTED CONFORMATIONAL STUDIES OF ß-GLUCAN BIOPOLYMERS WITH BIOLOGICAL ACTIVITY

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(1,3)-ß-D-glucans such as Laminarin (Lam) are known to behave as immunopotentiators. Triple-helix is the prevailing conformation in aqueous solution and can be denatured with NaOH as single or partially opened-helices conformers. Which of these conformers is biologically most active or, if complete strands separation actually occurs is unclear. Fluorescence resonance energy transfer (FRET) spectroscopy represents an indirect method to characterize conformational changes of NaOH-treated Lam. FRET phenomenon occurs when a donor excitation energy is transferred to an acceptor over a short distance (10-80 Å). In this work, Lam was derivatized with 1-aminopyrene (AP) as donor (λ 450 nm), and fluorescein-5-isothyocyanate (FITC) as acceptor (λ 520 nm). The chain opening degree was assessed by treating double-labeled Lam (Lam-AP-FITC) with different concentrations of NaOH (0•10 – 1•00 M). FRET results demonstrated that partially opened triple-helix rather than single-helix conformation would be formed upon NaOH treatment of Lam. Increasing degrees of strand opening were associated with increasing concentrations of NaOH. From 0•10 to 0•25 M, FRET showed a significant decrease, involving an actual separation between AP and FITC probes. A conformational change degree between closed triple-helix and partially opened-triple-helix would be more reliable than the complete strands separation.

SB-P05

CGTase FROM *Bacillus circulans* DF 9R: PRODUCT SPECIFICITY DETERMINANTS

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Cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19), a member of the GH13 family, catalyses starch conversion into cyclodextrins (CDs), an important industrial process. CDs are cyclic a-1,4-glycosidic-linked oligosaccharides, with 6 (a-), 7 (ß-) or 8 (?-) glycosil units. Three out of the 9 described CGTase substrate binding subsites are involved in product specificity. CGTases from different strains of *B. circulans* produce mainly ß-CD; however, that from the DF 9R strain produces similar amounts of a- and ß-CD. Multiple sequence alignment showed that, as expected, the enzyme contains the 5 CGTase domains, the 7 regions conserved in the glycoside hydrolase Family 13, the conserved catalytic triad residues and those required for carbohydrate and calcium binding sites. However, also contains 43 residues not present in any of the CGTase family members. Shannon entropy analysis indicates that only 13 of them have an entropy value higher than 65 % and those at positions 179, 247, 537 and 609 show non-conservative substitutions. The fully conserved Gly179 (-6 subsite) has been naturally changed for Gln. That is why we built two mutants (Q179G and Q179L) to compare their biological properties with those of the native enzyme. Results indicate that the residue at position 179 is involved in product specificity and can give rise to a rational design of new mutants biotechnologically relevant.

SB-P06

THE BIOLOGICAL ACTIVITY OF ppGalNAc-T2 IS REGULATED BY CHEMICAL ACETYLATION

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Acetylation is an important molecular regulatory mechanism on the biological activity of proteins. Polypeptide GalNAc transferases (ppGalNAc-Ts) are a family of enzymes which catalyze the initiation of mucin-type O-glycosylation. ppGalNAc-Ts are type II transmembrane proteins with a Golgi luminal region containing a catalytic domain and a C-terminal R-type lectin domain. We investigate the acetylation effect on the catalytic activity and the carbohydrate-binding specificity of ppGalNAc-T2. Mass spectrometry of purified human recombinant ppGalNAc-T2 acetilated *in vitro* revealed acetylation of K103, S109, K111, K363, S373, K521 and S529. The first five amino acids are located in the catalytic domain. As a consequence of acetylation, ppGalNAc-T2 reduced its specific catalytic activity by 95% to different peptide acceptors (MUC1, MUC2 and MU5Ac). K521 and S529, belong to the lectin domain, their acetylation modified the carbohydrate-binding ability of ppGalNAc-T2. Direct binding assays showed that acetylated ppGalNAc-T2 enhanced the interaction to GalNAc-MUC1. The GalNAc-binding form of lectin domain was also changed by acetylation as demonstrated by competitive assays. In conclusion, the biological activity of ppGalNAc-T2 was clearly regulated by acetylation. In addition, immunoprecipitation on HeLa cell lysate demonstrated the *in vivo* presence of acetylated ppGalNAc-T2.

SB-P07

STRATEGY FOR OVEREXPRESSION AND STRUCTURAL ANALYSIS OF GumB, AN OUTER MEMBRANE LIPOPROTEIN

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Membrane proteins play essential roles in different cellular processes as signaling among cells, transport across membranes, and energy transduction. Despite their importance, there are few known 3D structures and less is known about their mechanism of action compared to soluble proteins. The lack of knowledge in this field is due to difficulties in obtaining enough amount of protein to carry out structural studies. Here we describe a strategy to resolve the structure of GumB, an outer membrane lipoprotein involved in secretion of xanthan, which is hard to overexpress. In initial overexpression assays, we found that GumB resulted toxic to the cells. After several trials, we found a condition with an appropriate level of expression. However, the amount of protein was not enough to set up crystallization screenings. At the moment, we are performing different purification protocols in order to obtain homogeneous pure GumB, in adequate amount suitable for the beginning of crystallization trials. As an alternative, we overexpressed, purified, and crystallized a form of GumB that lacks the signal peptide. Thus, it is overexpressed as a soluble protein in the cytoplasm (GumBc). Currently, we are working on the resolution of the 3 Å GumBc structure in order to use it as template for molecular replacement and to determine the structure of the lipidated form of GumB localized in the membrane.

SB-P08

FIRST STEPS FOR STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF GumC, AN INTEGRAL MEMBRANE PROTEIN

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Xanthan is an exopolysaccharide synthesized by different species of Xanthomonas, which is involved in diverse biological functions and in a wide range of industrial applications. Despite biosynthesis of xanthan repetitive units is widely studied, its polymerization and secretion mechanism is poorly understood. Previous studies from our laboratory in *Xanthomonas campestris* suggest that polymerization and secretion of xanthan could be associated, and the integral inner membrane protein GumC is involved in these processes. Therefore, we are interested in understand its structure and molecular mechanism of action. Because obtaining sufficient amounts of high-quality pure membrane proteins is still the mayor bottleneck in membrane protein studies, the first challenge of this work was to obtain enough amount of pure GumC. We overexpressed GumC in *Escherichia coli* BL21(DE3) with a hexahistidine–coding sequence. After several trials, we purified GumC to homogeneity by a two–chromatographic–step protocol and obtained a good yield of pure protein (8 mg per liter of culture). Currently, we are carrying out biophysical assays and crystallization screenings. In addition, based on *in silico* analysis, we are performing functional studies by site-directed mutagenesis, which showed difference in xanthan production when some conserved amino acids are mutated.

SB-P09

CHARACTERIZATION OF THE INTERACTION BETWEEN MITOGEN-ACTIVATED PROTEIN KINASE: JNK1 AND ITS SCAFFOLD

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The JNK MAPKs are characterized by the dual phosphorylation motif T-P-Y within their activation loop. JNK was discovered by its ability to phosphorylate the N-terminal transactivating domain of the transcription factor c-Jun. Scaffold proteins, which bind signaling molecules thereby creating functional modules that ensure the specificity of signal transmission, can increase the efficiency of the MAPK activation process.

JNK binds their upstream regulators (MAPKKs), downstream targets and scaffold protein by surface interactions that are achieved through docking motif binding sites that are outside of the catalytic domain. However, it is not clear how the signaling modules can be formed when all MAPK protein-protein interactions are in the same binding region. This raise the following questions, are scaffolds, MAPKKs, and targets competing for the same binding region? Does JNK phosphorylation regulate binding affinities? The general objective was to study *in vitro* binding selectivity of JNK1 towards MKK7, JIP1 and c-jun. We present here a detail study of JNK1-JIP1 interaction by classical spectroscopic techniques (circular dichroism, fluorescence anisotropy) and advanced techniques like fluorescence correlation spectroscopy. This work serves as a starting point to understand the paradox that scaffold protein, upstream regulator and transcription factor bind JNK in a competitive way.

MI-P01

A MEMBRANE COMPLEX INVOLVED IN THE SYNTHESIS, TRANSPORT AND SUCCINYLATION OF CYCLIC β-1,2-GLUCANS

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Cyclic â-1,2-glucans (CâG) are periplasmic homopolysaccharides that have been shown to play an important role in several symbiotic and pathogenic relationships. *B. abortus* CâG synthase (Cgs) is an integral inner membrane (IIM) protein which catalyzes the four different activities (initiation, elongation, phosphorolysis, and cyclization) required for the synthesis of CâG. Once synthesized in the cytoplasm, CâG are transported to the periplasm by the CâG transporter (Cgt) and succinylated by the CâG modifier enzyme (Cgm). Cgt and Cgm, as well as Cgs, are IIM proteins. In this work, we used a bacterial two-hybrid system (BACTH) to study the interaction network between these three IIM proteins. Our results indicate that Cgs interacts with Cgt and Cgm, and that Cgt interacts with Cgm. We have also observed that each one of these proteins form homotypic complexes. Furthermore, an analysis carried out with Cgs truncated or in-frame pentapeptide insertion mutants reveal that Cgs-dimerization as well as Cgs interactions with Cgt and Cgm are mediated by the NH-terminal domain and may be modulated by the C-terminal phosphorylase domain of the protein. We propose that Cgs, Cgt and Cgm form an inner membrane protein complex where the interactions between these proteins may provide a mechanism to coordinate the synthesis, transport to periplasm and succinylation of CâG.

MI-P02

HALOPHILIC BACTERIA *Salinibacter ruber* EXHIBITS REACTIVE PROTEINS AGAINST ANTI-UBIQUITIN ANTIBODY

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Although ubiquitin is a key and conserved protein present only in eukaryotes, all domains of life contain ubiquitin-like proteins (Ubls) sharing similar folding and role with ubiquitin. Previously, we found several proteins that react with anti-ubiquitin antibody in halophilic archaea. The present work analyzed whether the halophilic bacteria *Salinibacter ruber*, as belonging to a different life domain, also posseses Ubls. For this, protein extracts from *S. ruber* cells were separated by 2D SDS-PAGE and subjected to Western blot using a polyclonal anti-ubiquitin antibody. Samples from the different growth phases displayed immune-reactive spots ranging from 51 to 112 kDa and pI 5-6. The spots were excised from the gel and subjected to protein sequence determination by MS of the tryptic fragments. The analysis of the obtained sequences showed that most of them are membrane proteins and some display a TonB-dependent receptor domain. A further analysis of some of the MS peaks showed that they matched with the small hypothetical protein SRU_2854. Although the statistical limits were not enough significant, SRU_2854 has a di-glycine motif at its Ct, as ubiquitin and Ubls. Also sequence alignment and structure prediction studies showed similarities to some Ubls. This protein could be interacting with a major protein that could be the recognized by the antibody.
Supported by CONICET and UNMdP.

MI-P03

CHARACTERIZATION OF A PROTEIN CONTAINING AN UBIQUITIN-LIKE DOMAIN FROM *Natrialba magadii*

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Ubiquitin-like proteins and Ubiquitin-like domain containing proteins have been found in both eukaryotes and prokaryotes. They belong to the â-grasp fold characterized by 4-5 antiparallel â-sheets with a middle á-helix region.
Searching for the presence of Ubls in halophilic archaea, we previously found by both in silico and FTIR analysis that P400, a polypeptide from *Natrialba magadii*, displays structural homology with Ubiquitin-like proteins. The aim of this work was to characterize the Nmag_2608 protein that includes P400. This conserved hypothetical protein of 262 amino acids is part of a group of orthologs from different halophilic archaea with no significant sequence likeness to known proteins. Secondary folding prediction showed that they are mainly composed by â-sheets. Also, bioinformatics analysis predicted that Nmag_2608 and most of its orthologs contain a signal peptide with a conserved LIPOBOX motif found in bacterial lipoproteins. Lastly, expression of Nmag_2608 in *N. magadii* cells allowed to detect, only in the stationary phase of growth, a 1.4 kbps transcript. All together, these results suggest that P400 is an Ubiquitin-like domain inside a membrane anchored protein expressed in *N. magadii* at late stages of growth.
Supported by UNMdP and CONICET.

MI-P04

MOLECULAR CHARACTERIZATION OF Bgl-A AND *Blg* GENES FROM S*hewanella* sp. G5

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Two genes that encode different â-glucosidases (âGs, EC.3.2.1.21) were partiality sequenced (EF141823 and DQ136044), from psychrotolerant *Shewanella* sp. G5 isolated from the intestinal content of *Munida subrrugosa* in the Beagle Channel, Argentina. âGs cold-active may be biotechnology interest for food processing at low temperatures and in a broad pH range. The aim of this study was obtained the complete sequence of the bgl-A and bgl genes in *Shewanella* sp. G5.
From NCBI database, genomes of four members of *Shewanella baltica* OS185 type (CP000753), OS223 type (CP001252), OS195 type (CP000891) and OS155 type (CP00563) were used to primers design. Within each genome, lower and upper regions to the bgl-A and bgl genes were used to design primers. Primers were design using DNA-MAN software and evaluated in silico software http://insilico.ehu.es/. Restriction tests through DNA-MAN software were also performed for both PCR products removing the regions that limit to both two genes. Genomic DNA extraction from *Shewanella* sp. G5 and PCR optimizations assays were performed. Amplification fragments of 2,934 pb and 4,100 pb of bgl-A and bgl respectively were obtained; and both amplicons were sequenced. In this study, we fine that bgl-A gene present a high similitude with *Shewanella baltica* OS223, but bgl gene have high similitude with *Shewanella baltica* Os185

MI-P05
UTILIZATION OF GLYCEROL BY *Rhodococcus* SPECIES
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Biodiesel production from animal fats and vegetable oils generates glycerol as the main by-product. Glycerol can be used for oil and biofuel production by oleaginous *Rhodococcus* bacteria. We investigated the ability of *Rhodococcus* strains to utilize glycerol. Strains belonging to *R.jostii* (6) *R.opacus* (2), *R.ruber* (1), *R.fascians* (5) and *R.erythropolis* (2) were used in this study. Most strains were able to grow on glycerol, with the exception of 4 *R.jostii* strains. The glpk gene (glycerol kinase) was amplified by PCR in these 4 strains, and their sequences were analyzed. We observed a deletion of the nucleotide 721 in glpk of strain 346, which may produce a frame shift in the sequence. Among the glycerol-grown strains, those belonging to *R.opacus, R.jostii* and *R.ruber* showed a lag phase of 7 days before growing; whereas *R.fascians* and R.erythropolis showed good growth. A genome-wide bioinformatic analysis of key genes of glycerol metabolism in *R.erythropolis* PR4 and SK121, R.opacus B4 and R.jostii RHA1 showed the occurrence of gplF genes encoding a Glycerol Uptake Facilitator (GlpF) in *R.erythropolis*, and their absence in *R.opacus* and *R.jostii* genomes, which is in agreement with the growth profiles on glycerol observed. The results suggested differences in the genetic potential for glycerol metabolism among *Rhodococcus* species, and the eventual occurrence of mutations in key genes

MI-P06
STRUCTURAL STUDY OF LIPOPOLYSACHARIDES FROM *Shigella flexneri*
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Shigella flexneri, a Gram-negative bacterium which causes dysentery, is classified into several serotypes on the basis of antigenic determinants on the lipopolysaccharide (LPS). Antisera produced by immunization of rabbits are used for the serotyping of different strains; however, these reagents may not able to cover all possible epitopes of the O antigen of *S. flexneri*. Therefore, the knowledge of the structure of the LPSs obtained from each serotype is essential. In this work we have performed a structural characterization of the LPSs obtained from *S. flexneri* 67X by mass spectrometry in order to compare its structure with strains with atypical serology.
Dried cells were extracted by the hot phenol-water method rendering a water phase LPS, named S-LPS and a phenol soluble R-LPS. Each of the LPSs was subjected to weak acid hydrolysis to split Lipids A from the oligosaccharide. Mass spectrometry analysis of the purified Lipids A revealed different degree of acylation and phosphorylation. The oligosaccharides obtained were hydrolyzed and the sugar components analyzed by HPAEC-PAD. Differences in the sugar relationship between both samples indicated the absence of the O-antigen in the R-LPS. When both LPSs were analyzed by mass spectrometry, ions showing a common Lipid A + core region were detected. In addition, signals corresponding to the O-antigen were characterized in the S-LPS.

MI-P07
PARTIAL PURIFICATION AND CHARACTERIZATION OF *Lactococcus lactis* TW34 BACTERIOCIN
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Bacteriocins are antimicrobial peptides and proteins produced by some bacteria to kill or inhibit the growth of other bacteria. Recently, bacteriocins have been considered for potential use in human health, veterinary medicine, and food preservation. The aim of this study was to purify and to characterize *L. lactis* TW34 bacteriocin, which shows a broad inhibition spectrum of Gram-positive fish pathogens. The cell-free supernatant was ethanol fractionated and subjected to ion-exchange chromatography (SP-Sepharose) by FPLC at pH 4.5. The collected fractions were evaluated for antimicrobial activity (agar well diffusion assay) against *Lactococcus garvieae*. Active fractions were pooled and analyzed by Tricine-SDS-PAGE, also detecting bacteriocin activity in the gel. Already described bacteriocin genes (nisin, lacticin 481, and lactococcin A) were assessed by PCR amplification using specific primers. TW34 bacteriocin was partially purified by cationic exchange chromatography. Its apparent molecular weight was about 4.2 kDa; a value close to that of nisin (3.5 kDa). Only nisin gene (898 bp partial sequence) was detected in *L. lactis* TW34. In addition, TW34 was resistant to nisin produced by the reference strain *L. lactis* subsp. lactis ATCC 11454. These results suggested that *L. lactis* TW34 produces a nisin-like bacteriocin.

MI-P08
GENE EXPRESSION AND SPECIFIC ACTIVITY OF THE BILE SALT HYDROLASE OF *Lactobacillus reuteri* CRL1098
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Bile acids (BA) act as biological detergent to emulsy and solubilize dietary lipids, facilitating their intestinal absorption. *Lactobacillus (L.) reuteri* CRL1098, a probiotic strain that showed to be effective in preventing hypercholesterolemia in mice, encodes a bile salt hydrolase (BSH) able to deconjugate the amino acid moiety from the conjugated BA. BSH belongs to the category of enzymes that act on carbon-nitrogen bonds other than peptide bonds in linear amides; other members of this enzyme category include the penicillin and cephalosporin acylases. In this work, the regulation of the CRL 1098 *hsb* gene and the specific activity of its HSB enzyme, against BA and alternative substrates, were studied. Real Time-PCR reactions showed no differences in the levels of *bsh*-mRNA transcripts, both in cells grew in the absence or in the presence of 1 mM conjugated BA (TDCA or GDCA), as well as in cells grew in MRS broth at different controlled pH values (4.5, 5.2 and 6.5). Cell free extracts of a *Lactococcus lactis* NZ9000 recombinant strain carrying the CRL1098 *bsh* gene showed hidrolizing activity towards all BA assayed, but not against penicillin V, ampicillin, ketocaproyl-homoserine lactone, and oxoctanoyl-homoserine lactone. These results suggest that BSH of *Lactobacillus reuteri* CRL 1098 is a constitutive enzyme and confirm its specificity against BA substrates.

MI-P09

BIOREMEDIATION OF METHOXYCHLOR BY NATIVE ACTINOMYCETES. EFFECT OF TEMPERATURE, pH AND CONCENTRATION

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Methoxychlor (MTX) is an organochlorine pesticide that was used in industrial and agricultural activities. MTX is a persistent, bioaccumulative, toxic chemical for which it was prohibited in many countries; nevertheless it is even found as environmental pollutant. The aims of this work were select an actinomycete strain available to biodegrade MTX and optimize the environmental conditions for this process.

Streptomyces sp. M7, *S. coelicolor* A3 and four actinomycetes isolates from a contaminated soil were cultivated in minimal medium with MTX (1.66 mg/L) as sole carbon source. Microbial growth, MTX remained and chlorine ions released were determined. The actinomycete selected was cultivated at different temperature, pH and MTX concentration conditions.

The isolates were characterized by 16S rDNA amplifications and sequenced and they were identified as members of *Streptomyces genus*. *Streptomyces* sp. A14 showed the best growing (0.39 mg/mL), MTX removal (100%) and chlorine released (ÄA540=0.14). Temperature of 30ºC and pH 7 were the best conditions for MTX biodegradation by Streptomyces sp. A14. Finally, these strain removed 97.6% of MTX at the higher pesticide concentration (16.60 mg/L). Our results suggest that *Streptomyces* sp. A14 has a big potential for bioremediation of MTX contaminated soils and these is the first evidence of aerobic MTX degradation.

MI-P10

PHYSIOLOGICAL ROLE OF THE C4 BIOSYNTHETIC PATHWAY IN STARTER AND NONSTARTER LACTIC ACID BACTERIA

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Lactic Acid Bacteria (LAB) are recognized as safe microorganisms that are capable to improve the quality of diary products. When the LAB *Lactococcus lactis* is employed as a starter for the production of fermented foods, high quantities of important aroma compounds such as diacetyl are generated by means of the C4 metabolic pathway. This route depends on the expression at low pH of the *als, aldB, aldC* and *butBA* genes encoding the enzymes involved in the conversion of pyruvate into aroma compounds. In the non-starter LAB *Enterococcus faecalis*, a bioinformatic search determined that only alsS and alsD (orthologous to *L. lactis aldB)* genes are present in its genome. Our studies showed that these genes compose a single operon which is maximally transcribed during the mid-exponential phase and further enhanced upon the addition of pyruvate to the medium. The disruption of the als gene in both microorganisms, produced C4 deficient strains sensitive to low pH and high pyruvate concentrations. The dramatic reduction in the growth parameters of mutant strains is accompanied by the inability to alkalinize external medium and it is reverted when medium pH is kept constant at higher values. These results suggest that the decarboxylation reactions related to the C4 pathway give to LAB a competitive advantage in a condition of intracellular pyruvate accumulation during growth at low pH.

MI-P11

RELATIONSHIP BETWEEN PURINE METABOLISM AND ANTIBIOTIC PRODUCTION *Streptomyces coelicolor*

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Purine metabolism in soil bacteria such as *Streptomyces* plays an important role in recycling nitrogen compounds from death organisms. However, only a parcial pathway for catabolism of GTP and ATP to allantoin has been reported. In this work we study a set of genes (sco6243, sco6247, sco6248) that is involved in the final conversion of allantoin to glyoxylate, ammonium and malate. In turn, we have also identified a negative transcriptional regulator for this set of genes, AllRStr, which is similar to the regulator protein of the allantoin pathway from *E. coli*, AllR. sco6243, sco6247 and sco6248 codify a putative malate syntase, allantoinase and allantoicase protein respectively. Growth analyses of mutant strains for each of these genes in minimal media containing purines or allantoin as the sole carbon and nitrogen source have demostrated that these genes are essencial for cell survival under these conditions.

Inactivation of AllRStr increases the expression of allantoin pathway genes and strongly impairs antibiotic production. We are currently disrupting these genes in an allRStr mutant background to determine if impaired antibiotic production is due to the overexpression of this metabolic pathway. This results may well suggest that availability of precursors for synthesis of (p)ppGpp or acetyl-CoA are a check point for the production of secondary metabolites in Streptomyces.

MI-P12

ROLE OF MALIC ENZYME IN THE METABOLISM OF *Streptomyces coelicolor*

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Antibiotics are products of secondary metabolism and are very important for human health. Soil bacteria *Streptomyces* produce 80% of total known antibiotics. A lot of effort has been made to understand biosynthesis and regulation of secondary metabolism in this genus. However, little attention has been paid to the central metabolic network which supplies building blocks for antibiotic biosynthesis.

Anaplerotic enzymes are very important in cell metabolism because they interconnect the main metabolic pathways. In previous work we characterized a NAD and a NADP dependent malic enzyme from *S. coelicolor,* these enzymes catalyze the oxidative decarboxylation of L-malate into pyruvate with reduction of NAD or NADP. Individual mutations in genes encoding for these enzymes resulted in no significant phenotype, however the double mutant strain showed a pronounced decrease in antibiotic and triacylglycerol production under different carbon sources tested. Metabolites analysis evidenced accumulation of TCA-precursors in the doble mutant but no differences in acetyl-CoA levels. These results indicate that these anaplerotic enzymes play an important role connecting energy-producing and biosynthetic pathways at transition phase between primary and secondary metabolism in *Streptomyces.* How the bacterium senses changes in metabolism and modifies the regulation of antibiotic production is under study.

MI-P13
TRANSCRIPTIONAL REGULATION OF FATTY ACID BIOSYNTHESIS IN *Streptomyces coelicolor*
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Fatty acids (FAs) and their derivatives are essential structural components of bacterial membranes and an important source of metabolic energy. Their biosynthesis represents one of the most energetically expensive processes to the cell. Thus, bacteria have evolved sophisticated mechanisms to finely control expression of genes responsible for the formation and degradation of FAs. In *Streptomyces coelicolor* genes encoding some of the core proteins of the FA synthase complex constitute an operon (*fab* operon, fabDHPF). *fasR*, an open reading frame located upstream *fabD*, encodes a positive transcriptional regulator of fab operon. Insertional mutation of *fasR* leads to a significantly decrease of bacterial growth rate and to a delay of morphological differentiation. Real time q-PCR revealed that the expression of the four *fab* genes was lower in the *fasR* mutant compared to the wild type strain. Transcriptional fusions to the *fab* operon promoter (Pfab) revealed that its expression was induced after inhibition of FA biosynthesis by cerulenin, and that this effect was FasR dependent. Therefore, it is tempting to speculate that FasR activity is controlled by a ligand that is an integral component of endogenous de novo FA biosynthesis. To identify this signal we are currently studying the effects of several putative ligands on FasR-Pfab interaction by EMSA and fluorescence anisotropy assays.

MI-P14
BACTERIOCIN PRODUCTION BY *Streptococcus uberis* INVOLVED IN BOVINE MASTITIS
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Streptococcus uberis is the main environmental causative agent of bovine mastitis in the world and a prolific producer of proteinaceous antibacterial substances (bacteriocins) targeting other strains. It is known that *S. uberis* produces ubericin A, a 5.3-kDa class IIa bacteriocin, which is encoding by the genetic locus designated as uba. The aim of this work was to examine the *ubaA* gene by PCR in 57 *S. uberis* strains isolated from bovine mastitis in Argentina. Additionally, the positive strains were tested for production of antimicrobial substances (AMS) against the most prevalent mastitis pathogen agents in the central dairy region of Argentina *(Staph. aureus, S. agalactiae,* coagulase negative staphylococci, *S. dysgalactiae, S. uberis* and *coliforms)*. Thirty seven strains were positive for *ubaA* gene. It was found AMS production against *Staph. aureus*, coagulase negative staphylococci and *E. coli*. The bacteriocins elaborated by *S. uberis* can also be exploited as promising new candidates to be either infused into the udder in the same way as antibiotic or used in solutions such as teat dips, as a preventive treatment against bovine mastitis.

MI-P15
CIPROFLOXACIN INCREASES MUTATION FREQUENCY OF HYPERMUTATOR AND WILD TYPE STRAINS OF *P. aeruginosa*
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Resistance of *P. aeruginosa* to the antibiotic ciprofloxacin (CIP) is mainly achieved through mutations in GyrA and ParC subunits of DNA topoisomerases, and the expression of the multidrug efflux pump MexCD-OprJ by mutations in its transcriptional repressor NfxB. We previously showed that for wild type and hypermutator strains of *P. aeruginosa* PAO1 (deficient in the Mismatch or 8-oxoguanine Repair Systems), *nfxB* mutations are dominant in cells selected at low drug concentration (SAIB 2009). In this work we show that the apparent mutation frequency of *nfxB* in wild type and hypermutator strains increases to very high values at subinhibitory concentration of CIP, and report the mutational spectra obtained. In order to identify *nfxB* mutants by luminescence and analyze with better precision this phenomenon, we worked with PAO1 strains carrying a chromosomal copy of MexCD-OprJ promoter fused to the *lux* operon. This allowed us to estimate that in the presence of subinhibitory concentration of CIP, *nfxB* was mutated in approximately 1/300 colonies in the hypermutator strains and 1/1200 colonies in the wild type strain. The absence of mutants in the inoculum and the presence of sectorized luminescent colonies containing mutated and non mutated cells, led us to conclude that the mutation process at very low CIP concentration occurs after the exposure of the bacterial cells to this drug.

MI-P16
GENOME-WIDE ANALYSIS OF HOMOPOLYMERIC TRACTS MUTATION RATE IN *Escherichia coli*
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Generation of genetic diversity through homopolymeric tracts mutation appears to represent a universal system for regulation of gene expression in prokaryotes. We examined the ability of genome context to affect the stability of homopolymeric tracts in *Escherichia coli*. The stability of a seven-repeat poly-A tract was measured using a reporter gene in which an extra A was introduced changing its reading frame. Isogenic wild-type and mismatch repair deficient (*mutS*) *E. coli* strains with the reporter gene inserted at 23 different locations in the genome were constructed. Rates of frameshift mutations that restore the reporter gene sequence were determined by fluctuation analysis. A difference of up to 13-fold in mutation rate was found among wild-type strains. If this difference was reflecting a context-dependence of mismatch correction, then it should be lost in *mutS* cells. However, although mutation rates were elevated in all *mutS* derivatives, the difference in rate among these strains was still 8.4-fold. This result shows a significant genomic context-dependence of the poly-A stability which is independent of the mismatch correction. We also found that neither the orientation, nor the distance, of the reporter gene relative to the replication origin, nor the density of GATC sites around the poly-A tract have a significant effect on the rate of homopolymeric tract mutation.

MI-P17

FUNCTIONAL ANALYSIS OF *Pseudomonas aeruginosa* MutlD(Q/M)HA(X)2E(X)4E CONSERVED MOTIF

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The DNA mismatch repair system (MRS) corrects mismatched base pairs caused mainly by DNA replication errors, increasing the accuracy of DNA replication 20–400-fold. Currently, two types of MRS have been elucidated. In *Escherichia coli* and closely related bacteria MutH is the MRS endonuclease, while in the rest of bacteria and in eukaryotes, MutL homologues carry out this function. A conserved $D(Q/M)HA(X)_2E(X)_4E$ motif in the C-terminal region of MutL has been implicated in the metal-binding/endonuclease activity of these proteins. However, the contribution of individual amino acids to these activities remains unclear. In this work we characterized the sequence–function relationship of the DMHAAHERITYE motif of *Pseudomonas aeruginosa* MutL. We generated several point mutation derivatives and analyzed their *in vivo* functioning and *in vitro* endonuclease activity. Complementation analysis let us identify a new amino acid essential for the *in vivo* functioning of MutL protein. Unexpectedly, the *in vitro* analysis showed that mutation of some essential amino acids for the *in vivo* function does not affect the endonuclease activity of the full length protein. Our results suggest that some conserved amino acids within the $D(Q/M)HA(X)_2E(X)_4E$ motif could also be important for the interaction with the N-terminal region of MutL, with other proteins of the MRS, or be involved in regulatory functions.

MI-P18

FUNCTIONAL ANALYSIS OF *Pseudomonas aeruginosa* MutS-β CLAMP INTERACTION

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We previously demonstrated that overexpression of the mismatch repair protein MutS in *P. aeruginosa* induces cell death and filamentation. These effects were not detected when overexpressed a C-terminal deletion MutS mutant. In addition, we found that the C-terminal domain copurified with the processivity factor â clamp (âC). The aim of this study is to determine if MutS interacts with âC and analyze the functional role of this interaction. First, we mutated the putative âC binding motif QSDLF located at the C-terminal domain of MutS generating the $MutS^{â}$ mutant. At difference of MutS, this mutant was not able to bind to âC *in vitro*. In concordance, overexpression of $MutS^{â}$ did not produce lethality and morphological changes. Since it has been proposed that âC could be involved in the repair of DNA replication errors, we analyzed the proficiency for mismatch repair activity *in vivo* of a mutant strain harboring the chromosomal *mutS*^{â} mutant gene. This mutant strain presented a mutation frequency similar to the parental strain in the presence or absence of the mutagen 2-aminopurine. On the other hand, the analysis of growth kinetic indicated that the *mutS*^{â} strain showed a more extended lag period and a lower exponential doubling time compared to the parental strain. Thus, ours findings suggest that the MutS-âC interaction may be implicated in the regulation of cell cycle in *P. Aeruginosa*.

MI-P19

ISAbs825 AS A MODULATOR OF BlaOXA-58 EXPRESSION AND CARBAPENEM RESISTANCE IN *Acinetobacter baumannii*

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Insertion sequences (IS) are mobile genetic elements responsible of gene inactivation, genome rearrangements, and enhancement of gene expression. We previously reported the insertional inactivation of the carO gene coding for an outer membrane protein in carbapenem-resistant A. baumannii clinical strains by a novel insertion sequence, ISAba825. We evaluated here ISAba825 functionality and its impact on the plasticity of the *A. baumanni*i genome. ISAba825 was tagged with a kanamycin resistance cassette and cloned in a suicide plasmid, and its transposition was evaluated by transforming this plasmid into *A. baumannii* ATCC 17978. ISAba825::Kn readily transposed in this host into endogenous plasmids, and sequencing analysis indicated its preference for short AT-rich targets generating 6-9 bp direct repeats. In the clinical *A. baumannii* strain Ab880, ISAba825 was found truncating the *tnpA* gene of an ISAba3-like element located upstream of a plasmid-borne blaOXA-58 gene. 5´-RACE analysis indicated that ISAba825 insertion generated an alternative hybrid promoter enhancing blaOXA-58 expression, and determination of minimal inhibitory concentrations for imipenem and meropenem indicated that this insertion was solely responsible for the generation of high levels of carbapenem resistance. Overall, a role for ISAba825 in the modulation of carbapenem resistance in *A. baumannii* is proposed.

MI-P20

INTERACTION OF RstBA AND RcscDB REGULATORY SYSTEMS FOR CONTROL OF THE *Salmonella typhimurium*

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The two-component regulatory systems are one of the most important mechanisms by which bacteria have managed to survive environmental changes, by modulating the gene expression. The aim of this study was to determine if there is an interaction between the RstBA and RcsCDB regulatory system to control the expression of different genes. This study was based on the observation that RstA (over-production only) negatively regulates *csgD*, whose product modulates those genes required for cell-cell interactions and their environment, like *bapA*. On the other hand, the *csgD* gene is positively regulated by RpoS, which in turn is repressed by RstA and induced by RcsB. This led us to think that RstBA and RcsCDB systems may be interacting in order to control genes, especially those required by *Salmonella* to establish systemic infection. In this work we investigated the effect of overexpression of RcsB on gene fusions controlled by RstA, such as *asr*, *narZ* and *spvA*. Our results indicate that RcsB and RstA competitively inhibit the *narZ* and *spvA* genes transcription. Furthermore, RcsB represses *asr* expression by inhibiting the *rstA* transcription.

MI-P21

EXPRESSION AND PURIFICATION OF *Salmonella typhimurium* RcsCDB SYSTEM PROTEINS

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The Rcs phosphorelay system involves the sensor protein RcsC, the cognate response regulator RcsB, and the histidin-containing phosphotransfer protein RcsD, which serve as an intermediary in the phosphoryl transfer from RcsC to RcsB. Previously, we reported that in the double mutant *rcsD rcsC*, the overproduction of RcsB regulator can not promote the Rcs system activation. These results suggested that only RcsB-P, the RcsB active form, is able to induce the RcsB-dependent genes modulation. We are interested to determine if RcsC or RcsD can independently transfer the phosphate group to RcsB, or if in this process is necessary that both proteins act together. In order to obtain soluble proteins, the full length *rcsB* gene and the sequences encoding the cytoplasmic domain of RcsC and RcsD, labeled with a His6 tag, were cloned into pT7-7 vector. The recombinant plasmids obtained were sequenced and the RcsB, RcsCcyt and RcsDcyt were expressed in *E. coli* BL21 DE3 strain. In the present work we performed the proteins purification step using Ni^{2+} affinity chromatography. The quality and quantity of the purified proteins were monitored by SDS-PAGE and BSA assay. The soluble proteins will be used an in vitro phosphorylation assays to determine the phosphorelay mechanism of the Rcs system.

MI-P22

SCREENING OF DesK MUTANTS IN *Bacillus subtilis* EXPLOITING A NOVEL REPORTER GENE ASSAY

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The histidine kinase DesK from *B. subtilis* is a membrane thermosensor that sense a decrease in membrane fluidity. Together with the response regulator DesR, constitutes a two-component system that regulates the transcription of the *des* gene, coding for the acyl lipid desaturase delta5-Des. To uncover the mechanism of membrane fluidity sensing we performed a random mutagenesis approach of the transmembrane segments of DesK. The phenotypic selection of mutants was carried out using the screening system that we have previously developed in *B. subtilis*, based on sporulation as reporter phenotype. So, as sporulation is impaired when RapA is expressed without its inhibitor, PhrA, we used a *rapA-phrA* null mutant expressing ectopically P*des-rapA* to build a derivative *des* and *desK* null mutant that expresses isotopically *desR* under P*xyl* promoter. The resulting strain, ARDCM2, showed Spo+ phenotype in sporulation medium after a temperature downshift to 23ºC. A vector carrying P*xyl-desK*C-end was built, which is suitable to clone *desK*N-end alleles upstream to *desK*C-end domain, regenerating full-length *desK*. ARDCM2 transformed with the vector expressing DesKwt showed reversion to Spo- phenotype. ARDCM2 transformants expressing mutated versions of DesK that showed Spo+ phenotype at 23ºC or Spo- phenotype at 37ºC were isolated.

MI-P23

TRANSCRIPTIONAL REGULATION OF *gbdR* THAT ENCODES A REGULATOR OF CHOLINE METABOLISM IN *P. aeruginosa*

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Choline is present in high concentrations in different tissues (lung, corneal and urinary epithelium) where *P. aeruginosa* causes infections. It is found as phosphatidylcholine, phosphorylcholine, acetylcholine or as choline-free and, in this way, it can be used as a source of carbon (C) and nitrogen (N) and favors the colonization of this pathogen. GbdR, a regulator that belongs to AraC/XylS family, is essential for the metabolism of choline and also NtrC and CbrB are involved. The aim of this work was to know the transcriptional regulation of *gbdR*. For that, we investigated if the metabolites related or not to choline affected the expression of *gbdR* and if its expression depended on other global regulators. To carry out this study, transcriptional fusions to *lacZ* were performed and inserted into the chromosome of *P. aeruginosa* WT, *ΔntrC, ΔcbrB* and *ΔrpoN* strains. The nitrogen depletion in the culture medium increased the reporter activity 3-fold times. The increase was higher (from 4 to 12 times) if histidine or choline were added as supplementary nitrogen source, respectively. Choline (no betaine or DMG) was the real inductor of *gbdR* expression. This expression also depended on the presence of RpoN and NtrC. The contributions made in this study will allow progress in molecular knowledge of the specific and global transcriptional regulation of choline metabolism in *P. Aeruginosa*.

MI-P24

SUNLIGHT RESPONSE OF *S. enterica* SEROVAR *Typhimurium* ATCC14028 AND LT2 STRAINS: ROLE OF RpoS

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Virulent ATCC14028 and non-virulent LT2 strains of *Salmonella enterica* serovar Typhimurium are closely related but, as it was reported, they exhibit different environmental stress tolerance. Whilst sensitivity to DNA damage and acid stress are increased in LT2, the effects of starvation and oxidative stress are similar in both strains. We recently described that kinetics of sunlight induced cell death was similar for ATCC14028 and LT2, except for a slightly increased sensitivity found in LT2, which could be ascribed to a low level of RpoS protein. To further analyze the role of *rpoS* in sunlight response, it was inactivated in both strains by introducing the mutation *rpoS*::Ap^R. Survival curves of stationary phase cells of the parental strains and their *rpoS* defective derivatives were obtained during natural sunlight exposures at 25-26°C with an irradiance of 860-868 Wm^2. In both genetic contexts, *rpoS* inactivation increased sensitivity, but at start of the irradiation the loss of viability was delayed in cells derived from ATCC14028. Since differences in the cell death kinetics of the mutants could not be explained by differences in their RpoS levels, the results suggest that the sunlight response of *S. Typhimurium* involves a mechanism beyond the RpoS control, and this mechanism is modified in LT2 strain with respect to ATCC14028.

MI-P25

ClpX2 REGULATES NifB AND NifEN LEVELS DURING NITROGEN FIXATION IN *Azotobacter vinelandii*

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Biological nitrogen fixation is mainly catalyzed by the molybdenum nitrogenase that carries at its active site the iron and molybdenum cofactor (FeMo-co). Among the genes that are required for the biosynthesis of FeMo-co nifB is remarkable because it is also essential for the biosyntheses of the cofactors all alternative nitrogenases. In this work, we show evidence suggesting the operation of a nitrogen source-dependent mechanism for the degradation of NifB and NifEN in A. vinelandii and that a duplicated copy of the ATPase component of the ClpXP protease (ClpX2) is involved in this regulatory pathway. clpX2 was induced under nitrogen fixing conditions and its inactivation slow-down NifB turnover and resulted in the accumulation of NifB and NifEN. clpX2 mutants displayed a defect in diazotrophic growth, especially when iron was limiting. Inactivation of other genes related to NifB activity (nifENX) also affected NifB degradation rates suggesting that NifB susceptibility to degradation might change during its catalytic cycle.
ANPCyT.

MI-P26

THE Pta-AckA PATHWAY IS REQUIRED FOR ACETATE ASSIMILATION IN *Bacillus subtilis*

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Bacteria have developed several mechanisms to sense and to adapt to constant environmental changes. When growing on abundance of nutrients such as glucose, many bacteria excrete partially oxidized carbon compounds, including acetate. Once these nutrients have been depleted, these cells undergo the acetate switch, a metabolic change in which acetate is assimilated, converted to acetyl-CoA and oxidized in the Krebs cycle.
In *Bacillus subtilis*, acetate is excreted through a two-step pathway involving the enzymes phosphotransacetylase (Pta) and acetate kinase (AckA); and its assimilation is believed to be catalyzed mainly through acetyl-CoA sinthetase (AcsA) activity. Additionally, AcsA is negatively regulated by acetylation, in a post-translational mechanism involving the acetylase AcuA and deacetylases AcuC and SrtN.
To further study these pathways, we obtained *B. subtilis* strains containing deletions in pta and acuA genes which conditionally express *acsA.* We evaluated their ability to incorporate acetate into lipidic and proteic fractions by labeling cultures with [1-14C]-acetate.
Our results indicate that pta strains do not efficiently incorporate acetate, even if *acsA* expression is induced, suggesting that the Pta-AckA pathway has a critical role in acetate assimilation in *B. Subtilis.*

MI-P27

COPPER STRESS TARGETS THE Rcs SYSTEM TO INDUCE *Salmonella enterica* MULTIAGGREGATIVE BEHAVIOR

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Copper is an essential cofactor required for a variety of cellular processes such as hydrolytic pathways, iron transport, respiration and defense against oxidative stress. However, it is also highly toxic and in consequence its intracellular concentration must be tightly controlled. Gram-negative bacteria rely on the cue regulon to regulate the intracellular copper concentration. In *Salmonella* the regulon is composed by genes coding for a MerR-like transcriptional regulator, CueR, an inner membrane copper-exporter, CopA, and two periplasmic proteins: the multicopper oxidase CuiD, and CueP, a copper-binding protein. We observed that *cuiD* strains form mucoid colonies on sublethal copper-containing solid media. We showed that this multiaggregative behavior is caused by the induction of extracellular colanic acid-polysaccharide in an Rcs phosphorelay-dependent manner and that Cu promoted the induction of the Rcs system in the *cuiD* strain. Expression of CuiD or of CueP abrogated the mucoid phenotype and repressed the Cu-dependent expression of the colanic acid biosynthetic operon, suggesting a role for CueP in alleviating the toxic effect of Cu-overloading by counteracting the mechanism that activates Rcs. We propose that the Rcs-dependent expression of exopolysaccharides is a defense mechanism to cope with Cu toxicity in conditions where the cue regulon is overcome.

MI-P28

A KEY RESIDUE FOR FUNCTION OF CheW, THE COUPLING PROTEIN IN BACTERIAL CHEMOTAXIS

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The coupling protein CheW is essential to transmit the signals sensed by chemoreceptors to the histidine kinase CheA. The precise role of CheW during signaling and its arrangement within the signaling complex have not been elucidated.
The conserved residue R62 in CheW has been proposed to function as a “light switch” by displaying important interactions with both CheA and chemoreceptors.
Cells expressing the point mutant CheW R62H do not mediate chemotaxis in soft agar plates. However, this mutant protein is able to mediate kinase activation *in vitro*. In order to identify its functional defect, we performed several *in vivo* assays aimed to characterize the ability of the mutant protein to modulate kinase activity, adapt to stimuli and mediate proper clustering of receptors at the poles of the cell. We found a slight defect in the clustering ability of the mutant protein, whereas no defects are apparent in the other signaling abilities. We also characterized previously isolated suppressors of R62H in both CheA and chemoreceptors.
In order to define the structural arrangement of CheW within the signaling complex, we introduced cysteine residues in the surface of both CheW and receptors and assessed their ability to form disulfides *in vivo*. We found a specific pair of cysteine replacements that get crosslinked.
Our results support the relevance of residue R62 in chemotaxis signaling.

MI-P29

COILED COIL CHEMORECEPTORS: IMPORTANCE OF SYMMETRIC INTERACTIONS BETWEEN HELICES FOR FUNCTION

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Bacterial transmembrane chemoreceptors (MCPs for methyl-accepting chemotaxis protein) transmit extracellular signals to an associated kinase in order to govern the chemotactic behavior of the cell. The highly conserved cytoplasmic domain of MCPs consists in a long alpha-helical hairpin that forms, in the dimer, a four-helix coiled coil bundle. A comparison between MCP sequences from many microorganisms allowed their classification into families based in the presence of symmetric insertions/deletions of seven aminoacids in their cytoplasmic domains.In order to test the importance of proper interaction between the two antiparallel helices within the monomer, we introduced a heptad deletion in the N-helix, the C-helix or both, in symmetric arrangement, of the serine-sensing MCP of E. coli, Tsr. We then tested the effect of these deletions on function and *in vivo* organization.We observed that the asymmetric constructions lost their function, while the symmetric one retained parcial function and maintained the organization of wild type Tsr. Moreover, point mutations in this latter construction restored full chemotactic abilities. These results suggest the need to preserve symmetric interactions between the antiparallel helices in the monomer, consistent with the observed evolutionary pattern of MCPs. Such interactions might also be relevant in other coiled coil signaling proteins.

MI-P30

INTRACELLULAR PROTEIN DENATURATION CAUSED BY SEVERE HEAT STRESS DOES NOT LEAD TO BACTERIAL DEATH

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Exposure of *E. coli* cultures to temperatures above 50ºC (severe heat stress, SHS) promotes cell death, an effect largely enhanced in cells lacking DnaK chaperones. Main SHS cell targets are not well defined, but intracellular protein denaturation/aggregation was proposed as a major heat inactivation factor. We studied whether SHS-induced protein denaturation compromises cell recovery from stress. Exposure of wild-type (wt) cells to 50ºC for short periods not causing substantial death in the population promoted growth arrest during subsequent incubation at 30ºC. Quali (SDS-PAGE) and quantitative (cell lysis) analyses indicated a rapid 7-10 fold SHS-mediated increase in intracellular protein denaturation, which was maintained during recovery at 30ºC and even after re-initiation of growth. Lack of DnaK caused massive death but only a slight increase in protein denaturation as compared to wt bacteria. Cell visualization using contrast phase microscopy showed neither morphological changes nor formation of protein aggregates due to SHS, a result also supported by buoyant density Percoll centrifugation analysis. The overall results indicate that even though SHS caused cytoplasmatic protein denaturation, it was not accompanied by massive formation of intracellular aggregates. Moreover, DnaK-mediated protein removal/reactivation was not necessary to maintain viability or to resume growth.

MI-P31

THE SbmA LOCUS IS INVOLVED IN THE SWARMING MOTILITY AND BIOFILM FORMATION

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SbmA protein is involved in the transport of MccB17, MccJ25, bleomycin and proline rich peptides into the *E. coli* cytoplasm. Although SbmA homologues were found in a variety of bacteria, the physiological role of this protein is unknown. The SbmA homologous BacA from *Brucella abortus* and *Sinorhizobium meliloti* is essential for the chronic infection of these pathogens. The swarming and biofilm formation are two important bacterial multicellular behaviors whereby the pathogen bacteria enhanced their chances of survival in competitive environments and infect the host. In this work we evaluated the possible importance of *sbmA* gene in biofilm formation and swarming motility. We observed that the sbmA absence produced a significant increase in both behaviors. On the other hand, we note that the mutation in the gene *yaiw,* which integrates an operon together *sbmA* , was not able to affect biofilm and swarming. Curiously, the inductor effect of the *sbmA* mutation was reverted when *tolC* or *trpE* mutation were added. Since in most pathogens the biofilm is often related to virulence, the capacity of sbmA *Salmonella* mutant to infect and replicate inside of macrophages was evaluate. The sbmA mutant showed an increased replication ability comparing with the wild-type after 48 hours. Taken together these results suggest an important physiological role of sbmA in the virulence phenotype.

MI-P32

METAGENOMIC ANALYSIS OF AROMATIC RING-HYDROXYLATING DIOXYGENASES FROM COLD MARINE ENVIRONMENTS

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Pollution, low temperatures and high UV-B radiation levels make intertidal sediments of Ushuaia Bay an interesting target for the construction of a metagenomic library, to study the microbial community from this extreme environment and its biodegradation potential. We performed molecular ecological analyses (catabolic gene library and qPCR) in the chosen sample, an important step for a targeted screening of the metagenomic library. PCR fragments of ring-hydroxylating dioxygenase genes were cloned into plasmids and sequenced. The library showed three different gene groups previously detected at this site (B, C and *nahAc*). The two most abundant genes in the library, C and *nahAc*, were estimated by qPCR to be at 10^5 and 10^4 copies/ì g DNA, respectively. In addition, *phnA1* genes from *Cycloclasticus* spp. were found to be at 10^4 copies/ì g DNA, and bacterial 16S rRNA genes were 4 orders of magnitude more abundant in the sediments. The purification of metagenomic DNA from the sediment was optimized to meet the requirements for the construction of a metagenomic fosmid library, where high yields, purity and an appropriate molecular weight is needed. DNA with an approximately size of 40 Kb was cloned into the fosmid vector Copy Control pCC2FOS (Epicentre). Currently, the screening for clones carrying PAH biodegradation pathway genes is being performed by both functional and molecular analyses.

MI-P33

DETECTION OF ACYLHOMOSERINE LACTONES IN CULTURES OF *Gluconacetobacter diazotrophicus* PAL5

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Gluconacetobacter diazotrophicus is an acid-tolerant nitrogen-fixing Alphaproteobacterium first found in association with sugarcane. It has also been isolated from rice, coffee and tea, among others crops. The recent sequencing of the *G. diazotrophicus* PAL5 genome shows the presence of one *luxI* homolog. These genes encode LuxI-type enzymes responsible for the synthesis of *N*-acylhomoserine lactones (AHLs), the main quorum sensing molecules in gram negative bacteria. The objective of this work was the detection and identification of AHLs produced by *G. diazotrophicus* PAL5. The strain was cultured aerobically, and extracts were prepared with acidified ethyl acetate. Samples were analyzed by thin layer chromatography developed with the biosensor *Agrobacterium tumefaciens* NTL4 (pCF218) (pCF372). Results show that *G. diazotrophicus* PAL5 produce at least two types of AHLs under the assayed conditions. Short-chain AHLs could be detected since early exponential growth phase, and medium-chain AHLs were detected in mid- and late-exponential growth phase. The results suggest that the *luxI* homolog in *G. diazotrophicus* PAL5 is expressed and quorum sensing molecules are produced and secreted. Similar to other bacteria, production of AHLs in *G. diazotrophicus* PAL5 could serves as a signaling mechanism among members of this genus or as inter kingdom signals.

MI-P34

SYNTHESIS OF QUORUM SENSING SIGNALS BY DIAZOTROPHS FROM THE RHIZOSPHERE OF STRAWBERRY PLANTS

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In the rhizosphere, interactions occur with both pathogenic and beneficial microorganisms. The latter group can stimulate plant growth by mean of phytohormone production, direct antagonism of pathogens or induction of systemic resistance in the plant. Quorum sensing (QS) systems from non symbiotic diazotrophic bacteria may play a role in the interactions with strawberry plants and with other organisms. The objective of this work was the characterization of *N*-acylhomoserine lactones (*N*-AHLs) produced by free living nitrogen fixing bacteria associated to roots of *Fragaria ananassa* Duch. cv. Camarosa. Strains were isolated and purified in nitrogen free semisolid media. Extracts were prepared with acidified ethyl acetate and samples were analyzed by TLC. *Chromobacterium violaceum* Vir07, *Agrobacterium tumefaciens* NTL4 (pCF218 pCF372) and *Pseudomonas putida* F117 (pKR-C12) were utilized as biosensor strains for the detection of QS molecules. Non-symbiotic diazotrophic bacteria producing QS molecules could be isolated from roots of strawberry plants. Production of short chain N-AHLs was detected in all strains. Only one strain produced long chain N-AHLs. Although biosynthesis of QS molecules is not widely distributed in diazotrophic strains from strawberry roots, secretion of short chain QS molecules seem to play a key role as signaling molecules.

MI-P35

PROTEOMIC ANALYSIS OF *Amycolaptosis tucumanensis* IN RESPONSE TO CHROMIUMN AND COPPER STRESS

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Heavy metal pollution is one the most serious environmental problems. Some heavy metals play an important biological role as trace elements but are toxic at higher concentrations. *Amycolatopsis tucumanensis* was isolated from contaminated sediments in Argentina. Its ability to remove Cu(II) and Cr(VI) has been reported.

The aim of this work was to analize the differential protein espression of *A. tucumanensis* under Cu(II) or/and Cr(VI) stress by two-dimensional polyacrylamide gel elctrophoresis.

Cells were incubated in minimal medium containing glucose and 10 ppm of Cu(II) or/and Cr(VI) during 72 h at 30ºC and 200 rpm. Cells grown in absence of heavy metals were used as control. The cells were chilled in liquid N2 and broken by physical technique. The supernatants of the cell lysates was used as protein sample. The proteome profiles were different, 18 spots were selected for the identification by mass spectrometry, and these included: dehydrogenase/reductase, alkyldroperoxidase Aph D, probable monoxigenase, RNA polimerase, cicloisomerase, some elongation factors, transcripcional regulator and several proteins with unknown function.

Cu(II) or Cr(VI) resistance mechanisms are not known clearly, for this reason is very important to study the functional analysis of these identified proteins and will allow to explain the tolerance of heavy metals by this bacterium.

MI-P36

DIFFERENTIAL REGULATION OF THE GENES INVOLVED IN RIBOFLAVIN BIOSYNTHESIS IN *Brucella abortus*

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Brucellosis is a worldwide zoonosis caused by closely related intracellular *Brucella spp* that affects livestock and humans. These species conserve an atypical riboflavin pathway, that presents the *ribH1* and *ribH2* genes, which encode enzymes with 6,7-dimethyl-8-ribityllumazine synthase activity, that synthesizes a riboflavin precursor. In *B. abortus*, *ribH2* is encoded in the chromosome II, contains an RFN riboswitch element in its 5' region, is expressed during the intracellular phase and is required for the virulence in mice; while *ribH1* is encoded in chromosome I and is dispensable for intracellular survival. These data suggest differences in the regulatory pathways for the *ribH* genes. In this work, we demonstrate that *ribH2* is specifically repressed by riboflavin and flavin mononucleotide. On the other hand, *ribH1* is transcribed polycistronically together with the riboflavin biosynthesis genes *ribD* and *ribE*, and the putative regulators *nrdR* and *nusB* genes. Overexpression of *nusB* produces a significative increase in the expression of *ribH1*, but not of *ribH2*. These results indicate the existence of two independent regulatory mechanisms for the *ribH* genes, probably acting at different stages of *B. abortus* life cycle.

MI-P37

c-di-GMP REGULATES MOTILITY AND BIOFILM FORMATION IN *Bordetella bronchiseptica*

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Cyclic diguanylate (or bis-(3'-5') cyclic dimeric guanosine monophosphate; c-di-GMP) is a ubiquitous second messenger that regulates diverse cellular functions, including motility, biofilm formation, cell cycle progression, and virulence in bacteria. Components of this regulatory network include GGDEF and EAL domain-containing proteins that determine the cellular concentrations of c-di-GMP by mediating its synthesis and degradation, respectively. In this work, we analyzed the presence of this network in *Bordetella bronchiseptica,* a pathogenic bacterium that causes respiratory infections in a wide variety of host. To this end, *B. bronchiseptica* was transformed separately with plasmids containing genes encoding *Pseudomonas aeruginosa* EAL or GGDEF proteins (Pa3497 and PA1120). The expression of these proteins could modify the levels of c-di-GMP if it is present in *B. bronchiseptica*. Biofilm formation and motility were evaluated in the recombinant bacteria to detect possible changes in c-di-GMP concentrations. As found in other organisms that contain high levels of c-di-GMP, we observed that *B. bronchiseptica* was able to form biofilm and reduce its motility only in the case that bacteria expresses the GGDEF domain-containing protein. These results demonstrate for the first time the presence of c-di-GMP regulatory network in *B. Bronchiseptica.*

MI-P38

CHARACTERIZATION OF THE *Ppx* GENE OF *Brucella abortus*

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Inorganic polyphosphate (polyP) is a linear polymer composed of orthophosphate residues (Pi) linked by high energy phosphoanhydride bonds. In bacteria, polyP is synthesized by the polyphosphate kinase (PPK) and hydrolyzed by exopolyphosphatase (PPX). We have cloned and mutagenized the putative *ppx* gene of the pathogen *Brucella abortus*. In a previous work we demonstrated that the *ppx* mutant is affected in J774 intracellular replication and in BALB/c mice infections. Further experiments were carried out in order to characterize the *ppx* mutant. The *B. abortus ppx* mutant showed an increased sensitivity to Sarkosyl detergent compared to the wild type. This result indicates that the absence of PPX affects the cell envelope stability of *Brucella*. SDS-PAGE and Western Blot analysis using outer membrane proteins (OMPs) monoclonal antibodies revealed differences between the *ppx* mutant and the wild type strain. In HeLa cells and in Bone Marrow-Derived Macrophages infections, the *B. abortus ppx* mutant showed a marked defect in intracellular survival during the initial phase of the curve compared to the wild type strain. These results suggest that the absence of PPX may affect the internalization of *Brucella* and/or the biogenesis of the intracellular replicative vacuole. Together, our results suggest that the virulence reduction of the mutant may be due to an altered bacterial membrane.

MI-P39

VIRULENCE FACTORS INVOLVED IN *Bordetella pertussis* SURVIVAL IN INTRACELLULAR NICHES

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Previous studies of our group have shown that *B. pertussis* (Bp), the etiologic agent of whooping cough, is able to survive and replicate inside human macrophages. The goal of this study was to gain a first insight into the virulence factors involved in Bp survival inside the immune cell. In this approach we investigated the ability of a virulent strain, avirulent mutants, and mutants deficient in specific virulence factors to enter and survive intracellularly in human macrophages in vitro. Confocal studies and Polymyxin B protection assays shown that among Bp virulence factors adenylate cyclase toxin (CyaA), previously described as involved in actin polimerization inhibition, is required to preclude Bp–containig vacuoles to undergo phagolysosome biogenesis which eventually leads to the bacterial survival within the first hours of infection. However, at longer times (48h of infection) neither CyaA nor other virulent factor seem relevant for bacterial suvival and duplication inside the macrophage. Accordingly, by comparing gene expression of infecting bacteria and bacteria that has been inside the macrophage for up to 48h using semi-quantitative RT-PCR we found that *cyaA* was downregulated in bacteria inside the cell suggesting that Bp modulates its expression once inside the macrophage. These results indicate that Bp undergoes an adaptative response to survive inside the cell.

MI-P40

STRONG ASSOCIATION OF ActA TO THE PEPTIDOGLYCAN PROMOTING *Listeria monocytogenes*

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The envelope of Gram-positive bacteria consists of a thick (20-80 nm) peptidoglycan layer decorated with a bulk of proteins and associated polymers. We used high mass accuracy spectrometry in a LTQ Orbitrap system to monitor the cell wall proteome of the Gram-positive pathogen *Listeria monocytogenes* in growth media containing different type of nutrients. A surface protein not previously known to strongly associate to peptidoglycan, the actin-assembly inducing protein ActA, was identified in peptidoglycan purified from bacteria growing in a chemically-defined minimal medium but not in peptidoglycan isolated from bacteria grown in a nutrient-rich medium. Multiple reaction monitoring (MRM) analysis of ActA-derived tryptic peptides revealed that the protein binds strongly to the peptidoglycan while maintaining its anchorage to the membrane. In this conformation, ActA is exposed on the cell surface and promotes efficient bacterial entry into non-phagocytic eukaryotic cells. ActA was also identified in peptidoglycan purified from intracellular bacteria upon infection of cultured epithelial cells, implying a strong ActA-peptidoglycan association when the protein is involved in recruiting actin and other cytoskeleton proteins. Altogether, our data revealed a novel association of ActA to the cell wall affecting important virulence traits such as the invasion of eukaryotic cells.

MI-P41

Serratia marcescens MODULATION OF AUTOPHAGY AND THE ROLE OF ShlA HAEMOLYSIN

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Serratia marcescens is an opportunistic pathogen important for public health. However, little is known about factors and mechanisms that contribute to *Serratia* pathogenesis. In this work, we demonstrated that the majority of the autophagic-like vacuoles in which *Serratia* resides and proliferates are non-acidic and have no degradative properties. No detectable increase of intracellular CFUs was detected when we induced autophagy by starvation; nevertheless, they were drastically diminished when wortmannin was used to block autophagy. In the last condition, microscopical inspection revealed that the scarce intracellular compartments harboring *Serratia* were positive for LC3 and packed with bacteria, exhibiting identical phenotype to the SeCVs. A *Serratia* mutant with impeded activation and secretion of the ShlA haemolysin was unable to induce autophagy from outside the CHO cells. Based on these findings, we propose that a) *S. marcescens* is capable to either delay or hamper the fusion to lysosomal compartments; b) ShlA expression is responsible for the autophagic phenotype induced by *Serratia* in epithelial cells; and c) wortmannin was unable to inhibit the autophagic process triggered by *Serratia*, although it could hinder bacterial internalization, indicating that PI3K activity is required for the *Serratia* internalization process into CHO cells.

MI-P42

CYCLOPHILINS PLAY AN IMPORTANT ROLE IN THE STRESS ADAPTATION AND VIRULENCE OF _Brucella abortus_

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Brucella is a facultative intracellular pathogen that causes brucellosis, a worldwide zoonosis that affects a wide range of mammals and humans. Virulence of this pathogen is associated with the ability to survive and replicate within professional and non professional phagocytes. We performed a comparative intracellular proteomic experiment to identify genes that were differentially expressed during intracellular life of *B. abortus.* The observation of up-regulation of two cyclophilins during *B. abortus* intracellular life prompted us to speculate about their potential significant role during the infection. To provide a framework in order to answer this question, we constructed a double mutant of BAB1_1117 and BAB1_1118 genes by in frame deletion and characterized it. The *B. abortus ppiase* mutant was impaired in the ability to grow at low temperature and showed an increased sensitivity to several detergents, to oxidative stress and low pH conditions. In addition, the mutant displays a reduced virulence in BALB/c mice and defective intracellular replication in HeLa cells. All defects were reverted to the wild type phenotype by ectopic expression of wild type copy of the genes. These findings suggest that these genes products make an important contribution to *Brucella* pathogenesis, probably by allowing *B. abortus* to adapt to the harsh environment encountered within the hosts.

MI-P43

BapA, A _Brucella abortus_ ADHESIN INVOLVED IN THE ORAL ROUTE OF INFECTION

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Brucella abortus is an intracellular pathogen and the causative agent of brucellosis, a worldwide zoonosis important for animal breading and of human health concern. The virulence of *Brucella* is dependent on the presence of specialized virulence factors that allow the bacteria to invade, persist and replicate within host cells. We used a bioinformatic approach to search for potential proteins that might play a role in bacterial virulence. The search identified a genomic region that harbours four open reading frames, one of which *(bapA)* has a domain with homology to the intimin of enterohemorragic *E. coli,* a receptor essential for attachment of this pathogen. Our results indicate that this virulence factor is involved in the adhesion to host cells by probably recognizing and binding a cellular receptor. In an attempt to identify this receptor we produced and purified a recombinant BapA and coupled it to an affinity column. Total HeLa extracts were injected into this column and proteins bound to it eluted with different stringent conditions and subjected to poliacrylamide gel electrophoresis. With this approach we have detected one major differential band that is currently being identified. Additionally, we have infected mice intraperitoneally and orally with wild type and *bapA* strain. Our results suggest that this adhesin might play a role in the oral route of infection.

MI-P44

LOW TEMPERATURE GROWTH REGULATION IN _Brucella abortus_ IS MEDIATED BY THE NOVEL SMALL PROTEIN CgpA

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Brucella abortus is a gram-negative, facultative intracellular pathogen that causes brucellosis, a worldwide zoonosis of veterinary and human concern. The *B. abortus* mutant *cgpA* (Cold Growth Protein A) has a growth defective phenotype at 23°C as a result of a diminished replication rate rather than a lack of cold resistance. We used a merodiploid strain with a 3x-Flag tagged *cgpA* gene copy to study expression at 8°C during several days without finding changes on the expression levels, indicating that cgpA is not a typical cold-shock protein. We also determined that the protein localizes in the outer membrane. Because part of the cold shock response implies remodeling membrane content, we hypothesized that there could be differences in the membrane protein pattern between *B. abortus* wild type and *cgpA.* We analyzed whole-membrane preparations from these two strains grown at 37°C and 23°C by SDS-PAGE and posterior MALDI-TOF to determine protein differences. We found a *cgpA*-dependent cold induction of a branched-chain alpha-keto acid dehydrogenase. This enzyme is possibly required for the synthesis of branched-chain membrane lipids which, in turn, could be modifying the membrane lipid composition to increase its fluidity as part of a putative cold-shock response mechanism. We postulate that *cgpA* is part of a signal transduction pathway related to cold-shock response regulation.

MI-P45

ANALYSIS OF TRANSCRIPTIONAL REGULATION OF AN ADHESIN IN *Brucella suis* AND *Brucella abortus*

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Species belonging to the *Brucella* genus are intracellular pathogens responsible of an endemic disease called brucellosis. Different *Brucella* species vary in their host preference. It has been shown that *Brucella* is able to adhere to epithelial cell lines. However, bacterial factors involved in adhesion and/or invasion remain unexplored. Recently, by heterologous and mutational approaches, we found that several members of the Type-V Secretion System autotransporter families of *Brucella suis* participate in bacterial adhesion to abiotic surfaces and HeLa cells. Our goal is to identify signals and regulatory pathways that control transcription of these adhesins in *B. suis* and *Brucella abortus.* Analyses of the adhesin genes and putative regulatory regions revealed differences in both promoter sequence and protein length between these species. By EMSA assays, we identified several regulators that interact specifically with the promoter region of one of the autotransporters BtaE. We have previously documented that these transcription factors also participate in the regulation of other important virulence factor of *Brucella*. By DNAase I footprinting, we identified their binding sites to the btaE promoter region. Differences in adhesin activity and/or expression between *Brucella* species may contribute to a differential tissue tropism.

MI-P46

SagA*, A NOVEL SECRETION ACTIVATOR FACTOR OF *Brucella abortus

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Brucellosis, a world-wide distributed zoonosis that affects a broad range of mammals and causes important economical loses, is caused by bacteria of the genus *Brucella*. The majority of the known species of this genus are pathogenic for humans and their pathogenicity is associated with the capacity to survive and replicate within host cells. With the purpose of identifying novel virulence genes we compared the genome of *Brucella abortus* with the rest of the sequenced species searching for differences between this and the less pathogenic ones. As a result of this search we identified a region of 15 Kb that is absent in *B. ovis*, the only species non-pathogenic for human. The structural characteristics of this chromosomal region, which we have designated Bab_0983, strongly suggest that it could have been horizontally acquired and, thus, is a strong candidate to harbor putative virulence genes. Analysis of this region indicates that it contains 20 ORFs, the majority of them with unknown function. One of these ORFs, Bab_1002 (designated *sagA*), codes for a protein with homology to peptidoglycan hydrolases and has been implicated in other microorganisms in the secretion of proteins through the outer membrane. Our results suggest that this gene is probably implicated in the secretion of proteins to the surface of the bacteria that mediate attachment and internalization into host cell.

MI-P47

THE Mce PROTEINS OF *Mycobacterium tuberculosis* ARE RELATED IN LIPID METABOLISM

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Mycobacterium tuberculosis is the causative agent of human tuberculosis, which remains a worldwide public health emergency. Extensively researches have been made to elucidate the bacterium-host interaction in which the Mce (mammalian cell entry) proteins are essential. The Mce proteins are codified in four *mce* operons. The *mce1* and *mce4* operons are related in the infection-survival of the bacterium within the macrophages and the cholesterol metabolism, respectively. Nevertheless, the physiological role of all *mce* operons is still unknown. In this study, we investigated the capability of *M. tuberculosis* Ä*mce1*, *2*, *3* and *3R* mutants to grow in the presence of different fatty acids as sole carbon source and we characterized their lipid profile, as an approach to investigate an environmental adaptation. We did not observe difference in the growth of the strains when were grown in palmitic and araquidonic acids. Interestingly, a different lipid profile was observed in the Ä*mce1* mutant when was grown in palmitic acid as sole carbon source. In all of the strains tested, the lipid patterns in the presence of estearic acid were similar. These results suggest that the *mce* operons would not be essential in the metabolism of these fatty acids, and instead they would be involved in the arrangement of inner membrane and cell wall lipids allowing an adaptation of the bacterium to the medium.

MI-P48

THE *mce4* OPERON OF *Mycobacterium smegmatis* IS INVOLVED IN CHOLESTEROL UPTAKE

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The *Mycobacterium smegmatis* genome contains six operons designated *mce* (mammalian cell entry). These operons, which encode membrane and exported proteins, are highly conserved in pathogenic and non-pathogenic mycobacteria. Although the function of the Mce protein family has not yet been established in *M. smegmatis*, the requirement of the *mce4* operon for cholesterol utilization and uptake by *Mycobacterium tuberculosis* has recently been demonstrated. In this study, we report the construction of an *M. smegmatis* knock-out mutant deficient in the expression of all six *mce* operons. The consequences of these mutations were studied and, importantly, we found that the mutant strain showed reduced cholesterol uptake when compared to the wild type strain. Further cholesterol uptake studies using single *mce* mutant strains showed that the mutation of operon *mce4* was reponsible for the cholesterol uptake failure detected in the sextuple *mce* mutant. This finding indicates that, similarly to what happens in *M. tuberculosis*, the Mce4 proteins are involved in the transport of cholesterol by *M. Smegmatis*.

MI-P49

INCREASED IL-17 EXPRESSION IS ASSOCIATED WITH PATHOLOGY IN A BOVINE MODEL OF TUBERCULOSIS

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The identification of bovine tuberculosis (bTB) biomarkers in specific stages of the disease will contribute to a better understanding of the immunopathology associated with tuberculosis and to improve the disease diagnosis and prognosis. The aim of this study was to understand the changing profile of the immune responses during the course of infection and to identify biomarkers associated with pathology. Here we describe the immune response developed in experimentally infected cattle with field *Mycobacterium bovis* strains. Blood samples were taken from each animal at different time points after *M. bovis* intratracheal infection and lymphocyte subset activation and cytokine mRNA expression were determined from peripheral blood mononuclear cells in response to purified protein derivative (PPDB). We found that CD4 and CD8 activation during the early stages of infection, together with IL-17 gene expression, were positively associated with pathology. The results of this study provide evidences of the role of IL-17 in the immunopathology of tuberculosis and support the use of IL-17 as a potential biomarker with predictive value of prognosis in bTB.

MI-P50

MabR, A TRANSCRIPTIONAL REGULATOR INVOLVED IN THE CROSSTALK BETWEEN THE FAS SYSTEMS IN *Mycobacteria*

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Mycolic acids (MA) are major components of the cell envelope of mycobacteria and play an important role in its architecture and interaction with the environment. Synthesis of MA is carried out by two fatty acid synthases working in concert: FASI, capable of *de novo* synthesis of medium-chain fatty acids, and FASII, responsible for their elongation. MabR is a novel transcriptional regulator, whose binding to the FASII promoter region (pfasII) was demonstrated *in vitro* and *in vivo*. Under *mabR* over expression conditions in *M. smegmatis*, down-regulation of *fasII* and *fas* transcription was observed and further correlated with a deficiency in MA and in *de novo* FA biosynthesis. Furthermore, MabR increased concentrations resulted in accumulation of C24 fatty acids probably reflecting an alteration in the crosstalk between FASI and FASII. In order to understand the coordinate regulation of the FAS systems we used a series of biochemical and spectroscopic techniques to analyze the effect of acyl-CoA and acyl-ACP molecules on MabR-DNA binding. In addition, using *lacZ* fusions we identified new *cis* elements in FasII operon that might also be involved in the coordinated transcriptional regulation of this pathway. These results represent an important step toward understanding the complex regulatory network involved in maintaining lipid homeostasis in mycobacteria.

MI-P51

PUTATIVE MALONYL-CoA BIOSYNTHESIS PATHWAYS IN *Mycobacteria*

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In mycobacteria, two acyl-CoA carboxylases, ACCase5 and 6, carboxylate both acetyl- and propionyl-CoA to produce malonyl- and methylmalonyl-CoA, the building blocks for fatty acid, mycolic and mycocerosic acid biosynthesis. *In vivo* experiments demonstrated that the essential ACCase6 enzyme complex is the dedicated acetyl-CoA carboxylase involved in the biosynthesis of malonyl-CoA. To find out a metabolic pathway that could overcome the lack of ACCase6 in a *M. smegmatis accD6* conditional mutant, we supplemented media with malonate. We found that malonate supplenentation overcomes malonyl-CoA deficiency, probably by a putative malonyl-CoA synthetase encoded in the *M. smegmatis* genome. Alternatively, malonate decarboxylation could also increment acetyl-CoA pools which could be carboxylated by ACCase5. However, acetate supplementation did not complement the lack of ACCase6. To further understand the physiological relevance of the putative malonyl-CoA biosynthesis pathways, we constructed an *accD5* conditional mutant, finding that AccD5 is an essential carboxyltransferase component of the ACCase5 enzyme complex. Double knock-out *accD5-accD6* mutants will provide the tools to understand the physiological role of ACCase5. Also, we are generating mutants in the putative malonyl-CoA synthetase, in order to find out the gene(s) responsible of generating malonyl-CoA in the absence of ACCase6.

MI-P52

REGULATION OF FATTY ACID BIOSYNTHESIS IN *Mycobacteria*

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The most relevant lipids in *Mycobacterium* cell envelope are mycolic acids, unusual fatty acids that are essential for *M. tuberculosis* survival and virulence. The biosynthetic pathway of these mycolic acids involve two fatty acids synthases (FAS-I and FAS-II) which should be strictly co-regulated to maintain lipid homeostasis in mycobacteria. We discovered a new transcriptional regulator that controls the expression of *fasII* genes, named MabR. Remarkably, overexpression of MabR in *M. smegmatis* leads to the transcriptional repression of the *fas* gene, suggesting that this transcription factor could also be involved in the regulation of FAS-I. However, we could not demonstrate direct binding of MabR to P*fas* by EMSA assays. The recently detection of specific DNA-binding activity to P*fas* in extracts of *M. smegmatis* prompted us to seek for a possible new regulator of the *fas* gene, which could be working in conjunction with MabR to maintain lipid homeostasis in mycobacteria. Purification and future characterization of this protein should help us to elucidate the complex regulatory network of mycolic acids biosynthetic pathway, an attractive target for the development of new antimycobacterial drugs.

MI-P53

THE *Brucella abortus* HIGH AFFINITY CHOLINE TRANSPORTER IS INVOLVED IN VIRULENCE

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Phosphatidylcholine (PC) is one of the major *Brucella* cell envelope lipids, which is involved in virulence. In a previous work, we demonstrated that in *Brucella*, PC synthesis occurs exclusively through the pcs pathway, implying that exogenous choline uptake is a prerequisite for its synthesis. However, a choline transporter has not been described for this pathogen to date. Bioinformatic and biochemical approaches were carried out in order to characterize choline transporter candidates. Three putative choline ABC transporters were detected by *in silico* screening of the *B. abortus* genome. Single and double deletion mutants were generated in the periplasmic binding protein of each system. By using synthesis of PC as a read-through of choline uptake, we detected that a mutant in BAB1_1593 was unable to form PC when cultured in choline-supplemented media. Radiolabeled choline uptake confirmed that BAB1_1593 is the only high affinity periplasmic choline binding protein encoded by *B. abortus*. Mice and cell culture infection assays showed that the absence of choline transport impairs virulence of *Brucella*. This phenotype paralleled the defect of a mutant unable to form PC, which confirmed the relevance of PC for the intracellular stage and, hence, for virulence of this pathogen. These results open up the possibility to evaluate choline transport as a new therapeutic target for brucellosis.

MI-P54

***Cohnella sp.* AR92, XYLANOLYTIC BACTERIA FROM SUGAR-CANE BAGASSE**

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Over 300 bacteria were isolated from samples of sugar-cane bagasse and the liquor that flows through the bagasse pile during its pre-treatment for paper production. From them, the xylanolitic bacteria *Cohnella* sp. AR92 was able to degrade xylan as a major extracellular enzimatic activity.
The isolate is a Gram-positive spore-forming bacteria, and was taxonomically characterized by 16S rDNA sequence, ITS-PCR fingerprinting and morphological and biochemical analyses. These led us to conclude that AR92 isolate should be further described as a new species.
Protein composition of the crude enzyme preparation was analyzed by SDS-PAGE and zymogram, which showed xylanase activity bands within the range of 440 to 30 kDa.
Enzymatic binding of the crude enzyme preparation of *Cohnella* sp. AR92 to insoluble birchwood and sugar-cane bagasse xylans was tested and results indicate that *Cohnella* AR92 has major affinity to sugar-cane bagasse xylan: the crude extract was found to bind to both insoluble substrates at 40% to 50% and 65%, respectively. The adsorption of enzymes to insoluble substrate plays an important role in the efficiency of the enzymatic hydrolysis of the substrates, which could be the reason of higher enzymatic activities observed towards sugar-cane baggasse xylan, as well as an indication of an adaptation to its natural environment.

MI-P55

ANTIFUNGAL ACTIVITY OF BACTERIA ISOLATED FROM BAT GUANO ON PATHOGENIC FUNGI OF STRAWBERRIES

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Tucumán is the principal producer of strawberry of Argentine. The major phytosanitary disadvantages of this crop are the diseases produced by soil fungi. One of the most serious and common is the anthracnose caused by fungal of genus *Colletotrichum*. They produce the major losses both in the fruit and plant production. In previous works 6 chitinolitc bacteria isolated from bat guano (*Taradita brasilensis*) were characterized as belonging to *Bacillus* and *Streptomyces* genus.
The aim of this work is to study the antifungal activity of these chitinolitc bacteria on fungus of *Colletoctichum* genus *in vitro*. The 6 bacteria were incubated in 3 different media: SPI- colloidal chitin, SPI-pupa and Standar nutrient during 72h, 200 rpm and at 20, 25 and 28 ºC. Supernatants of these cultures were used in inhibition tests on *Colletoctichum acutatum* and *C.fragariae* grown on potato dextrose agar.
Only the bacteria of the genus *Bacilllus* showed to possess antifungical activity. Supernatants of *B.atrophaeus* PA 14 had the major capacity of inhibition of both fungi. The results suggest that this bacterium produce extracellular antifungal metabolite. *B.atrophaeus* PA 14 is a potential antifungical biocontrol agent for anthracnose in strawberries. This will allow the decrease of the use of chemical products and will favor the sustainable production.
This work was supported by grant PIP-CONICET 297.

MI-P56

SOIL MICROBIAL COMMUNITIES FROM ARGENTINIAN YUNGAS AS AFFECTED BY AGRICULTURALACTIVITY

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To evaluate deforestation and long-term agriculture effect on soil microbial communities we sampled soils from two pristine montane forests (M1 and M2), a pristine pedemontane forest (P) and both a 40-year and a 100-year sugarcane monoculture (S40 and S100, respectively). Acid phosphomonoesterase activity (PA) and soil basal respiration (SR) were measured. We also calculate the metabolic quotient (qCO2) or SR per microbial biomass unit, based on data from previous phospholipid fatty acid (PLFA) analysis. Further, community level physiological profiling (CLPP) was used as an indicator of microbial heterotrophic functionality. PA was significantly ($p<0,05$) higher in pristine soils and also discriminated among them (M1>M2>P). Similarly, pristine soils showed a lower qCO2 value, which evidences microbial communities with higher K-strategists proportion and energetic efficiency. SR could only differentiate M2 from S100, M2 displaying a higher basal activity. CLPP did not show differences between land use, which may suggest the presence of microbial communities with high functional redundancy. Finally, integrated PCA of physico-chemical and biochemical data explained 71% of their variability and showed that AF, qCO2, pH and available P content allowed discriminating pristine from agricultural soils. We propose these variables as suitable soil quality indicators in this region.

MI-P57

ABUNDANCE OF POLYCYCLIC AROMATIC HYDROCARBON-DEGRADING POPULATIONS IN PATAGONIAN COASTAL SEDIMENTS

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The aim of this project was to quantify polycyclic aromatic hydrocarbon (PAH)-degrading bacterial populations in coastal sediments of Northern Patagonia. We analyzed the relative abundance of various genes encoding PAH dioxygenases by qPCR, in DNA extracted from intertidal sediment samples collected at Fracasso Beach, a protected area, and Córdova Cove, a chronically polluted site. We quantified the following catabolic genes: *phnA1* (*Cycloclasticus* spp.), *nahAc* (*Pseudomonas* spp.), *phnAc* (â-*Proteobacteria*) and C, a novel dioxygenase gene previously detected in Subantarctic sediments. Additionally, we quantified 16S rRNA genes using a universal primer set. Physico-chemical parameters, including ORP, granulometry, organic matter, ammonium and hydrocarbon concentrations, were also measured in these samples. *phnA1* genes were abundant in polluted sediments, and their abundance was found to be two orders of magnitude higher in sediments with twice the concentration of 3-ring PAHs. The other analyzed genes were found to be present, although below quantification limit for this technique. These results suggest that *Cycloclasticus*, a marine obligate hydrocarbonoclastic bacterium, plays an important role in the biodegradation of low molecular weight PAHs in coastal sediments of Patagonia. Laboratory scale studies are being performed to analyze population dynamics after crude oil or PAH exposure7

MI-P58

STATISTICAL EVALUATION OF MEDIUM COMPONENTS OF ENTOMOPATHOGENIC PROTEIN PRODUCTION

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Bacillus thuringiensis produces proteins with entomopathogenic action. Advances in the production of bio-insecticides involve the application of suitable fermentation technologies, especially with the use of appropriate media, overcoming the metabolic limitations of the microorganisms. The use of agro-industrial residues as substrate in the production by fermentation can significantly reduce the final price and add value to low-cost materials. The objective of the present work was to screening substrates that have a positive impact on the production of entomopathogenic proteins from *Bacillus thuringiensis* RT. Evaluation was performed by Plackett-Burman experimental design. Eleven medium components were evaluated. Mortality bioassays were carried out using 3rd instar larvae of *Spodoptera frugiperda*. The most significant variables affecting positively the production were starch, milk powder and whey. Medium with highest protein production (0,8 mg/ml) was 4 times better than Luria Bertani medium (0,2mg/ml). There were significant differences in mortality with the media tested. In conclusion, many substrates were identified to improve production, which could be considered for optimization medium and studies in bioreactors.

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MI-P59

PROTECTION OF OILSEED RAPE (*B. napus*) TOWARD FUNGAL PATHOGENS BY STRAINS OF BIOCONTROL PGPR

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The aim of this work is to evaluate the effects of treatment of oilseed rape (*Brassica napus*) with indigenous bacterial strains isolated from soybean rhizosphere. Antifungal activities of two of these isolates, previously identified and designated as *Pseudomonas fluorescens* BNM296 and *Bacillus amyloliquefaciens* BNM340, were assayed by dual culture with two serious fungal pathogens to *Brassica* species, *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Oilseed rape seeds were inoculated with each of the bacterial suspensions and colonization of seedling roots was evaluated. In addition, the treated plants were assessed for their resistance to the fungal pathogens using detached leaves assays. The dual bacterial culture of BNM340 inhibited the myceliar growth of *B. cinerea* (66.4±1%) and *S. sclerotiorum* (71.1±5.2%) with respect to each of the fungi growing alone. In the case of BNM296, the relative growth of *B. cinerea* and *S. sclerotiorum*, in the presence of the bacteria, were also inhibited 46.1±4.6% and 37.6±6%, respectively. The quantification of bacteria adhering to the surface of inoculated *B. napus* seeds and roots showed that both of tested bacteria effectively colonized them. Leaves of treated plants with BNM296 and BNM340 exhibited an enhanced state of resistance against *S. sclerotiorum* and *B. cinerea*, demonstrating that these bacterial suspensions are capable of inducing ISR.

MI-P60

MOLECULAR IDENTIFICATION OF *Enterococci* ISOLATED FROM ARTISANAL CHEESES

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Enterococci are relevant for improving and developing the flavor and quality of cheese. *E. faecalis* is the commonest specie isolated from artisanal cheeses. Strains of *E. faecium* (EVR) are also recovered from artisanal cheeses, being the reason of human infections. EVR develops multi-resistance to different antimicrobials such as aminoglycosides, ß lactams and glycopeptides.

Aims: To correlate the phenotypic and genotypic identification of the enterococci isolates from artisanal cheese manufactured in farms settle in Tandil, BA province.

Methods: 21 artisanal cheeses were processed. The phenotypic characterization was performed by the API System, whilst the molecular identification of the strains was undertaken by PCR amplification of *tuf* and *sodA* genes.

Results: 13 strains of enterococci were isolated from cheeses, by which 9 strains were phenotypically identified as *E. faecalis,* whilst 4 isolates were not typified. PCR revealed that all the strains tested were identified as *E. faecium*.

Conclusions: The phenotypic characterization is not a reproducible and reliable method to identify enterococci. In contrast, the genotyping techniques, allows a fast and accurate identification of those strains. In artisanal dairy products is pivotal to identify other species than *E. faecalis* that may become as emergent pathogens in human (EVR).

MI-P61

PHYSIOLOGICAL ROLE OF THE C4 BIOSYNTHETIC PATHWAY IN STARTER AND NONSTARTER LACTIC ACID BACTERIA

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Lactic Acid Bacteria (LAB) are recognized as safe microorganisms that are capable to improve the quality of diary products. When the LAB Lactococcus lactis is employed as a starter for the production of fermented foods, high quantities of important aroma compounds such as diacetyl are generated by means of the C4 metabolic pathway. This route depends on the expression at low pH of the *als, aldB, aldC* and *butBA* genes encoding the enzymes involved in the conversion of pyruvate into aroma compounds. In the non-starter LAB *Enterococcus faecalis*, a bioinformatic search determined that only alsS and alsD (orthologous to L. lactis aldB) genes are present in its genome. Our studies showed that these genes compose a single operon which is maximally transcribed during the mid-exponential phase and further enhanced upon the addition of pyruvate to the medium. The disruption of the als gene in both microorganisms, produced C4 deficient strains sensitive to low pH and high pyruvate concentrations. The dramatic reduction in the growth parameters of mutant strains is accompanied by the inability to alkalinize external medium and it is reverted when medium pH is kept constant at higher values. These results suggest that the decarboxylation reactions related to the C4 pathway give to LAB a competitive advantage in a condition of intracellular pyruvate accumulation during growth at low pH.

MI-P62

EFFECT OF CYPRODINIL+FLUDIOXONIL FUNGICIDES ON FERMENTATIVE PROCESS DURING WINEMAKING

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Different chemical compounds have been used to control fungus diseases in grapevines. Recently, new antifungal products have been introduced in the market to control these diseases. One of the most used products is composed by cyprodinil and fludioxonil (commercial name: Switch), which is characterized by a very short pre-harvest interval (PHI; 1 day). The winemaking process can be affected by fungicide residues present in grape musts. The aim of this study was to evaluate the effect of cyprodinil+fludioxonil fungicide on the alcoholic and malolactic fermentations during the winemaking process. The experiments conducted included combinations of different fungicide doses (0.05, 0.5, 1, 2, 5 mg/L), yeast strains and malolactic bacteria. During fermentation, microorganism viability, fermentation performance and degradation of fungicides were analyzed. The fungicide concentration present in the must remained stable during fermentation and in the finished wines. Only the highest fungicide doses (5 mg/L) reduced yeast viability and arrest the alcoholic fermentation. When compared at the maximum residue level, 1mg/L (MRL; SENASA Res. 507/08) for grape musts, no differences were found between yeasts on residual sugars and yeast viability. The malolactic fermentation and lactic bacteria viability were not affected at the MRL of 1 mg/L.

MI-P63

MICROBIAL DIVERSITY ANALYSIS IN ARGENTINE AGRICULTURAL SOILS USING MASSIVE PARALLEL PYROSEQUENCING

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Microbial communities in soils have an extremely important role in economic sustainability and in global nutrient cycling. Pyrosequencing technology allows a deep analysis of these communities. To test the hypothesis that bacterial communities change with land use and to analyze their seasonal dynamics, we used 454 pyrosequencing of V4 region of 16s rRNA gene (Pyrotags) on two different types of soils, no- till farming and undisturbed. Samples were collected in three independent sites of La Pampa Ondulada region in Buenos Aires, Argentina providing triplicates, and on four different time points during one year, in one of the sites. We obtained a total of 1,059,702 pyrotags. Taxonomical classification was done using a personalized version of the PANGEA pipeline. We found variations in community composition over time even at the phylum level. Shannon- Weaver diversity Indices were calculated as well as rarefaction curves. In disagreement with previous knowledge, we observed no significant differences on biodiversity indices between no- till farming and undisturbed soils from phylum down to genus level. Moreover, Shannon Indices were higher for no-till farming than for undisturbed soils at the species level. This work is the most comprehensive examination to date of bacterial diversity in agricultural soils and suggest that no-till farming is compatible with soil sustainability.

MI-P64

MINERAL PHOSPHATE SOLUBILIZATION BY *Burkholderia tropica*

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Conversion of soil insoluble phosphates to a form available for plants is a necessary goal to achieve for sustainable agricultural production. *Burkholderia tropica* strains have been reported to posses the ability to solubilize insoluble phosphates. Mineral phosphate solubilization (MPS) activity is usually related with the expression of a periplasmic glucose dehydrogenase (GDH), responsible for the conversion of glucose into gluconate. In the present work it was checked the ability of *B. tropica* to express an active GDH under different culture conditions and their relation with MPS activity. Plate assays using tricalcium phosphate (TCP) and different carbon sources, showed that *B. tropica* was able to solubilize inorganic phosphates with aldoses as carbon source but no solubilization was observed when other sugars were used. Batch cultures carried out with glucose as C-source showed GDH activity and gluconic acid production only in cultures using TCP. Soluble phosphorous concentration in the media increased together with the gluconic acid. These results indicate that phosphate solubilization was due to acidification via GDH activity and it seems that this activity is regulated by phosphate starvation.

MI-P65

COLONIZATION OF DIFFERENT CROPS BY THE PLANT GROWTH PROMOTING-BACTERIUM *Burkholderia tropica*

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The use of biofertilizer and biocontrol organisms is being considered as an alternative or a supplementary way of reducing the use of chemicals in agriculture. These beneficial bacteria are able to colonize roots and, some of them, are also able to colonize plant tissues (endophytes). The understanding of bacterial colonization patterns is a critical prerequisite for the development of effective inoculants. In the present study, colonization patterns of the plant growth promoting bacteria *Burkholderia tropica* has been monitored by dilution plating assays and microscopic localization in crops of *Lycopersicon esculentum*, *Brassica napus* and *Sorghum bicolor* inoculated with this organism. Microscopic localization of *B. tropica* (labeled with a stable plasmid carrying the gen for green fluorescent protein) showed that seedling inoculation led to extensive root surface colonization in these plants. This is in accordance with the high epiphytic population densities found in all plants tested (~6-7 log CFU/g fresh weight). Root endophytic bacterial enumerations were also similar for the different crops (~5 log CFU/g fresh weight) and aerial tissues were colonized efficiently (~4 log CFU/g fresh weight). This study provides evidence that *B. tropica* is able to colonize other plants than its original host and also establish stable associations, at least under our experimental conditions.

MI-P66

A T3SS MUTANT OF *Mesorhizobium loti* HAS AN INCREASED COMPETITIVENESS FOR NODULATION ON LOTUS TENUIS

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Type III secretion system (T3SS) is a multiprotein complex through which effectors proteins are delivered into the host cell where they modulate cellular functions such as defense response. *M. loti* has a functional T3SS that is involved positively in the nodulation process on *Lotus spp*.

Here we inserted a gentamicin cassette into the *mlr8765* gene of *M. loti* which codified for a protein with tetratricopeptide repeats. In pathogens, these repeats were found in the chaperones of the translocator component of the T3SS that is necessary to deliver effectors into the host cell.

We analyzed proteins secretion patterns of bacteria under induction of the T3SS genes and we observed that in the case of the mutant there was no secretion of the T3SS normally secreted proteins. These results could not correspond to a simple mutation in the translocator´s chaperone then we suspect that the upstream gene was also affected by the mutation. This gene codified for the RhcV protein, central component of the T3SS.

In co-inoculation assays with a mixture 1:1 of wild type and mutant, the 100% of the induced nodules were occupied only with the mutant strain. This result suggests that the T3SS is involved in multiple mechanisms that could either improve or decrease the ability to compete for an effective modulation. Further studies are necessary to determine the affected mechanism in the mutant.

MI-P67

ANALYSIS OF SECRETED PROTEINS BY *Mesorhizobium loti* T3SS AND ITS ROLE IN SYMBIOSIS ON *L. japonicus*

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Mesorhizobium loti posses a functional type three secretion system that is involved in the nodulation process on *Lotus* spp. In bacterial pathogens this system translocates effectors proteins directly into host cells where they modulate cellular functions.

We made a translational fusion into a moderate copy number vector of the N-terminal protein fragment to a reporter peptide. This allows the determination of the ability to direct the secretion of the fused protein through the T3SS for two putative *M. loti* effectors. As the expression from a plasmid of moderate copy number has a potential for non-physiological level of protein accumulation, that could alter the behaviour of the secretion hierarchy, we decided to integrate the translational fusions into the genome of *M. loti* as a single copy. We determined that the secretion of this fusions could be detected better under calcium deprived culture conditions, and we demonstrated that the N-terminal fragment of the protein codified by *mlr6316* and *mlr6331* also have the capacity to direct its secretion through *M. loti* T3SS.

We also determined the nodulation phenotype of a T3SS mutant on *Lotus japonicus* and attributed to the *M. loti* T3SS secreted proteins a direct positive role on the nodulation process because double and triple mutants on these proteins have a negative effect on this process.

MI-P68

COPPER DEPENDING INHIBITION OF *Xanthomonas sp* BY THE SIDEROPHORE PYOCHELIN

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In a screening for metabolites produced by soil and rhizosphere bacteria capable of inhibiting X*anthomonas citri pv citri* we identified strains from the *Pseudomonas* genus able to inhibit this pathogen. After purification we were able to identify the active compound as pyochelin. Since this compound is a siderophore capable of chelating metal ions, we tested if the inhibitory effect could be dependent on metal ion chelation. We observed no reduct ion on MICs when Fe+3, Zn+2, Mg+2 were added but we significantly reduced its inhibitory effect by adding copper. Since pyochelin is active against *Xanthomonas* species but not active against other gram negatives such as *E. coli* and *Salmonella*, we thought that resistant strains might have siderophores that would allow them to overcome the presence of pyochelin. Indeed, we observed that *E. coli* became sensitive to pyochelin when the biosynthesis of enterochelin was impaired by a mutation in the entE gene. In addition, we observed *Xanthomonas* protection against pyochelin when we added partially purified enterochelin. This E. coli siderophore is reported to be involved in copper reduction making it more available for cell growth, so we hypothesized that a reducer agent as ascorbate would mimic enterobactin in reducing Cu+2 to Cu+1 and therefore would protect sensitive strains to pyochelin. We observed such protection in sensitive strains.

MI-P69

hrpM IS INVOLVED IN GLUCAN SYNTHESIS, BIOFILM FORMATION AND PATHOGENICITY OF _Xac_

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One of the most devastating citrus diseases is bacterial citrus canker caused by *Xanthomonas axonopodis* pv. citri (*Xac*). Previous work from our laboratory demonstrated that *Xac* is able to form biofilm communities in biotic and abiotic surfaces. To obtain new genes involved in Xac biofilm formation we performed a screening using a library of 6000 tn5 mutants. Mutants with less adherence than the wild type strain to polystyrene surface after 24 hours of growth in Y-minimal medium were chosen. One of the mutant obtained from the screening corresponded to an insertion in *hrpM* gene, encoding a glucosyltransferase enzyme. According to *hrpM* gene homology with identified sequences in other bacteria, it is a candidate for the cyclic â-1, 2-glucan synthesis in *Xac*. Here we demostrated that the disruption of this gene compromised the production of the cyclic â-(1,2)-glucan. Moreover, this mutant showed reduced swimming motility and biofilm development. Bacterial suspensions were inoculated in lemon and grapefruit plants and no disease symptoms were observed in leaves infected with the *hrpM* strain.

MI-P70

BIOINFORMATICS CHARACTERIZATION OF REGULATORY ELEMENTS IN TRYPANOSOMATID GENOMES

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The Kinetoplastid parasites *Trypanosoma cruzi, Trypanosoma brucei* and *Leishmania major* are unique microorganisms which mainly use post-transcriptional mechanisms to regulate gene expression, including control of mRNA turnover and translational efficiency. In many cases these processes depend on the presence of certain elements located at the 3' untranslated regions (3'UTRs) of mRNAs that are recognized by RNA-binding proteins (RBPs). We have previously showed that trypanosomal transcripts encoding genes with functions in different cellular processes contain conserved structural elements in their UTRs that are involved in RNA-binding. Using information from the KEGG metabolic pathways database (http://www.genome.jp/kegg/pathway.html) we grouped mRNAs based on their mapping to broad metabolic categories. We next tested whether the UTRs in each set of mRNAs contained a common sequence element using motif discovery tools, and obtained common secondary RNA elements enriched in the 3'UTR of groups of functionally linked transcripts. The model that emerges from our results is in line with the results of an increasing number of studies that suggest coordination of gene expression by binding of RBPs to different categories of related mRNAs, thus defining a post-transcriptional operon.

MI-P71

IMPORTIM-LIKE PROTEINS INVOLVED IN NUCLEAR TRANSPORT ARE ESSENTIAL IN _Trypanosoma brucei_

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Trypanosomatids are protozoan parasites with a complex life cycle that alternate between hosts and differentiation stages. Therefore, they are subjected to environmental changes that must necessarily be compensated by a capable regulatory mechanism. The nuclear envelope provides a physical separation between the nucleus and the cytoplasm allowing precise control of many cellular processes through the selective regulation of transport between the two compartments. While small proteins can pass through the nuclear pore complex (NPC) in a passive way, those of larger size require a nuclear localization signal and specific transporters engaged in active transport across the NPC. We previously reported the finding and cloning of two genes with sequence identity to the karyopherins, importin alpha and importin beta, two well-characterized transporters in other species but with unknown function in trypanosomatids. Here we show, by RNAi, that both importins are essentials in *T. brucei* procyclic forms. We also generated fusions to fluorescent proteins and were able to study the localization dynamics of these inportins. Since orthologous proteins have been crystallized, it was possible to model the three-dimensional structure of the proposed importins in trypanosomatids. So far our experimental data is consistent with the suggested cellular function.

MI-P72

POSITIONAL SCANNING SYNTHETIC LIBRARIES TO THE STUDY OF _T. cruzi_ RECOMBINANT HUMAN ANTIBODY SPECIFIC

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It is well established that protective immune mechanisms against *T.cruzi* are constituted in part by strong antibody (Ab) responses to the parasite. However, it has also been proposed that some parasite Abs can cross-react to human antigens, suggesting that autoimmunity may have a role in the pathogenesis of Chagas disease. The aim of this study was to elucidate the antigen specificity of relevant human *T. cruzi* antibodies. Using phage display technology, two single chain variable fragments (scFvs) B6 and A1 were isolated from Chagas heart Disease patients. Western-blot analysis revealed that scFv B6 and scFv A1 recognized *T.cruzi* proteins of 175 and 47 kDa respectively. Interestingly, scFv A1 also identified a 47 kDa protein in mouse brain lysate. The antigen specificity of scFv B6 and scFv A1 was examined using a positional scanning hexapeptide library, which was screened by competitive ELISA for its ability to inhibit Ab binding to a parasite lysate. This proteomic information together with a positional scanning based biometrical analysis that systematically compares the screening results of the library with selected protein databases will result on the identification of candidate *T.cruzi* antigens as well as cross-reactive antigens from the human and murine species.

MI-P73

GLUCOSYLCERAMIDE SYNTHASE IN PARASITES, A KEY ENZYME OF THE GLYCOSPHINGOLIPID PATHWAY

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Sphingolipids are important components of eukaryotic cells, many of which function as bioactive signaling molecules. Although for mammalian cells most metabolic products of the sphingolipid via have been largely studied and a lot of the enzymes involved have been well-characterized, the panorama in parasitic protozoa is practically unknown. The core structure of the glycosphingolipids, glucosylceramide, is synthesized by a UDP-glucose: ceramide glucosyltransferase (GCS). In a previous work, we have shown the presence of an active GCS enzyme in the intraerythrocytic stages of *P. falciparum* with a substrate specificity different from that of the mammalian counterpart. Later on we showed that epimastigote forms of *Trypanosoma cruzi* present an active GCS with the same substrate specificity of the mammalian enzyme. GCS purified from *T. cruzi* was used to obtain a specific polyclonal antibody developed in mice. This antibody was able to detect the presence of GCS in lysates of *P. falciparum*, *Toxoplasma gondii*, *Leishmania amazoniensis* and *Trypanosoma brucei*. Furthermore, activity assays with NBD-Ceramide and NBD-Dihydroceramide were performed using lysates from the different parasites as the enzyme source. Differences between this key enzyme of the glycosphingolipid pathway and the mammalian enzyme would allow considering GCS as a new chemotherapeutic target.

MI-P74

EFFECT OF INTERACTION BETWEEN LIPOSOME PREPARATIONS AND *Trypanosoma cruzi* STAGES

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One of the major public health problems in Latin America is Chagas' disease which is caused by the kinetoplastid protozoan *Trypanosoma cruzi*. Transmission occurs mainly by triatominae insect vectors, but also by blood transfusion and congenital routes. Although successful programs in vector control and banked-blood screening have led to a decline in transmission, neither total eradication nor effective treatment of the disease has been achieved. The only current treatment relies on nifurtimox and benznidazole, both drugs show toxic side effects and limited efficacy. The main challenges in pharmacotherapy of Chagas' disease are to increase effectiveness of drug treatment and to prevent adverse effects. Nano-drug delivery systems (nanoDDS) represent useful means to selectively deliver the drug to intracellular targets. We assayed the interaction of different lipid composition and sizes of liposomes with epimastigotes, blood stream tripomastigote and amastigote form of *T.cruzi*. As phosphatidylcholine (PC) is widely used used and the main component of lipidic nanoparticles formulations, we prepared 100% egg PC, 100% soy bean PC and 50% egg PC-50% soy bean PC nnDDS. The source of PC, the nnDDS composition preparation as well as liposome size modifies the effects against parasitic cell. The main conclusion is that liposomes themselves are effective against *T. cruzi* cells.

MI-P75

MOUSE SIGLEC-E BINDING TO SULFATED GLYCOPROTEINS EVIDENCE MODULATORY EFFECTS OF *T. cruzi*

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Siglec-E (sialic acid recognizing Ig-super family lectins), is an ortologue protein of human siglec-9. In order to investigate if sulfated oligosaccharides are directly involved in the modulatory effects of *T. cruzi* , immunological, cell biological and molecular biological techniques using mouse Siglec-E-Fc fusion molecules were performed showing that:Binding of purified Cz and C-T from epimastigotes to Siglec-E showed a significant decrease after desulfation treatment of these molecules, sugesting that sulfates enhance the interacción; binding of membrane proteins from trypomastigotes obtained by extraction with Triton X-100 0.1% from a virulent strain to Siglec-E resulted higher than the obtained from the non-virulent strain tested; similarly, a microsomal enriched fraction obtained by using a different methodology showed the highest binding to Siglec-E as compared to the nuclear, large and small granules and soluble fractions obtained. The results obtained will allow to study the immune modulatory effect of *T.cruzi* on phagocytic and APCs cells, which play an important role both in the innate immune response and in the dissemination of the parasite, based on siglec-cruzipain interaction, as well as the involvement of sulfated structures, in the host-parasite interaction to understand the biology of *T. Cruzi*.

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MI-P76

FUNCTIONAL STUDIES OF CYTOCHROME P450 REDUCTASES FROM *Trypanosoma cruzi*

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Trypanosoma cruzi encounters extreme fluctuations in environmental conditions during its digenetic life cycle. Modifications in lipid composition of membranes are made during metacyclogenesis in order to achieve relevant morphological and functional changes in the parasite for adaptation to the environment. Moreover, depletion of ergosterol causes trypanosomal cell death, and enzymes of this biosynthetic pathway have been proposed as targets for an antiparasitic therapy. In our laboratory, we have identified and characterized a gene family consisting of three putative Cytochrome P450 reductases (CPR) in *T. cruzi* named *Tc*CPR-A, *Tc*CPR-B and *Tc*CPR-C. Ergosterol content was significantly increased in *Tc*CPR-B and *Tc*CPR-C overexpressing parasites. Nevertheless, overexpression of *Tc*CPR-A was lethal, displaying aberrant cells, with abnormal morphology and ultrastructural alterations. Interestingly, it was recently reported that Tah18, a yeast orthologue for *T. cruzi* CPR-A, plays a "pro-death" role in response to oxidative stress, inducing cell death pathways. We are nowadays also studying a possible participation of this CPR in stress induced apoptosis mechanisms in *T. cruzi*. In order to establish the particular role of these enzymes in the mentioned processes, complementation assays are being performed using a cpr knock-out *Saccharomyces cerevisiae* strain (cpr-).

MI-P77

CLONING AND CHARACTERIZATION OF A POLY(ADP-RIBOSE)GLYCOHYDROLASE IN *Trypanosoma brucei*

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Poly-ADP-ribose (PAR), generated by poly(ADP-ribose)polymerase (PARP) in the presence of DNA strand breaks (DSB), is common to nuclear processes related to DNA metabolism, such as structural chromatin remodeling during DNA repair, transcription, DNA replication and cell death pathways. Poly(ADP-ribose)glycohydrolase (PARG) is the main PAR hydrolyzing activity in the cell. *T. brucei* evades the immune system by antigenic variation of surface glycoproteins (VSG), which requires recombination sites within DNA inducing transient DSB. To further investigate this process, here we present the cloning and characterization of TbPARG. Southern-blot analysis confirmed a single copy of this gene in *T. brucei* genome. Sequence alignments of the catalytic domain showed the PARG signature containing the three essential acidic residues (D-E-E) conserved within trypanosomatids. Expression of the 60 kDa protein in procyclic stage was demonstrated by Western-blot. Nuclear localization of the enzyme in the basal state of the parasite, as well as after hydrogen peroxide treatment, was observed by IFI. PAR generation after a genotoxic insult was also detected in the nucleus by using antiPAR antibodies. Currently, we are working on TbPARG assay in procyclic extracts and of the recombinant protein rTbPARG-His, expressed in bacteria.

MI-P78

***Trypanosoma cruzi* "HIGH MOBILITY GROUP B" PROTEIN IS A CHROMATIN ARCHITECTURAL FACTOR**

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High mobility group B (HMGB) proteins are abundant non-histone chromatin proteins that play important roles in the execution and control of many nuclear functions. TcHMGB is an HMGB family member from *Trypanosoma cruzi,* the causative agent of Chagas disease. TcHMGB has two HMG box domains, like mammalian HMGBs, but lacks the typical C-terminal acidic tail and, instead, bears a 110 amino-terminal domain that seems to be unique of kinetoplastid HMGBs. Despite these differences, TcHMGB maintains HMG box architectural functions: it binds distorted DNA structures like cruciform DNA and it is also able to bend linear DNA. TcHMGB is present in the nucleus in all *T. cruzi* life cycle stages. The protein content, however, is not constant, being higher in replicative forms (amastigotes and epimastigotes) than in the non replicative trypomastigote form. This is consistent with the lower heterochromatin content and higher transcription rates previously reported in replicative forms. In contrast to H1 histone, HMGB proteins cause chromatin to be more relaxed, and concomitantly more accessible to transcription factors, remodeling complexes and other nuclear proteins. In this context, TcHMGB may alter chromatin structure between life cycle stages, but also in different genome regions, taking part in epigenetic control of nuclear processes like transcription, recombination, replication and repair.

MI-P79

EFFECT OF NORDIHYDROGUAIARETIC ACID IN LYMPHOID AND PROMONOCYTIC HIV-1 PERSISTENTLY INFECTED CELLS

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Nordihydroguaiaretic acid (NDGA), a polyhydroxyphenolic compound isolated from Larrea divaricatta, described as an antiviral drug, is a strong antioxidant and an inhibitor of various signaling pathways. In order to eliminate virus present in cellular HIV-1 reservoirs, such as macrophages and CD4+ T lymphocytes, novel therapeutic approaches are required. The objective was to investigate the action of NDGA on HIV-1 persistently-infected lymphoid and monocytic cells. Lymphoid (H9+, H9/HTLVIIIB) and pro-monocytic (U1) HIV-1 persistently-infected cell lines were treated with different concentrations of NDGA during 48 hs and compared with uninfected counterparts, H9 and U937 respectively. Different parameters were measured: (a) viral yield as p24 antigen in cell supernatants by ELISA, (b) cell viability by trypan blue assay, (c) the redox state by the oxidation of dichlorodihydrofluorescein to a fluorescent compound, (d) cytokine production (TNF-alpha and IL-6) in cell supernatants by ELISA. In lymphoid cells, the presence of 2ug/ml NDGA inhibited: (a) to 46% and (b) to 80%; while no change in (c) was observed, and (d) were diminished to 60-70%. In U1 no change in (a,b or c) was observed. Thus, NDGA effect on virus yield in persistently infected lymphoid cells is not related to the redox state but to unknown cell signalling that might be influenced by an immunomodulatory activity.

MI-P80

EVALUATION OF NATURAL AND SYNTHETIC COMPOUNDS AGAINST HIV-1 IN PERSISTENTLY INFECTED CELL LINES

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Since antiretroviral therapy effectively suppresses but does not eradicate HIV-1 infection, methods to purge the viral reservoirs are required. Many strategies involve the reactivation of latently HIV-infected cells by exposing it to cytokines or upregulating cellular transcription. The 1-cinnamoyl-3,11-dihydroxymeliacarpin isolated from Melia azedarach L., and synthetic steroids derived from the stigmasterol (1:(22S,23S)-3â-bromo-5á,22,23-trihydroxystigmastan-6-one and 2: (22S, 23S)-22, 23-dihydroxystigmast-4-en-3-one) display antiviral and immunomodulatory activities, affecting NF-kB activation and the production of cytokines. Here, we evaluated the antiretroviral activity of these compounds in lymphoid (H9/HTLVIIIB) and pro-monocytic (U1) HIV-1 persistently-infected cell lines. Cells were treated with different concentrations of these compounds and cellular viability and p24 viral antigen were evaluated by trypan blue and ELISA, respectively. In contrast to Zidovudine (ZDV), all compounds exhibited a high cytotoxicity in lymphoid cells with respect to monocytic cells. However, similar to ZDV behavior, no effect on p24 production was detected. Thus, sensitivity of lymphoid cells could be related to the immunomodulating properties of the compounds.

MI-P81

A N-TERMINAL COILED COIL MOTIF IS ESSENTIAL FOR TACARIBE VIRUS NUCLEOPROTEIN FUNCTIONALITY

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Arenaviruses,such as Tacaribe virus (TCRV) are enveloped viruses with a negative-sense RNA genome, which encodes four proteins:the viral RNA polymerase (L protein), a matrix protein (Z), the precursor (GPC) of the envelope glycoproteins and the nucleocapsid protein (N).The N protein tightly binds to genomic and antigenomic RNAs forming nucleocapsids, which act as templates for both transcription and replication of viral genomes mediated by the virus polymerase.Besides, the ability of N to take part in multiple protein-protein interactions may be important in several steps of the viral life cycle. We have shown that the N-terminal region of N is responsible for homotypic N-N interactions.Here, we have defined a newly recognized coiled coil motif domain as being essential for N self-interactions. Key hydrophobic amino acids within the putative coiled coil motif, were replaced by the polar amino acid Glutamine. The ability of point mutants to self-interact was evaluated by coimmunoprecipitation and their capacity to support viral RNA synthesis was assessed using a TCRV minireplicon system.The relevance of the coiled coil motif in the interaction between N and the L polymerase will be discussed. It is concluded that residues 92-119 of the TCRV N protein may fold as a coiled coil, which is crucial for N to sustain N-N interactions as well as for its role in viral genome replication.

MI-P82

PRODUCTION AND PURIFICATION OF RECOMBINANT MEASLES VIRUS HEMAGGLUTININ TO MEASURE HUMORAL IMMUNITY

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Measles virus, a member of the Paramyxoviridae, is capable of causing acute and persistent infection. The acute infection is following by life-long immunity in which neutralizing antibodies against the viral haemagglutinin (HA) play an important role. The HA protein is a type II surface glycoprotein, and is involved in the attachment to the host cell.
Purification and evaluation of recombinant HA protein expressed in baculovirus system and production of polyclonal antibodies. Evaluation of neutralizing antibodies in infected and vaccinated individuals using the recombinant HA protein.
A truncated version Measles HA protein (without transmembrane hydrophobic region) derived from Edmonston strain, was expressed using baculovirus System. The HA expressed was purificated with Ni-Nta agarose .The purified HA was evaluated by EIA, SDS-PAGE and western blot. The recombinant HA was used in capture EIA assay to evaluate neutralizing antibodies. The EIA results were compared to those obtained with a micro-neutralization assay. The purified recombinant HA protein was detected by EIA SDS-PAGE and Western blot. Moreover, recombinant HA was detected by EIA using human serum from infected and vaccinated individual. These results suggest that the recombinant HA can be used in evaluation of immunological parameters.

MI-P83

EXPRESSION OF GROUP C ROTAVIRUS VP6 PROTEIN IN BACTERIAL, BACULOVIRAL AND HERPES VIRUS BASED SYSTEMS

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Rotavirus belongs to the Reoviridae family. The viral particle possesses a structural protein named VP6 that is of particular interest, since it forms part of the internal capsid, is not glycosylated and is the most inmunogenic of the structural proteins of the virion. In addition, based on the antigenic and genetic similarities of VP6 these viruses have been classified in groups A-G.
Group C rotaviruses were detected in cases of diarrhea in humans and it has been demonstrated that infection and disease rates due to this agent are underestimated. It is necessary to emphasize that the lack of commercially available methods to detect the virus greatly contributes to this situation. In this context, the immunogenicity of VP6 protein and his preserved aminoacidic sequence turn it into a potentially excellent diagnostic tool.
Specific primers were designed and the codifying region of human group C rotavirus VP6 gene was amplified by RT-PCR. The original material was clinical samples available in our laboratory. The amplified fragments were cloned in three different vectors according to the expression system to be used (bacterial, baculoviral and a herpes virus based system). The expression level of VP6 protein was evaluated by SDS-PAGE, Western blot and ELISA.
This recombinant protein could be used in the development of diagnostic methods to detect this virus in diarrheal samples.

MI-P84

DIAGNOSTIC AND STRAIN IDENTIFICATION OF CANINE PARVOVIRUS IN ARGENTINA BY MOLECULAR BIOLOGY TOOLS

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Canine Parvovirus (CPV) is a very contagious and fatal viral disease. Although live attenuated vaccines are available, viral evolution leads to several episodes of CPV throughout the world even in vaccinated animals. The original strain CPV2 was replaced in dog populations by two antigenic variants: CPV2a and CPV2b. Recently, a new antigenic variant carrying the aa substitution Asp426Glu, located at the major antigenic region, and called CPV2c, has spread all over the world. As diagnosis of CPV is difficult because clinical signs may be confused with other canine enteric diseases, the objective of the present work was to develop a specific diagnostic tool to detect and characterize CPV in clinical samples. A total of 75 samples were submitted to the laboratory. Samples were taken from domestic dogs between 2008 and 2010. Diagnosis was made by PCR amplification of a 583 bp fragment of VP2 gene which includes the 426 aminoacidic position and strain determination was performed by sequence analysis. CPV DNA was detected in 51 of 75 (68%) samples. 39 of the 51 positive clinical specimens (76%) were obtained from vaccinated dogs. Out of 51 positive samples, 46 (90%) belonged to the CPV-2c variant, 3 to CPV-2b, and the remaining 2 were CPV-2a. These results, suggest that the newest genotype CPV-2c has spread and replaced earlier variants becoming the predominant strain among dogs.

MI-P85

F1 MOTIF OF DENGUE VIRUS POLYMERASE IS INVOLVED IN INITIATION OF VIRAL RNA SYNTHESIS

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Dengue virus (DENV) is the most important mosquito-borne viral human pathogen worldwide. The viral genome is a single strand RNA molecule of positive polarity of about 11 kb long. The RNA encodes a single polyprotein that results in ten viral proteins. The coding sequence is flanked by highly structured 5' and 3'UTRs. NS5 is the largest of the DENV proteins. It contains an N-terminal methyltransferase domain (MT) and a C-terminal RNA polymerase domain (RdRp). The RdRp activity depends on the presence of the RNA promoter (SLA), present at the 5' end of the genome. We used recombinant DENV NS5 proteins and genetically modified viruses to investigate the relevance of positive-charged regions of the RdRp on RNA binding, polymerase activity, and viral replication in cell culture. Using in vitro activity assays, we analyzed 14 mutants of NS5. We found 4 mutants that were unable to synthesize RNA with any RNA template. In addition, we found one mutant located in the F motif, which showed a specific defect in RNA synthesis only when the viral RNA was used as template. These and other observation led us to propose that the F1 region of the F motif is involved in specific initiation of viral RNA synthesis. Furthermore, we investigated the replication of viruses carrying mutations using mammalian cells and found different phenotypes associated to specific NS5 changes.

MI-P86

***Trichoderma* spp. AS A BIOINDICATOR OF SOIL QUALITY IN THE YUNGAS REGION IN NW-ARGENTINA**

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This study analyzes the use of *Trichoderma* spp. in pristine and agricultural soils as a potential microbiological indicator of soil quality. Soils from a 23-year (S23) and two 3-year soybean monocultures (PR3 and I13, respectively), a pristine pedemontane forest (M) and a montane forest (SMR) were sampled. To remove fungal spores from soil, samples were washed following the methodology of Bissett and Widden (1972). Washed soil particles were placed on PDA-rose bengal agar plates and the number of *Trichoderma* spp. colonies was determined after 8-day at 25ºC and a 8 h of day light period (2,300 lux). High *Trichoderma* number was observed in the largest (>1.2 mm) soil aggregates. The number of *Trichoderma* spp. was similar in the two pristine soils (SMR and M), while in agricultural soils only S23 and PR3 showed significant ($p<0.05$) differences between them, being higher in PR3 than in S23. The frequency of *Trichoderma* spp. was significantly ($p<0.05$) lower in S23 than that in PR3 and M. Our results suggest that the frequency of *Trichoderma* spp. in these soils is affected by the length of time under agricultural use. Nevertheless, further studies on *Trichoderma* diversity are necessary to determine if one or more species might be used as soil quality indicators in this region.

MI-P87

EFFECTS OF SOLAR ULTRAVIOLET RADIATION (UVR) ON MOLECULAR DIVERSITY OF PLANKTON FROM THE CHUBUT RIVER ESTUARY

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Within the framework of a project designed to evaluate the impact of UVR upon estuarine plankton, we present here a molecular analysis of plankton diversity. Water samples were exposed to three radiation treatments (PAR, PAR + UV-A and PAR + UV-A + UV-B) in microcosms for ca 10 days during the Austral summer. At the beginning (t_0) and at the end of the experiment samples were filtered through 20, 10, 5 and 0.22 µm pore sizes. The DNA amount retained in each filter indicated that most of the plankton biomass was in the 0.22-5 µm fraction at t0. In contrast, at the end of the experiment this proportion changed according to the radiation treatment and big cells (> 20 µm) dominated. An rDNA library was obtained from the DNA corresponding to the 0.22-5 µm fraction. There was no relationship between treatments and the number and frequency of restriction genotypes. Analyses of 27 clones fraction from t_0 indicated the presence of three genera of Rhodobacteraceae, one genus of Rhodospirillaceae, one SAR11 genus, one genus of Bacillaceae, an unclassified sequences of Alphaproteobacteria, Actinobacteria and Rhodospirillaceae. Also, there were six sequences similar to *Ostreococcus tauri* (Mamiellales). Even though the sequence analyses are still ongoing, our initial data suggest a big impact of UV-B radiation in the amount and composition of the plankton community towards big cells.

ST-P01

AGONIST AND CHOLESTEROL MODULATE THE ALPHA7 ACETYLCHOLINE RECEPTOR IN NON-NEURAL ENDOTHELIAL CELLS

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The "neuronal" α7-type nicotinic acetylcholine receptor (α7AChR) is found in various non-neural tissues, including vascular endothelium, where its peculiar ionotropic (high Ca^{2+} permeability) and metabotropic (Ca^{2+}-mediated intracellular cascades) properties may play important roles in angiogenesis, inflammation and atherogenesis. Molecular properties of the α7AChR, its response to nicotine stimulation, and its cellular distribution were studied here using a combination of pharmacological, biochemical and fluorescence microscopy tools. α7AChRs in rat arterial endothelial cells (RAEC) were found to undergo agonist (nicotine)-induced up-regulation (from 53±16 to 385.2±46.8 fmol/mg protein with 50 μM nicotine), increasing their cell-surface exposure. α7AChRs occurred predominantly in the "non-raft" subcellular fractions, yet cholesterol depletion mediated by cyclodextrin treatment reduced the number of cell-surface α7AChRs. Nicotine was found to increase the affinity of the α7AChR for crystal violet, an open-channel blocker. Under basal conditions, α7AChRs in endothelial cells displayed a high-affinity, presumably desensitized conformation (Kd ~0.76 nM), and both nicotine and cyclodextrin affected their cell-surface expression.

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ST-P02

1,25(OH)$_2$-VITAMIN D3 -DEPENDENT ACTIVATION OF AKT IN SKELETAL MUSCLE CELLS

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We previously reported that 1,25-dihydroxi-vitamin D3 [1,25(OH)$_2$D3] induces non-transcriptional rapid responses through activation of Src and MAPKs in the skeletal muscle cell line C2C12. However, there is no information on the regulation of the PI3K/Akt signaling pathway by the hormone in these cells. We report here that 1,25(OH)$_2$D3 promotes Akt phosphorylation in Ser473 (activation) in a time-dependent manner (5-60 min). When proliferating C2C12 cells were pre-treated with methyl-beta-cyclodextrin or caveolin-1 expression was silenced with siRNA, 1,25(OH)$_2$D3-induced activation of Akt was suppressed, indicating that the hormone exerts its effects in cell membrane calveolae. PI3K, ERK1/2, p38 MAPK and PKC were shown to participate in 1,25(OH)$_2$D3–dependent activation of Akt. We also demonstrated c-Src involvement in Akt phosphorylation by 1,25(OH)$_2$D3 using the inhibitor PP2 and antisense oligodeoxynucleotides. During the early stages of differentiation of C2C12 cells we observed that the hormone increases phosphorylation of Akt without affecting its expression. Src and PI3K, were involved in Akt activation and heavy chain myosin and myogenin expression induced by 1,25(OH)$_2$D3. These results suggest that Src and Akt are required during myogenesis triggered by the hormone.

ST-P03

ALPHA-2M/LRP1 SYSTEM INDUCES CELL MIGRATION AND PROLIFERATION BY INTRACELLULAR SIGNALING ACTIVATION

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Alpha2-Macroglobulin (α2M) is a broad specific proteinase inhibitor, which is recognized by LDL receptor-related protein 1 (LRP1), an endocytic receptor belonging to the LDL receptor gene family. Previously, we demonstrated that α2M/LRP1 system induces intracellular signaling activation characterized by the activation of PKC α/ß, MAPK-ERK1/2 and NFkB, which downstream mediated the MMP-9 expression in macrophage-derived cell lines. It is know that these intracellular signaling pathways are involved in cell migration and proliferation. Thus, in this work we investigated whether α2M/LRP1 system is mediating these cellular events using different cell lines (RAW 264.7 and HT-1080). By wound-healing assays we observed that α2M increased the cellular motility of RAW 264.7 cells. In addition, by BrdU (5-bromo-2-deoxyuridine) and flow cytometry we show that α2M induced cellular proliferation of HT-1080 cells. Both cellular events were fully blocked by pharmacological inhibitors of PKC α/ß, MAPK-ERK1/2 and NFkB (GÖ-6976, PD98059 and BAY) as well as by negative dominant mutants of intracellular signaling intermediates such as RAS N17 and MEK AA. In conclusion, our data demonstrate that α2M/LRP1 system promotes the cell migration and proliferation by downstream activation of multiple intracellular signaling pathways

ST-P04

RhoA, JNK AND p38 MAPK PARTICIPATES IN APOPTOSIS MEDIATED BY ANG II AT$_2$ RECEPTORS

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Ang II AT$_2$ receptors are abundantly expressed in fetal tissues, suggesting a role of these receptors in growth and organogenesis. Here we report a possible signaling pathway of apoptosis induced via AT$_2$ receptor activation, as a primary mechanism of tissue differentiation. In Hela cells overexpressing AT$_2$ receptors we studied the apoptotic mechanism. After stimulation with Ang II (10^{-7}M), we evaluated apoptosis by different methods. Immunofluorescence staining and confocal microscopy, showed apoptotic cells after 30 min stimulation. Similarly, JNK phosphorylation and activation appeared after 30 min of Ang II treatment. We evaluated activation of caspases 8 and 3. Cleavage of both caspases increased in a time dependent manner beginning after 30 min stimulation. In order to study RhoA participation in apoptosis induction, we performed co-transfection assays with RhoA V14, N19 and wild type mutants. These experiments suggest the participation of RhoA GTPase, since apoptosis features appeared earlier in cells co-expressing AT$_2$ and RhoA V14, than in those expressing only AT$_2$ R. We also studied p38 MAPK participation in the signaling pathway activated by AT$_2$ and find that inhibition of this protein elicits the apoptotic process. In summary, the present results suggest the participation of RhoA, JNK and p38 MAPK in the signaling pathway mediating apoptosis induced by Ang II AT$_2$ receptors.

ST-P05
MEASUREMENT OF EFFECTIVE SCAFFOLD PROTEIN AFFINITY TO MEMBRANE BINDING SITES BY *IN VIVO* SCATCHARD

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The yeast, *Saccharomyces cerevisiae*, pheromone response pathway is one of the best understood signal transduction systems. All key components of the system and their interactions have been identified by more than 30 years of genetic and biochemical investigation. In recent years modeling efforts have been undertaken to try to describe the behavior of the system in a quantitative manner. These models usually contain a large number of parameters to be determined by fitting to a dataset of experimental results. Direct measurement of these parameters can be used to constrain these models and obtain more accurate and predictive descriptions of the pathway.
In this work we describe a microscopy imaging technique developed to measure protein relocalization in yeast in a quantitative manner. Using this technique we measured membrane recruitment of the scaffold protein of the mating pathway Ste5, an early event that occurs upon pheromone stimulation. Varying the amount of Ste5 by genetic manipulation allowed us to perform an *in vivo* Scatchard to measure the effective binding affinity of the scaffold protein with its membrane associated binding sites. Using this parameter we will refine our mathematical model of the onset of the pheromone response pathway.

ST-P06
Akt ACTIVITY REGULATES THE UNFOLDED PROTEIN RESPONSE IN CULTURED MAMMALIAN CELLS

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Accumulation of unfolded proteins inside the ER triggers the UPR (Unfolded Protein Response), an homeostatic response involving the activation of three parallel pathways (IRE1, PERK and ATF6), aimed at reducing unfolded protein levels. The UPR promotes cell survival in the short term but stimulates apoptosis if unfolded protein levels remain high. For both pro- and anti-apoptotic functions UPR regulates Akt, the central component of the key mammalian survival pathway, a fraction of which localizes to the ER membrane. Here we study the interconnection between Akt and UPR in 293 and MCF7 cells. To do so, we developed fluorescent protein-based reporters of IRE1, ATF6, Akt1 and Akt2. These reporters allowed us to monitor simultaneously UPR and Akt status in individual cells. Interestingly, blocking Akt phosphorylation with AKT inhibitor IV in unstimulated cells resulted in loss of ER localization of Akt1. Surprisingly, this treatment triggered the UPR, as measured by our ATF6 and IRE1 reporters. Akt localization to the ER seems critical for UPR control, since treatment with the PI3K inhibitor LY294002, which did not alter the ER localization of Akt1, did not activate UPR. We confirmed these results by RT-PCR and western blots. Thus, our evidence indicates that not only can UPR activate Akt but also Akt activity can inhibit UPR, suggesting the operation of complex feedback regulation.

ST-P07
ENERGY METABOLISM MODULATES THE PHEROMONE RESPONSE GPCR PATHWAY IN THE YEAST *S.cerevisiae*

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Oscillatory mechanisms control aspects as diverse as seasonal breeding, circadian rhythms and early embryogenesis. Mating pheromone in yeast activates a conserved GPCR-MAPK cascade pathway. In high pheromone, yeast form a mating projection (shmoo) to fuse with a partner. If no mating occurs, cells abandon that shmoo and "search" in another direction, making a new shmoo every 100 minutes. The oscillatory mechanism underlying this searching behavior is unknown. Here we screened, by microscopy, the complete deletion collection of non-essential kinases and phosphatases to find mutants that form less shmoos. We selected 29 out of the 130 strains. These mutants grouped in different classes. Interestingly, one class included 3 enzymes that regulate PDA1, the catalytic subunit of the pyruvate dehydrogenase complex, suggesting that respiration might modulate shmoo formation. Given that yeast exhibit metabolic oscillations with a period of about 50 minutes, we hypothesize that the oscillations propagate, perhaps via ethanol levels, to the pheromone response pathway. Supporting this idea, decoupling pheromone signaling from respiration by addition of ethanol (the end product of yeast fermentation) or deletion of PDA1, greatly inhibited formation of multiple shmoos. We speculate that integration of signaling with the core metabolism might be a general strategy to elicit complex behaviors.

ST-P08
INTEGRATING SIGNALS: ENVIROMENT LINKS MAPK PATHWAYS IN *S. cerevisiae*

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Cells must integrate information from multiple external inputs to respond appropriately. Here, we study the interaction between the pheromone response (PR) and high osmolarity glycerol (HOG) pathways using quantitative fluorescent cytometry and western blots. Despite sharing some of their components, these MAPK cascades show no cross-activation when stimulated individually. However, a high osmolarity shock to cells pre-exposed to pheromone, leads to a rapid dephosphorylation of the PR MAPK, which lasts for the duration of HOG activation. Surprisingly, addition of pheromone to cells adapted to high osmolarity causes persistent activation of HOG at the level of phosphorylation of the MAPK Hog1, its nuclear translocation and induced transcription. Our data suggest that the PR activates HOG by a two branched mechanism: a) opening the glyceroporin Fps1, leading to reduction of internal turgor pressure, and b) activation of Rgc1 (Regulator of Glycerol Channel 1), required for HOG dependent transcription. This hypothesis is supported by a) the observation that wt cells adapted to 1M glycerol (instead of sorbitol) or Δfps1 cells do not phosphorylate Hog1 and b) Δrgc1 cells do phosphorylate Hog1 but fail to induce gene expression. Finally, we found the activation of HOG by pheromone is relevant, since its disruption leads to significantly slower turgor pressure recovery from an osmotic shock.

ST-P09

DYNAMICS OF GRADIENT SENSING IN SINGLE CELLS

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To choose a mating partner the yeast *Saccharomyces cerevisiae* detects mating pheromones produced by yeast of the opposite sex using reversible binding to a GPCR. The non-motile yeast determine the direction of a potential mating partner by measuring on which side there are more bound receptors. This sensing modality can only work when external pheromone does not saturate the receptors. However, the behavior of yeast in dense mating mixtures indicates that, even when pheromone concentration is much higher than the equilibrium constant of pheromone with its receptor, yeast polarize in the correct direction. How might yeast accurately determine gradients at high concentrations? Using a systems biology approach based on mathematical modeling and numerical simulations we show that the actual binding dynamics of pheromone to the receptor is such that enhances gradient determination in near saturating conditions. We used the output of our simulations as input to a published model of spontaneous emergence of cell polarity, and show that the pheromone binding dynamics results in significantly more precise polarization (in the direction of the gradient) than other binding rates. Our results apply to other biological systems in similar binding dynamics.

ST-P10

***Candida albicans* TPK1P AND TPK2P ISOFORMS DIFFERENTIALLY REGULATE PSEUDOHYPHAL DEVELOPMENT**

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Candida albicans PKA catalytic subunit is codified by two genes (TPK1 and TPK2) and the regulatory subunit by BCY1 gene. It is known that both Tpk isoforms play positive roles in vegetative growth and filamentation, though distinct roles have been found in virulence, stress response and glycogen storage. However, little is known regarding their participation in pseudohyphal development. This point was addressed using several TPK mutants in different BCY1 genetic backgrounds. We observed that under hypha-only inducing conditions, the pseudohypha/hypha ratio was higher in strains devoid of TPK2. Under pseudohypha-only inducing conditions strains lacking TPK2 were prone to develop short and branched pseudohyphae. In tpk2Δ/tpk2Δ strains, biofilm architecture was less dense, with reduced adhesion ability to abiotic material like silicone catheters, suggesting a significant defect in cell adherence. Immunolabeling assays showed a decreased expression of adhesins Als1p and Als3p only in the tpk2Δ/tpk2Δ strain. Complementation of this mutant with a wild-type copy of TPK2 restored all the altered functions: pseudohyphae elongation, biofilm composition, cell aggregation and adhesins expression. Our study suggests that the Tpk2p isoform may be part of a mechanism underlying not only polarized pseudohyphal morphogenesis but also cell adherence.

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ST-P11

INVOLVEMENT OF VIMENTIN IN THE REGULATION OF THE ERK CASCADE

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The intermediate filament protein vimentin was identified as an ERK1/2-interacting protein; however, the molecular mechanism by which this protein regulates ERK1/2 is not fully understood. The aim of this study was to investigate the involvement of vimentin in the ERK signaling pathway. We found that knocking down vimentin by siRNA resulted in a reduced ERK1/2 activation by EGF, whereas overexpression of vimentin induced a sustained activation of this MAPK. The phosphorylation of RSK, an ERK1/2 downstream target, was not affected by changes in vimentin expression levels. Using fractionation assay, we also found that reduced vimentin expression retains ERK1/2 in the cytoplasmic fraction. This effect of vimentin was supported by immunocytochemical staining as well. However, coimmunoprecipitation experiments after vimentin silencing or overexpression suggested that this protein does not directly interact with ERK1/2. In conclusion, vimentin affects ERK1/2 activity and translocation of this MAPK into the nucleus. This effect on translocation may be mediated by changes in ERK activity and may be due to vimentin interaction with upstream regulators of the cascade.

ST-P12

***Trypanosoma cruzi*: INVOLVEMENT OF CLASS I PI3K AND NA^+/H^+ EXCHANGER IN cAMP-PKA SIGNALING PATHWAYS**

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Transmission of Chagas disease depends on *T. cruzi* development and differentiation within its insect vector. Different parasite forms have the ability to respond to environmental changes in life cycle through signal transduction pathways. In this regard, earlier studies have indicated a role of cAMP in *T. cruzi* differentiation processes, including activation of protein kinase A (PKA) as regulator of stage differentiation. However, very little is known about the targets proteins for PKA involved in this process. In this work we studied two possible effectors downstream of PKA activated during the transition to intermediate forms -between epimastigote and trypomastigote- of *T. cruzi*: class I phosphatidylinositol 3 kinase (PI3K) and Na^+/H^+ exchanger. The activities of these proteins, containing PKA phosphorylation sites, were regulated by an activator (cAMP non-degradable analog) and an inhibitor (KT5720) of PKA, suggesting a direct relationship between cAMP-mediated processes and activation of both targets. Moreover, the transition to intermediate forms was regulated by LY294002 and EIPA, PI3K and Na^+/H^+ exchanger inhibitors respectively. Taken together, our results suggest involvement of PI3K and Na^+/H^+ exchanger as downstrem targets of cAMP-PKA signaling pathway activated in response to environmental changes that occur in the insect vector where parasite differentiation takes place.

ST-P13

N-TERMINAL DOMAIN OF THE REGULATORY SUBUNIT OF PKA FROM FUNGI IN DIMERIZATION AND TETHERING

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Among fungi both ascomycetes and basidiomycetes have only one isoform of regulatory subunit (R) of PKA, while zygomycetes have four isoforms. A bioinformatic comparison of the divergent amino termini of the R subunits from fungi with those of mammals showed a conserved region with characteristics of dimerization domains (D/D) both in primary and secondary structure. A subphyllum of ascomycetes, exemplified by *Neurospora crassa*, lacked this region and showed to have a monomeric R subunit. We studied in detail the N-terminus from *Saccharomyces cerevisiae* (ascomycete) and *Mucor circinelloides* (zygomycete). In the *S.cerevisiae* dimeric R (Bcy1) subunit the deletion of the D/D yielded a monomeric isoform. A comparison of the D/D region of Bcy1 and the four R subunits from *M. circinelloides* with the D/D domains of RI and RII predicts possible interacting surfaces for these subunits with other proteins. By pull-down assay and mass spectrometry, Bcy1 was shown to interact with several proteins; from these some were selected making theoretical predictions of putative AKAPs domains. Determinants and strength of the interactions with Bcy-1 and the role of the N-terminus in these interactions were assayed by peptide array. The best interactors were Ira2 and Hsp60, being positive charges necessary for high affinity interaction, marking a difference with mammalian AKAPs.

ST-P14

R SUBUNIT ISOFORMS OF PKA FROM *Mucor circinelloides*: DIFFERENT ROLES AND UBIQUITINATION

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In *Mucor circinelloides* PKA is a tetramer composed of two regulatory subunits (R) and two catalytic subunits (C). There are 4 isoforms of R (PKAR1-4) which have differential expression pattern during the fungal growth and differentiation. We found that the apparent molecular weight (MW) of R subunits in SDS-PAGE is higher than the MW estimated from their aminoacidic sequences. By western blot and inmunoprecipitation assay, a 120 KDa ubiquitinated isoform of R was detected, which is associated with kinase and binding activities demonstrated by biochemical analysis. In cAMP-agarosa purifications, the 120 KDa protein was not detected by anti-R antibody suggesting that this modification could be changing its capacity to interact with cAMP, however the ubiquitination does not alter its capacity to interact with C. Another protein of 60 KDa was also detected ubiquitinated and it was identified as the PKAR2 isoform. We concluded that R isoforms are modified post-translationally by ubiquitination, particularly we defined multiubiquitinated high MW isoforms and the monoubiquitination of PKAR2. The ubiquitinated isoform is more abundant in old cultures, suggesting also a differential role during fungal growth. By the construction of knock out strains lacking PKAR1 and PKAR2, we demonstrated that they have different roles in fungal growth and differentiation.

ST-P15

EXPRESSION REGULATION OF C SUBUNIT OF PROTEIN KINASE A FROM *Saccharomyces cerevisiae*

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One mechanism that yeast cells use to protect the internal system from the effects of environmental variation is to initiate a common gene expression program to protect the cell and many of these genes can be related to specific signaling pathways. The aim of this work is to study the expression regulation of two catalytic isoforms of protein kinase A, Tpk1 and Tpk3, in *S.cerevisiae*, under different conditions of stress and carbon source availability. The activity of TPK1 promoter is high in the diauxic-shift, while TPK3 promoter activity remains constant. In the presence of glycerol, both promoters activities are higher than in glucose presence. We demonstrated that TPK1 and TPK3 promoters activities are inhibited by PKA and this regulation is isoform-specific. Under heat shock and osmotic stress conditions, TPK1 promoter activity increases while TPK3 promoter activity remains constant. By *in silico* analysis and using different mutants we demonstrated that Msn2/4, stress transcription factors, regulate positively TPK1 but not TPK3 promoter activity. ChIP assays showed a decreased Msn2 occupancy of TPK1 promoter, during heat shock, but an increase of Pol II occupancy indicating that the promoter was transcriptionally active. These results are in agreement with "black widow" model.

ST-P16

1,25(OH)$_2$D3 INHIBITS GROWTH OF TRANSFORMED ENDOTHELIAL CELLS BY DOWN-REGULATION OF NFkB PATHWAY

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We have previously demonstrated that 1,25(OH)$_2$-Vitamin D3 has antiproliferative effects on the growth of endothelial cells transformed by the viral G protein-coupled receptor associated to Kaposi sarcoma (KSHV-GPCR). In this work, we have investigated whether 1,25(OH)$_2$D3 exerts its growth inhibitory effects by interfering with the NFkB pathway which is activated by vGPCR and serum in endothelial cells (SVEC). Time (4-48 h) and dose (0.1-100 nM) response studies showed that the hormone decreases NFkB and increases IkBa protein levels in SVEC and SVEC-vGPCR cells, the highest response taking place at 16 h and 10 nM 1,25(OH)$_2$D3. Moreover, inhibition of NFkB translocation to the nucleus was observed and occurred by a mechanism independent of NFkB association with vitamin D3 receptor (VDR). IkBa protein synthesis was decreased in presence of cycloheximide, demonstrating that de novo IkBa synthesis is induced by 1,25(OH)$_2$D3. The role of Akt and MAPK pathways on hormone modulation of NFkB was investigated. The results indicated that inhibition of Akt by LY249002 mimics 1,25(OH)$_2$D3 induction of IkBa protein levels without changes on NFkB. Inhibition of MAPKs did not modify protein levels of NFkB or IkBa. Altogether, these results suggest that the antiproliferative effects of 1,25(OH)$_2$D3 on endothelial cells and SVEC transformed by vGPCR occurred by down-regulation of the NFkB pathway.

ST-P17

ROLE OF A FYVE DOMAIN IN A cAMP PHOSPHODIESTERASE INVOLVED IN OSMOREGULATION IN *T. cruzi*

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Intracellular levels of cyclic nucleotide second messengers are regulated predominantly by a large superfamily of phosphodiesterases (PDEs). *Trypanosoma cruzi*, the causative agent of Chagas disease, encodes four different PDE families. One of these PDEs, *T. cruzi* TcrPDEC2, has been previously characterized in our laboratory as a FYVE-domain containing protein. Here, we report a novel role for TcrPDEC2 in osmoregulation in *T. cruzi* and reveal the importance of its FYVE domain. Our data show that treatment of epimastigotes with TcrPDEC2 inhibitors stimulates the recovery of the parasites exposed to hyposmotic stress. In addition, TcrPDEC2 localizes to the contractile vacuole complex, a key organelle involved in osmoregulation. Furthermore, parasites overexpressing a truncated version of TcrPDEC2 without the FYVE domain show a failure in its subcellular localization and a marked decrease in PDE activity. Finally, we have previously characterized a phosphatidylinositol 3 kinase (TcVps34) in *T. cruzi*, which is also involved in osmorregulation and has been reported to interact with TcrPDEC2, and we are currently studying the role of TcVps15, a ser-thr protein kinase related to Vps34. Taking together, these results strengthen the importance of the cAMP signaling in *T. cruzi* and aim to reveal the network of the enzymes involved in the homeostasis in this parasite.

ST-P18

DOWN-REGULATION OF WNT/ ß-CATENIN SIGNALING IN DIABETIC INTESTINE

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Wnt pathway controls diverse biological processes during embryonic development and is required for adult tissue maintenance. In the intestine, the canonical Wnt signaling cascade plays a crucial role in driving the proliferation of epithelial cells. Despite, little is known about this pathway in the muscle layer of normal and diabetic adult intestine.

We examined the Wnt signaling profile in the intestine of an experimental model of diabetes in rodents. The expression of Wnt1 and 3a, as well as the intracellular signal transducers ß-catenin and Tcf-4 was analyzed by quantitative real-time (q)RT-PCR, western blot and immunohistochemical studies. Our analysis showed high expression of these signaling components in normal adult intestine. In normal muscle layer, ß-catenin was localized in the smooth muscle and neuronal cells. Diabetes produced a dramatic fall of the intracellular transducers β-catenin and Tcf-4 with apoptotic process in the myenteric neurons. In addition, the analysis of the Wnt target gene Cx43, also demonstrated a reduced expression of this protein suggesting an altered gap junction coupling.

All findings togheter showed a down-regulation of Wnt/ ß-catenin signaling in diabetic intestinal muscle layer.

NS-P01
EFFECT OF TNFRp55 DEFICIENCY ON THE CIRCADIAN PROFILES OF IMMUNOENDOCRINE SIGNALS
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The biological rhythms in the peripheral clocks are regulated by neuroimmunoendocrine signals proceeding, directly or indirectly, from the master clock in the suprachiasmatic nucleus. TNF is a pleiotropic inflammatory cytokine and exhibits its biological properties upon binding to its cognate membrane receptors TNFRp55 and TNFRp75. Our objective was to assess the impact of TNFRp55 deficiency on the circadian profiles of the rest-activity cycle, IL-17 and progesterone (Pg) levels in C57BL/6 wild type and TNFRp55-/- mice. Circadian locomotor activity was registered under constant darkness during 20 days using the Archron system. IL-17 and Pg levels were determined by ELISA and RIA, respectively. We observed nocturnal activity decreased and desynchronized in the TNRFp55-/- mice. IL-17 levels vary throughout a day in serum, joint, ovary and liver in the wild type strain. Pg also showed a circadian profile in the same tissues except liver. Interestingly TNFRp55 deficiency phase shifted most of those temporal oscillations. These results suggest that TNF would play a role in the circadian modulation of endogenous rhythms through its p55 receptor.

NS-P02
VITAMIN A DEFICIENCY MODIFIES THE CIRCADIAN EXPRESSION OF BDNF AND RC3 IN THE RAT HIPPOCAMPUS
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A role for vitamin A has been established in phenomena related to cognitive function. Some evidence points out retinoids as regulators of clock activity through their nuclear receptors. Objectives: 1) to investigate circadian variation of the brain-derived neurotrophic factor (BDNF), Neurogranin (RC3) and CLOCK genes expression in the rat hippocampus, and 2) to assess the effect of a vitamin A-deficient diet on the clock-controlled locomotor activity and the oscillating patterns of Bdnf, Rc3 and clock genes. Male Holtzman rats from control and vitamin A-deficient (DE) groups were maintained under constant darkness during 10 days before the experiment. Hippocampi were taken every 4 h. Scanning of Bdnf and Rc3 gene regulatory region for RAREs and E-boxes was carried out using MatInspector (Genomatix). Rest-activity cycles were registered using the Archron system. RARá and RXRâ mRNA levels were quantified by Real-time PCR. Bdnf, Rc3, Bmal1 and Per1 transcript levels, were determined by RT-PCR and BMAL1 and PER1 protein levels by immunoblotting. We observed Bdnf and Rc3 expression display a circadian rhythmicity in the rat hippocampus. RXRâ mRNA levels were reduced, nocturnal activity decreased and circadian rhythms of Bdnf and Rc3 expression were attenuated in the DE group following changes in BMAL1 levels.
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NS-P03
ENDOGENOUS RHYTHMS OF ANTIOXIDANT ENZYMES IN HIPPOCAMPUS ARE MODIFIED BY THE VITAMIN A DEFICIENCY
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Daily profiles of GPx and CAT have been reported in different tissues. Some evidence points out retinoids as regulators of clock genes activity through its RAR and RXR receptors. Our objectives were to investigate whether GPx, CAT, and clock genes display a circadian expression pattern in the rat hippocampus and evaluate to which extent vitamin A deficiency (VAD) could modify the circadian profiles of Bmal1, Per1, CAT and GPx in that brain area. Male Holtzman rats from control and vitamin A-deficient groups were maintained under 12h-Light:12h-Dark or 12h-Dark:12h-Dark conditions during 10 days before the experiment. Rest-activity cycles were registered using the Archron system. Hippocampi were taken every 4 or 5 h. Transcript levels of Bmal1, Per1, CAT and GPx were determined by RT-PCR and protein levels by Western blot. Regulatory regions of CAT and GPx genes were scanned for clock- and retinoid-responsive sites using MatInspector (www.genomatix.de). E-box and RARE sites were found on regulatory regions of GPx and CAT genes, which display an endogenously-controlled circadian expression in the rat hippocampus. VAD reduced the nocturnal activity of the rats and modified the circadian rhythmicity of CAT and GPx, probably, by altering BMAL1 and PER1 oscillation.
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NS-P04
THE LIGHT/DOPAMINE RELATIONSHIP ON THE CHICKEN RETINAL ENZYME N-ACETYLSEROTONIN ACTIVITY
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Circadian physiology in the vertebrate retina is regulated by light and diverse signals such as melatonin and dopamine (DA), which act as mutually inhibitory factors. We previously described that chicken retinal ganglion cells (RGCs) contain circadian oscillators synthesizing melatonin with higher levels during the day in antiphase to the nocturnal photoreceptor cell (PRC) rhythm. Here, we investigated the effect of DA on the activity of the melatonin synthesizing enzyme N-Acetylserotonin (AA-NAT) in PRC and RGC preparations. Animals were kept under a 12:12 h light-dark cycle (LD) for 7 days, released to constant darkness (DD) for 24 h and then exposed to light pulses of different durations (30-90 min) during the subjective day (circadian time: CT 3) or night (CT 17.5), or injected with DA (50 nmol/eye) in one eye and vehicle in the other (contralateral eye) at CT 17.5 in DD. A light pulse of 30-60 min at CT 3 or CT 17.5 caused a significant decrease in AA-NAT activity of PRCs while it has no effect on RGC enzyme activity. Likewise the DA treatment in the absence of light significantly inhibited AA-NAT activity in PRCs but not in RGCs. The results strongly suggest that in the chicken retina DA mimics the effect of light on AA-NAT activity of PRCs; however, light and DA have no effect on RGCs implicating that responses seen in these cells are mostly driven by the circadian clock.

NS-P05

EFFECTS OF UNSATURATED FATTY ACIDS ON THE CONFORMATIONAL STATE OF NICOTINIC ACETYLCHOLINE RECEPTOR

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Free fatty acids (FFA) are non-competitive antagonists of the nicotinic acetylcholine receptor (AChR) and their site of action is supposedly located at the lipid-AChR interface, where lipids can be annular or non-annular. It is known that the cis-unsaturated FFA, and not trans-unsaturated FFA, produce conformational modifications in the AChR resting state. Using T. californica receptor-rich membranes, we studied the changes in AChR conformational state generated by differences in the double-bond position of monounsaturated FFA. Using the higher affinity of the fluorescent AChR blocker crystal violet for the desensitized than for the resting state, it was observed that a double bond in positions ù 6 or ù 9 increased the KD values of the AChR in the desensitized state whereas no effect was observed in ù 11 or ù 13. Only FFA with an ù 9 double bond changed the KD values in the resting state. DPH and Laurdan fluorescence studies showed that fluidity increased the most in FFA with ù 9 and ù 11 double bonds and that ù 6 and ù 13 had less effect. Fluorescence resonance energy transfer experiments showed that the FFA with an ù 6 double bond remained as an annular lipid whereas all the others also interact at non-annular sites. Thus, the location of the unsaturated double bond appears to be of critical importance for FFA-AChR interaction.

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NS-P06

PHOSPHATIDYLSERINE MODULATION OF AChR LEVELS

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Nicotinic acetylcholine receptors (AChR) are modulated by their lipid environment. The present study was designed to investigate whether AChR function is affected by plasma membrane phosphatydilserine (PS) levels. For this purpose, a mutant PS-deficient cell line, PSA-3, was produced by stable transfection with cDNAs coding for the adult mouse AChR subunits and a plasmid selecting for geneticin. Total RNA was extracted and RT-PCR reactions were performed to verify complete genomic insertion. [125I]á-bungarotoxin radioligand binding assays and fluorescence microscopy studies were employed to study cell-surface and total expression levels and the functional and pharmacological properties of the AChR. Receptor cell-surface expression in the new PS-deficient cell line was stable and depended on PS levels, being reversibly reduced under PS-deficient conditions. Equilibrium and kinetic [125I]á-bungarotoxin binding properties in PS-deficient cells were the same as in control cells. Centrifugation analysis showed a higher proportion of unassembled AChR in PS-deficient cells, which also exhibited higher levels of internalization than normal cells. In conclusion, cell-surface AChR levels are modulated by PS levels.

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NS-P07

THE ANTICONVULSIVE DRUG OXCARBAZEPINE IS A NICOTINIC ACETYLCHOLINE RECEPTOR CHANNEL BLOCKER

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Oxcarbazepine is an anticonvulsive and mood-stabilizing drug used in the treatment of some forms of epilepsy. Here, we tested the effect of the drug on the ion channel properties of the nicotinic acetylcholine receptor (AChR). Electrophysiological recordings using the single-channel recording patch-clamp technique were used to evaluate AChR function in the presence or absence of different concentrations of oxcarbazepine. The main effects caused by the drug were a concentration-dependent decrease in channel mean open time, an increase in one of the components of the mean burst duration, ôburst, concomitant with a decrease in the duration of the second ôburst component, and the appearance of a new closed-channel component. The duration of the latter remained constant in the range of concentrations tested, although its relative contribution showed concentration-dependent behavior. It is concluded that oxcarbazepine blocks the AChR channel, allowing it to reopen quickly, through a mechanism compatible with that of channel blockers.

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NS-P08

DIACYLGLYCERIDES AFFECT DISTRIBUTION AND BINDING PROPERTIES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR

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The effects of exogenous and endogenously-generated diacylglycerides (DAG) on the density, affinity, distribution and single-channel properties of the nicotinic acetylcholine receptor (AChR) in CHO-K1/A5 cells were evaluated by a combination of techniques. [125I]-á-bungarotoxin ligand binding assays showed that cells incubated in the presence of palmitoyloleoylglycerol and dioctanoylglycerol (DOG) for 30 min to 3 h augmented cell-surface AChR levels, with a concomitant decrease in the affinity for the?toxin. Longer exposures (18-48 h) decreased AChR density to values between 60 and 80% of those found in control cells. The remaining AChRs accumulated at intracellular compartments. Electrophysiological studies revealed that DOG treatment (30 min) decreased the mean open time of the AChR channel. Increasing the intracellular DAG pool with the diacylglycerol kinase inhibitor D5794, alone or applied together with DOG, promoted a 50 and 100% increase of the cell-surface AChR, respectively. In the latter case, a 50% decrease in the intracellular pool of the receptor was also observed. We conclude that exogenous and endogenous DAGs modulate the expression, distribution and single-channel properties of the muscle-type AChR.

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NS-P09

STATISTICAL ANALYSIS OF ACETYLCHOLINE RECEPTOR CLUSTER ORGANIZATION

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The study of protein clustering in cell membranes is constrained when the size of the molecular aggregates is below the resolution of the microscopy used. Classical tests (Ripley, Poisson) only make use of the (x,y) spot coordinates, unresolved clusters being considered a single and dimensionless point. Nor do the tests take cluster size/brightness into account, potentially giving rise to misleading interpretations. The image analysis procedure presented here includes cluster size, extending previous work (Wenz & Barrantes, Biocell 33:150) aimed at establishing whether the membrane organization of the acetylcholine receptor is linked to cytoskeletal integrity. The analysis is based on the frequency distribution of euclidean distances calculated from 3D coordinates [i.e. x,y, brightness] between all pairs of spots in microscopy images. The frequency distributions of control and cytochalasin D and jasplakinolide treated CHO-K1/A5 cells were contrasted with simulated random and nonrandom patterns having similar numbers of spots. Comparison of the frequency distributions of the euclidean distances indicates that cytoskeleton-disrupting drugs affect receptor aggregation at the cell surface and that such effect is related to the size/brightness of the clusters.

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NS-P10

ESTRADIOL INDUCES MEMBRANE LOCALIZATION OF THE ESTROGEN RECEPTOR AND ASSOCIATION WITH SHC IN NEURONS

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Estradiol (E2) has important physiological effects in the central nervous system. Previous studies from our laboratory have demonstrated that E2 induces axonal growth on male hypothalamic neurons by a membrane-initiated mechanism involving Ca2+, PKC and ERK signaling. We have also found that ERá can be detected from the cell exterior as a biotinylated cell-surface protein. To corroborate the presence of ERá in the cell-surface of hypothalamic neurons we studied the membrane distribution of the receptor in intact cells by immuno-electron-microscopy. After 48 h of E2 treatment, male hypothalamic neurons were incubated with anti ERá, labeled with protein A-colloidal gold complex and processed for electron microscopy. Gold-particles adhered on the surface of the plasma membrane and attached to cytoplasmic vesicles membranes were observed, confirming that ERá is expressed on the cell-surface. We also analyze if ERá might mediate effects of E2 by direct association with the Shc adapter protein. We immunoprecipitated Shc protein and detected ERá on Western blots, and found that ERá is associated to Shc in control conditions. In the presence of E2 the amount of ERá co-immunoprecipitated with Shc increases two folds. Together our results suggest that the cell-surface expressed ERá is associated to the adapter protein Shc constituting a possible linkage of rapid E2 action to MAPK activation.

NS-P11

TRANSMEMBRANE DOMAIN SELF-ASSOCIATION OF NEURONAL MEMBRANE GLYCOPROTEIN, M6A

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GPM6A is a stress/antidepressant-responsive gene expressed mainly in neurons of the hippocampus and it is believed to be involved in human depression. This gene is down regulated in both physically and socially stressed animals. M6a is a protein with four transmembrane domains (TMs), two extracellular loops and N- and C- terminal extensions toward the cell cytoplasm. M6a regulates neurite/filopodium outgrowth through an unknown mechanism. It is well known that self-association of membrane proteins is important in the regulation of extra and intracellular signaling pathways. Establishing a possible M6a-homologous interaction in rat hippocampus could give us a clue of how this protein exerts its function. In order to test the possibility that the M6a-TMs may be able to associate with itself we employed the TOXCAT system, which allows to monitor and asses TM-TM interactions in biological membranes. Additionally, to determine if M6a is organized as a monomer or an oligomer chemical crosslinking assays in rat hippocampus homogenate and cell lines were performed. The TOXCAT assay showed that there is self-interaction among all M6a transmembrane domains. Moreover, we found that endogenous M6a shows different oligomerization states. In conclusion, our data suggests that self-association of M6a-TMs could be involved in oligomers formation.

NS-P12

MORPHOLOGICAL AND BIOCHEMICAL CHANGES INDUCED BY ACUTE OXIDATIVE STRESS IN OLIGODENDROCYTES (OLG)

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Aims 1) To characterize the morphological changes induced by reactive oxygen species (ROS) in primary cultures of OLG 2) To analyze the OLG proteome after acute ROS induction 3) To study the role of CRMP2, a protein involved in axonal guidance, in this process. Methods 1) Exposure of primary OLG cultures to 3-nitropropionic acid (3-NP) for 1 h. Detection of ROS and cell viability by fluorescent dyes. Confocal microscopy (CM) of myelin basic protein (MBP) and tubulin expression. Morphological analysis by Branching Index (BI) and intersections at distance from soma 2) Proteomic analysis by 2D-DIGE, trypsin digestion and mass spectrometry. 3) Characterization of CRMP2 isoforms and phosphorylation by Western blot. Cellular expression of CRMP2 by CM. Results 1) 3-NP induced ROS production and reduced the BI and intersections at the periphery, indicating a significant shortening of processes. 2) Proteomic analysis showed increased CRMP2 and vimentin (fold 1.75 and 2.19) and reduced ribosomal protein SA (fold 0.2) in 3-NP treated OLG as compared to control. 3) After 3-NP treatment there was an increase of phosphorylated CRMP2 isoforms and the BI reduction was reverted by Y27632, a Rho kinase inhibitor. Conclusions Rho kinase pathway activation and CRMP2 phosphorylation may be involved in the retraction of OLG peripheral processes induced by acute, non-lethal oxidative stress.

NS-P13

EFFECT OF NITRIC OXIDE ON ASTROCYTIC GLYCOGEN METABOLISM

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In CNS glycogen (G) is localized in astrocytes, where it is metabolized to lactate and delivered to neurons for the supply of energy substrate. Thus, any event affecting G metabolism, will affect the normal function of neurons. Several CNS disorders are characterized by excessive nitric oxide (NO) production. The exposure to NO results in nitrosylation of protein thiols which can alter protein function. It was previously established that S-nitrosoglutathione (GSNO) is a viable intercellular S-nitrosylating agent. In the present work we have studied the effect of NO on astrocytic G metabolism. For this, C6 cells were stimulated for either, G accumulation or G degradation. In the presence of GSNO, both, G accumulation and G synthase activity were inhibited. However S-nitrosylated proteins (PrNOs) were not detected, probably due to antioxidant events preventing nitrosylation. PrSNOs did form and could be detected during G degradation in the presence of GSNO, but degradation was unaffected. These preliminary results points to both, a NO damage dependence upon the cell energetic charge, and G metabolism regulation by NO in the astrocyte. Since astrocyte G plays an important role in supporting neuronal activity, this study would be relevant to those CNS diseases characterized by excessive NO production like multiple sclerosis, stroke, Parkinson's and Alzheimer's diseases.

NS-P14

DNA DAMAGE RESPONSE IN TEMOZOLOMIDE TREATED HUMAN GLIOBLASTOMA CANCER INITIATING CELLS

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Gliobalstoma multiforme (GBM) are aggressive tumors that respond poorly to treatments. GBM are currently treated by surgical removal followed by radiotherapy and chemotherapy with alkylating agents. Temozolomide (TMZ) the most used oral drug is a DNA methylating agent that induces cytotoxicity primarily through the formation of O6-methylguanine lesions. During DNA replication O6-methylguanine mispairs with thymine leading to mismatch repair system activation and subsequent outcomes such as double strand breaks. The aim of this work was the isolation and propagation of glioma cancer initiating cells (GCIC) from surgical samples to study the cellular response towards TMZ. GCIC were isolated following an adherent culture protocol comprising adhesion on laminin and incubation with EGF and bFGF. Initially, we confirmed that after several passages, GCIC displayed the same genomic alterations that the GBM biopsy. Vimentin, Sox2, Nestin and CD44 expression were determined by immunocytochemistry. qPCR analysis of TMZ treated GCIC revealed the induction of ATM,ATR,E2F1 and p21 in a dose dependent manner. By flow cytometry we observed an increase in G1/S population when cells were exposed to TMZ during different times of treatment. The isolation of Individual GCIC lines would provide a powerful system to elucidate the molecular mechanisms responsible for the drug resistance displayed by GBM.

NS-P15

HUMAN EMBRYONIC STEM CELLS DERIVED-NEURONS: A SUITABLE MODEL TO STUDY TOXICITY TRIGGERED BY 6–OHDA

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Human embryonic stem cells (hESC) are an attractive source of cells for use in regenerative medicine. They have unlimited proliferative properties and possess the potential to differentiate into all cell types including dopaminergic neurons (DN). Parkinson's disease (PD) is characterized by the progressive loss of DN in the substantia nigra. Much PD research relies on drug models that mimic the selective DN degeneration that occurs in this disorder. One of the agents widely used for this purpose is the 6-hydroxydopamine (6-OHDA). First, we derived DN from WA09 hESC line cultured according to Zhang, SC protocol. The resultant cells expressed Map2, Tuj1 and TH. Then, we evaluated if these DN were susceptible to 6-OHDA. XTT assays revealed that this neurotoxin induces loss of cell viability. qRT-PCR analysis showed that Bcl-xL and Bcl-2 expression decreased after 24h of 6-OHDA exposure in a dose dependent manner, while Bad and Bax mRNA levels remained unaffected. Importantly, a marked induction of the transcription factors involved in apoptosis regulation, Nur77 and E2F1, was observed in 6-OHDA treated cells. Similar results were obtained with post-mitotic human SH-SY5Y cells possessing many qualities of DN. We propose that the hESC-DN would represent a suitable experimental model for studying the molecular and cellular mechanisms underlying the pathophysiology of PD.

NS-P16

PROLACTIN, THROUGH PERIPHERAL NEURAL PATHWAY, HAS LUTEOLYTIC EFFECT IN LATE PREGNANT RATS

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Prolactin (PRL) performs dual effects (luteotropic and luteolytic) on the different types of corpora lutea. We investigate, using the *ex vivo* celiac ganglion-superior ovarian nerve-ovary (CG-SON-O) system of 21-day pregnant rats, whether PRL via NOS is able to modulate luteal regression. The system was incubated in Krebs Ringer at 37ºC, keeping the CG and the ovary connected by the SON, in separate compartments. PRL (10^{-7}M) was added in the ganglionic compartment (PRL)g, Controls were not stimulated. Periodic extractions of the ovary incubation liquid were carried out at 30, 60, 120, 180 y 240 min to subsequently measure progesterone (P), oestradiol (E2), PGF2á concentrations by RIA and nitrites by the Griess method. At 240 min, the luteal mRNA expression of 3â-HSD, 20á-HSD, aromatase, PGF2áR, iNOS, bcl-2 and bax were analysed by RT-PCR. ANOVA 1 followed by the Tuckey test with a statistical significance of $p < 0.05$ was used. (PRL)g decreased the release of P without affecting the expression of 3â-HSD and 20á-HSD and decreased only the release of E2 at 240 min of incubation, without affecting the expression of aromatase. (PRL)g increased the release of nitrites without affecting the expression of iNOS and increased the release of PGF2á, whose receptor showed a tendency to decrease, while the ratio bcl-2:bax decreased. PRL, via the SON, promotes luteal regression at the end of pregnancy in rats.

PL-P01

ANTIOXIDANT POLYPHENOLS IN PROCESSING POTATO VARIETIES CULTURED IN ARGENTINA

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Plants produce a variety of secondary metabolites with beneficial effects on human health. Compounds such as antioxidantpolyphenols have been detected in native potatoes but there is little knowledge about their levels in processing varieties. QTLassisted breeding allows for the introduction of genotypic features of native potatoes to processing varieties resulting in the increase of levels of beneficial polyphenols in these varieties. In order to optimize breeding, a profound knowledge of the regulation of polyphenol production is required. Previous studies indicate that the production of polyphenols is regulated at both the transcriptio nal and metabolic level. We quantified levels of chlorogenic acid, flavan3ols and anthocyanins as well as the antioxidant capacity in pulp and peel of tubers of 12 processing potato varieties. The content of chlorogenic acid and flavan3ols was higher in peel than in pulp whereas anthocyanins levels were below the detection limit for most varieties. There appeared no direct correlation between the content of polyphenols and antioxidant capacity although also the antioxidant capacity was higher in peel as well as transcript levels of key enzymes involved in the polyphenolbiosynthesis. A comparative analysis between processing and native potatoes will be performed in order to clarify how the metabolism of polyphenols works.

PL-P02

SALICYLIC ACID AS MODULATOR OF Cd TOXICITY IN SOYBEAN PLANTS

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In this study, the possibility of enhancing Cd stress tolerance of soybean plants (*Glycine max* L.) by exogenous application of salicylic acid (SA) was investigated. Salicilic acid was applied in the Hoagland solution at various concentrations ranging from 62 to 500 µM for 48 h. After SA pre-treatment, plants were subjected to 25µM Cd for 5 days. Our results showed that 125 µM SA provided significant protection against Cd compared to controls, diminishing TBARS levels (40%) and increasing GSH content (36%) respect to Cd treated plants.
Pre-treatment with 125 µM SA did no elevate catalase (CAT) activity, but enhanced heme oxygenase-1 (HO-1) (24%) activity and gene expression. These findings indicate that HO-1 acts not only as the rate limiting enzyme in heme catabolism, but also as an antioxidant enzyme. Results show that SA, which is a well known phenolic phytohormone with roles in plant growth and development, could be used effectively to protect soybean plants from the damaging effects of Cd pollution in soils by promoting heme catabolism leading to the production of the highly antioxidant biliverdin and carbon monoxide, without any adverse effect on the plant growth.

PL-P03

MRCV PROTEINS P6, P9-1, P9-2 AND P10 ARE ABLE TO INTERACT WITH COMPONENTS OF THE INSECT CYTOSKELETON

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Mal de Río Cuarto virus (MRCV, *Reoviridae*, *Fijivirus*) causes the most important maize disease in Argentina. The virus multiplies in phloem cells of various grasses as well as in planthoppers that transmit the disease. MRCV genome consists of 10 dsRNA segments that putatively encode for 13 viral proteins, most of which have unknown functions. The detection of interactions between MRCV proteins and those of its hosts will shed light on their role within viral infection. In particular, possible interactions with host cytoskeleton components could unravel mechanisms underlying intracellular and intercellular virus movement. Yeast two-hybrid (Y2H) assays revealed than MRCV P6, P9-1, P9-2 and P10 were able to interact with *Spodoptera frugiperda* (Sf9) actin and tubulin proteins. Bioinformatics analysis suggested that P6 contains a coiled-coil motif with homology to the *Myosin tail 1* domain family. P9-1 is the major component of the viroplasm and P10 is the major outer capsid protein. Next we established that P6 and P10 co-localized with tubulin upon transfection assays of Sf9 insect cells. Interestingly, P6 and P10 as well co-localized in this system. In the context of the Wheat Interactome Project directed by Jorge Dubcovsky (UC Davies), we are currently evaluating the interactions of MRCV proteins with a wheat protein Y2H library.

PL-P04

STUDY OF THE ROLE OF A HARPIN FROM *Xanthomonas axonopodis* pv. *citri* IN CITRUS CANKER

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Phytopathogenic bacteria colonize their hosts through the secretion of effector proteins by using the Type III Secretion System. This system is encoded by the *hrp* cluster (*h*ypersensitive *r*esponse (HR) and *p*athogenicity), which is essential for pathogenicity in host plants and induction of HR in non-host plants since it mediates the translocation of effector proteins to the plant cell. HR is characterized by a local rapid programmed cell death that is induced after recognition of the pathogen and slows the spread of infection. The *hrp* cluster of *Xanthomonas axonopodis* pv. *citri* (Xac) contains a gene that codes for a harpin protein called Hpa1. Harpins are glycine-rich heat stable proteins that can form pores in membranes and can induce HR in some plants. In order to study the functionality of Hpa1 protein from Xac, it was expressed and purified in a recombinant form. We observed that Hpa1 produces HR in tobacco and pepper plants. To study the effect of Hpa1 during the disease we constructed a Xac Δhpa1 mutant by marker exchange. Infiltration of this mutant caused less number of cankers relative to the wild type bacteria. Moreover, co/infiltrations of Xac WT with the recombinant protein also produced a greater number of cankers in the presence of the protein. These results indicate that Hpa1 is involved in the elicitation of HR in non-host plants as well as in virulence in host plants.

PL-P05

RELATIONSHIP BETWEEN STRESS TOLERANCE AND FLAVODOXIN LEVELS IN TRANSGENIC TOBACCO PLANTS

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Ferredoxins (Fd) are iron-sulfur proteins involved in different one-electron transfer pathways. Fd levels decrease under adverse environmental conditions in photosynthetic organisms. In cyanobacteria, this decline is compensated by induction of flavodoxin (Fld), an isofunctional flavoprotein. Fld is absent in higher plants but transgenic *Nicotiana tabacum* lines accumulating *Anabaena* Fld in plastids displayed increased tolerance to oxidative stress. As the tolerance degree was correlated with Fld dose in plastids, the aim of this work is to evaluate whether the protective effect of Fld saturates above certain threshold of Fld content, if it continues to improve or if Fld overaccumulation becomes detrimental due to perturbation of redox homeostasis. Therefore, we prepared tobacco plants expressing Fld by transplastomic. Photosynthetic performance of plants with different Fld doses was evaluated under normal growth conditions. To estimate stress tolerance, these plants were exposed to methyl viologen, an herbicide which generates oxidative stress in chloroplasts. Photosynthesis efficiency, antioxidant and chlorophyll levels, and cellular damage were determined. Highest stress tolerance was observed in plants expressing 70 pmol Fld/g fw. Overaccumulation of Fld did not improve its protective effect; however, it did not affect negatively photosynthetic and metabolic performance.

PL-P06

ROLE OF AUXIN-AMIDOHYDROLASES IN SOLANACEAE PLANTS

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Auxins regulate plant development, growth and responses to the environment. A cDNA clone coding for *StIAR3*, an auxin-conjugate amidohydrolase, was isolated from a potato library in our laboratory. *StIAR3* is up-regulated upon different biotic stress suggesting its role in plant-pathogen interactions. In order to analyze the involvement of *StIAR3* in the resistance mechanism of *Nicotiana benthamiana* plants we carried out a functional genomic strategy using Virus-Induced Gene Silencing (VIGS) to knock-down amidohydrolase gene expression. Phenotypical analysis evidenced an altered growth pattern in *IAR3* silenced plants. It was correlated with reduced *StIAR3* transcript levels measured by RT-PCR. In addition, bioassays performed with *Phytophthora infestans* were carried out in order to evaluate the hypersensitive response (HR) in silenced and control plants. A differential HR susceptibility was evidenced 5 days after inoculation by examining visible necrotic spots between non-silenced and silenced tobacco leaves. These results will allow us to evaluate the impact of amidohydrolases and auxin balance in the plant defense response to fungal pathogens.
Supported by UNMdP and CONICET.

PL-P07

REGULATION OF PUTATIVE P1 TARGETS IN LEAVES OF HIGH ALTITUDE MAIZE LANDRACES UNDER UV-B CONDITIONS

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P1 is a R2R3-MYB transcription factor that regulates the accumulation of a specific group of flavonoids in maize floral tissues; among them the flavones and phlobaphenes. At present, only a few genes that encode for enzymes in the last steps of flavonoid biosynthesis have been demonstrated to be P1 targets. Recently, RNA-Seq have identified 777 genes significantly higher in red maize pericarps expressing the P1-rr allele, than in white P1-ww pericarps. This list includes all known P1 regulated genes, as well as other new putative targets. In this list, a number of transcription factors, enzymes of the cell wall biosynthesis and other pathways were identified. In this work, we have analyzed the transcriptional regulation of these genes by UV-B in leaf tissues of different maize high altitude landraces that express different P1 alleles that are expressed in leaves and are also UV-B regulated, and in a low altitude line (W23 b, pl) as a control. Our results show an induction of almost all the analyzed genes in the different lines by UV-B. Also, in the high altitude landraces, some transcripts have already higher levels in the absence of UV-B than in the W23 line (e.g. RHS1, PAL3, JAC1). In conclusion, P1 is probably involved in an overall adaptive response to UV-B radiation in maize leaves, regulating the expression different targets besides the well characterized flavonoid pathway.

PL-P08

BEHAVIOR OF A GERMIN-LIKE PROTEASE INHIBITOR IN WHEAT SEEDLINGS UNDER SALT STRESS

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Abiotic stress causes morphological and biochemical changes in plants. Previously, we isolated from the wheat leaf intercellular fluid (IF) a protein belonging to the Germin-like protein family with protease inhibitor activity (GLPI), which takes part in the defense mechanisms against septoriosis. Subsequently, we detected that it also has both superoxide dismutase (SOD) and phosphodiesterase activities. This work evaluated the behavior of GLPI on wheat tolerance against salt stress. For this, wheat seedlings were grown under several NaCl concentrations. The first leaf displayed a normal content of GLPI in FI, but increased in the cell wall. Conversely, GLPI decreased in the FI of the second leaf, but remained unchanged in the cell wall. The activities of both SOD and GLPI accompanied these changes. Also, salt stress did not significantly change the size of the first leaf, but caused an increase in the content of both chlorophyll and free proline. In contrast, the size, fresh weight, and chlorophyll content decreased in the second leaf. These results suggest that wheat plants have several mechanisms to provide tolerance to salt stress, including location of GLPI into the cell wall.
Supported by CONICET and UNMdP.

PL-P09

CASPASE- 3 LIKE ACTIVITY IN *Solanum tuberosum-P. infestans* INTERACTION

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Caspases are key components in animal programmed cell death. In the last decade, caspase like activities were described during different plant developmental and defense responses. Here we present a new plant-pathogen system where caspase-3 like activity was detected. Protein extracts from *Solanum tuberosum* leaves infected with *Phytophthora infestans* for 0, 1, 6, 12, 24 and 48 h were prepared. Higher relative DEVDase activity was detected at pH 5,2 and 37ºC after 24 h of *P. infestans* infection. This activity was significantly inhibited (85%) when extracts were pre-incubated for 1 h with specific DEVDase inhibitor (Ac-DEVD-CHO 50 µM). General protease and caspase inhibitors had no effect (2,5 mM PMSF, 100 µM E-64, 10 µM E-64, 0,5 mM Pepstatin A and 50 µM Z-VAD-FMK). An *in silico* prediction analysis of potential caspase-like cleavage site (DEVD) restricted to *S. tuberosum* revealed 7 predicted proteins in GenPep, 16 in TrEMBL and 1 in Swiss Prot databases with at least one DEVD motif. Most of these proteins correspond to apoptosis related proteins and transcription factors. Finally, this DEVDase activity appears to be related to animal caspases, since it is insensitive to broad-range general protease inhibitors. However, in contrast to animal caspase 3 which is active at pH 7, DEVDase ativity was active at acidic pH, possibly suggesting a vacuolar or mitochondrial intermembrane localization.

PL-P10

PLPKI AS A POTENTIAL BIOCHEMICAL MARKER INVOLVED IN HORIZONTAL RESISTANCE TO *P. infestans*

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Late Blight (LB) is the main disease that affects potatoes around the world. Due to the unavailability of an efficient chemical control method, the use of genetic resistance to LB represents a safe strategy. Two forms of genetic resistance to *Phytophthora infestans* (Pi) in potato species have been described, vertical resistance (VR) or horizontal resistance (HR). VR is characterized by interactions between products of dominant R genes in the host and corresponding avirulence genes in the pathogen. In contrast, HR is assumed to be multiple gene-based. This type of resistance is durable and thus commercially more attractive than VR. In our lab, we have isolated and characterized a serine proteinase inhibitor specific for proteinase K (PLPKI) which was induced in potato leaves of a cultivar with a high degree of HR upon infection with Pi. The aim of the present work was to establish a correlation between the degree of HR with PLPKI accumulation in *Solanum tarijense* clones (*tjr*), which are part of the Argentinean Potato Breeding Program. Both total proteinase K inhibitor activity and western blot analysis with an antibody raised against PLPKI showed the highest accumulation of PLPKI in a *tjr* clone which has been described as highly resistant. These results suggest that PLPKI can be potentially used as a biochemical marker to assist breeders in the selection of HR cultivars.

PL-P11

CATALASE RESPONSE IN ROOTS OF WHEAT PLANTS SUBJECTED TO ABIOTIC STRESS FACTORS

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Adverse environmental growth conditions are related to oxidative stress production in plant cells. Catalase, CAT, (H_2O_2: H_2O_2 oxidoreductase; EC 1.11.1.6) is one of the most relevant antioxidant enzymes. It is found in all aerobic organisms and catalyses the dismutation of H_2O_2 to O_2 and H_2O. In *Triticum aestivum* (wheat) two genes codes for this enzyme: *cat1* and *cat2*. In the present work, CAT activity, isoforms and gene expression were studied in wheat roots under diverse abiotic stressors. Treatments with 1 and 10 µM $CdCl_2$, 0,5 µM paraquat (PQ), 1 mM H_2O_2 and water deprivation (22% polyethylene glycol) were performed using a floating culture system. Cd^{2+}, PQ and H_2O_2 decreased specific CAT activity (15, 20, 26 and 10% for 1 and 10 µM Cd^{2+}, 0,5 µM PQ, 1 mM H_2O_2 respectively, respect to control values). On the other hand, water deficit increased absolute CAT activity, but this rise was related to an increment in root protein content. Two bands of CAT activity, corresponding to different isoforms, were detected in native PAGE. The number of CAT isoforms did not change in treated roots respect to control. Nevertheless, *cat1* and *cat2* gene expression were modified by treatments. Taken together, all these results indicated not only that catalase is an enzyme sensitive to abiotic stress factors but also its has a complex regulation that depended on the type of stress.

PL-P12

THE ROLE OF ARABIDOPSIS COX ASSEMBLY FACTORS IN COPPER HOMEOSTASIS AND STRESS RESPONSES

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Cytochrome c oxidase (CcO) requires copper (Cu) as a cofactor for its activity. Recent findings have proposed that mitochondrial proteins involved in CcO assembly may have additional functions in plants. In this work, we overexpressed (OE) three CcO assembly factors, COX17-1, COX17-2 and COX19, to investigate their role in Cu homeostasis and the oxidative response. The expression of miR398 and Cu transporters was increased in OECox17-2 and OECox19, suggesting the existence of a Cu-deficiency response, although Cu levels were not significantly modified. Plants showed a decrease in the number and size of rosette leaves, in cell size and in plant height, inhibition of root growth and a delayed germination time. Some of these phenotypes were reversed by the inclusion of Cu in the growth medium. Total dark respiration was similar to wild-type, but the alternative respiratory pathway activity and transcript levels of the alternative oxidase gene AOX1a were increased in OECox17-2. OECox19 and OECox17-1 showed a significant decrease in transcript levels of BCS1, encoding a mitochondrial protein responsive to oxidative stress. Altered transcriptional responses of AOX1a, BCS1 and the ferritin gene FER1 were observed under stress conditions. The results suggest that Cox17-2 and Cox19 participate in Cu homeostasis and that the three proteins influence the response of the plant to stress.

PL-P15

THERMOSPERMINE CAN PERFORM SIMILAR FUNCTIONS TO SPERMINE IN *A. thaliana* DEFENSE AGAINST PATHOGENS

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Polyamines (PAs) are natural aliphatic polycations with multiple functions. Previously, our group demonstrated that the accumulation and oxidation of the common tetraamine spermine (Spm) protect plants against the biotrophic bacterium *Pseudomonas viridiflava*. In this work, we analyzed the functional specificity of Spm and its structural isomer, the uncommon thermospermine (TSpm). The ability of *P. viridiflava* to colonize *A. thaliana* plants harboring mutations in the Spm and TSpm biosynthetic genes (*ACL5* and *SPMS*) was compared to wild type plants (WT). Both mutants showed increased colonization by *P. viridiflava*, which was reverted by TSpm addition. The patterns of global changes in gene expression induced by Spm and TSpm were analyzed by transcript profiling of transgenic plants that constitutively over-express *ACL5* and *SPMS*, which were previously reported to be more resistant to *P. viridiflava* than WT. Genes whose expression was significantly changed in both transgenic lines, exhibited an enrichment in ontology groups related to pathogen perception and defense responses. It can be concluded that TSpm can, at least partly, play similar roles to those of Spm in plant defense. Further analysis of the relationship between the differential localization of ACL5 and SPMS and their roles in plant defense will increase our understanding of the physiological functions of TSpm and Spm.

PL-P14

TRANSCRIPTOME ANALYSIS OF *Portulaca oleracea* UNDER DROUGHT STRESS CONDITIONS

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Drought stress is undoubtedly one of the environmental types of stress that more limits growth and crop yields, producing a 50% reduction in the performance of the main crops. Therefore, it is very important the identification of new elements involved in the adaptation to this type of stress. *Portulaca oleracea* is a C4 plant that adapts to drought stress by changing its photosynthetic metabolism. In the present work, the "differential display" technique was used to identify transcripts showing changes in their expression in leaves of plants under the following conditions: well-watered, after withholding water during 21 days and after 21 days of re-watering. Two-hundred and two differentially expressed transcripts were obtained of which 53% were induced under stress conditions, the 25 % were repressed by drought and 22% were differentially expressed only after re-watering. Eighty percent of the identified sequences corresponded to unigenes. Half of these sequences corresponded to unknown sequences. The results obtained in the present work offer the possibility to identify new molecular factors involved in the adaptation to drought stress conditions.

PL-P16

TRANSCRIPTION REPROGRAMMING IN *Lotus japonicus* PLANTS UNDER FOLIAR INFECTION BY *Pseudomonas syringae*

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Legume plants have the ability to establish symbiotic interactions with rhizobia bacteria and mycorrhizal fungi, a trait that make them able to growth in poor soils. It is known that, at least partially, the development of symbiosis is regulated by plant defense genes that are equally activated by natural pathogens. Therefore, the identification of these genes is an interesting technological aim. Unfortunately, the molecular mechanisms that participate in the defense of legume plants to pathogens remain still unclear. To add more light to this field, the main aim of this work was to characterize the transcriptomic changes that occur in both, susceptible and resistant ecotypes of the model legume *L. japonicus* upon the infection caused by *P. syringae*. We present here an optimized infection protocol and our first results of an Affymetrix Gene chip analysis on a susceptible cultivar. Our experiments showed a notable transcription reprogramming in infected leaves, where most of the down-regulated genes correspond to those related to photosynthesis and starch metabolism. On the other hand, in the up-regulated group those responsible for flavonoid biosynthesis are predominant (mostly chalcones and anthocyanins). In addition, we identified several transcription factors that could play an important role in plant defense. The similarities with previously published reports are discussed.

PL-P17

EXPRESSION PROFILE ANALYSIS OF MALIC ENZYME TRANSCRIPTS DURING DIFFERENT STRESS CONDITION IN MAIZE

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The *Zea mays* genome contains seven genes encoding putative Malic enzymes (ME): two plastidic NADP-MEs (ZmME1 and ZmME2); three cytosolic NADP-MEs (ZmME3, ZmME4 and ZmME7); and two mitochondrial NAD-MEs (ZmME5 and ZmME6). To elucidate the role of each ME, maize plants were exposed to different stress conditions, like UV light; root incubation with Na_2CO_3 pH 8 or 11; or cellulose. After the treatments, the expression level of each ME transcript, along with ME activity, were analyzed. After UV treatment, a five-time increased expression of ZmME1 was found. Meanwhile, a repression of two-times was observed for ZmME3. ZmME2 increased three-times in leaves by incubation with Na_2CO_3 pH 8, while it was repressed two-times in roots. At pH 11, this gene was induced 4-times in roots but repressed in the same level in stems. The gene for ZmME7 was repressed five-times at pH 8. Root incubation with cellulose, increased 4-times ZmME4 and ZmME7. Finally, the transcripts encoding NAD-ME (ZmME5 and ZmME6) did not significantly changed in any of the treatments evaluated, suggesting that mitochondrial NAD-ME might be involved in metabolic pathways not related with these type of stress. Overall, the results obtained indicate that each ME fulfils particular roles in vivo, probably related to the specific kinetic properties and metabolic regulation of each isoform.

PL-P18

CHARACTERIZATION OF ACID PEPTIDASES SECRETED BY PLANT-PATHOGENIC FUNGUS *Botrytis cinerea*

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Botrytis cinerea causes gray mold, a devastating disease of various plants of economic interest. This necrotroph grows at moderate temperatures but also as low as 4°C at which crops are stored. Infection with *B. cinerea* depends on cell wall degrading enzymes such as endopolygalacturonases (ten Have et al., 1998). Cell walls also contain protein, hence peptidases might also contribute to virulence. *B. cinerea* secretes aspartic peptidases (APs, Espino et al., 2010; ten Have et al., 2004) which act at low pH values, in correspondence with the low pH reported during *B. cinerea* infection (van Kan, 2006). Espino also reported a secreted glutamic peptidase (GP) and six secreted sedolisins. It is not known how these enzymes contribute to the secreted peptidase activity. We used a BcAP8 knock-out mutant, which shows 80% reduction in secreted peptidase activity to study the spectrum of other peptidase activities. We detected an inducible GP activity and data suggest the presence of a sedolisin activity. *In planta* analysis showed mostly AP activity and finally we show AP activity at low temperatures.

PL-P19

STRUCTURAL ALTERATION OF PLANT CELL WALL IN TRANSGENIC *Arabidopsis thaliana*

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Carbohydrate-binding modules (CBM) are non-catalytic domains, classified in 59 families, which are found being part of non-catalytic proteins, transport proteins and enzymes involved in polysaccharide metabolism (i.e. the dynamic plant cell wall metabolism). The SBD123 (Starch Binding Domain) from the N-term region of starch synthase III from *A. thaliana* was classified into the CBM53 family and this was the first CBM described in a biosynthetic enzyme. In vitro adsorption assays show that SBD123 has a promiscuous binding to starch and to other plant cell wall polysaccharides such as xylane, pectin and cellulose. Considering the evolutionary advantage of SBDs to posses two binding sites in regard to the rest of the CBMs, we decided to evaluate this behavior in vivo. Thus, we carried out the expression of de SBD123 protein targeted to the plant cell wall by the transformation of *A. thaliana* plants with a chimeric construct containing the signal peptide of Expansin 8 from *A. thaliana* fused to SBD123. The phenotype of transgenic plants was evaluated by transcriptomic, metabolomic and fluorescence confocal microscopy. We found a decrease of cell wall polysaccharide content with alterations in cell wall integrity, decrease of xylose and galactose (hemicellulose compounds) and increased mRNA levels for CESA 4, 7, 8 (involved in secondary cell wall synthesis) compared to wild type plants.

PL-P20

COMPLEMENTATION OF LETHAL CONDITIONAL MUTANT YEAST BY RPL10 FROM *Arabidopsis thaliana*

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Ribosomal protein L10 (RPL10) is a key factor in joining the 40S and 60S ribosomal subunits into a functional 80S ribosome. In yeast, a mutation in *Rpl10* demonstrated that it is essential for viability. The *Arabidopsis* genome contains three *RPL10* genes encoding RPL10A-C. Previously, we showed that *A. thaliana RPL10*s are not functionally equivalent, being involved in plant development, translation and UV-B response. On the basis of the high degree of amino acid sequence identity between *Arabidopsis* and yeast RPL10s (67%); the objective of this work was to analyze the ability of AtRPL10s to complement a yeast lethal conditional mutant. We used the AJY2104 strain where *RPL10* is expressed under a GAL1 promoter, consequently, this strain is unviable under repressing condition on glucose unless a functional *RPL10* is episomally provided. First, cDNAs encoding to *At*RPL10A-C from *A. thaliana* were cloned under control of a constitutive GDP promoter. After transformation with these constructs, complementation was evaluated. Serial dilutions of the *AtRPL10*s expressed in AJY2104 (*GAL1::RPL10*) showed growth onto selective plates under repressing condition indicating complementation by *At*RPL10s. This result was also supported by growth assay in liquid medium. Future studies of polysome profiles will allow analyzing the 60S biogenesis and translation in this complemented strain.

PL-P21

ROLE OF POLYAMINES IN THE MODULATION OF NADPH OXIDASE AND CATALASE IN *Nicotiana tabacum* LEAVES

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Polyamines (PAs) are molecules implicated in plant growth, development and cell death. We had observed that 1mM putrescine (Put), spermidine (Spd) or spermine (Spm) increased O_2^- and H_2O_2 and cell death in tobacco leaves. The aim of this work was the study of NADPH oxidase involvement in O_2^- generation and H_2O_2 removal by catalase (CAT). Enzymatic activity, protein and gene expression of cat1 and Ntrboh genes were studied. Leaf discs of SR1 tobacco plants were treated for 2 and 24 h with 0.1mM or 1mM Put, Spm or Spd. All PA concentrations increased NADPH oxidase expression at 2 h, but decreased it at 24 h. The enzymatic activity decreased between 50% and 80%, with both concentrations of the 3 PAs at both times. CAT gene expression increased at 2h with 1mM PAs, but decreased at 24 h. PAs diminished CAT activity at 2h, but protein expression raised over 50% and 30% with 1mM Put and 0.1 mM Spm, respectively, and it was significantly reduced at 24 h by Spd and Spm. These results show that at short times, the 3 PAs had a positive modulation of gene expression but a negative effect on both enzymatic activities. This suggests a post-transcriptional regulation of the enzymatic activity or a direct action over the enzyme. At long times, 1 mM Spm and Put had opposite effects, suggesting that ROS generation and detoxification in tobacco could be regulated in a different way for each PA.

PL-P22

INSIGHTS INTO THE HSP100-TRANSITE PEPTIDE RECOGNITION PROCESS

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Molecular chaperones of the Hsp100 family have been identified in all kingdoms of life. It has been proposed that these proteins play a key role in protein folding assistance, disaggregation, proteolysis and precursor import into chloroplasts. Some of these activities have been previously observed investigating an Hsp100 form *E. coli* called ClpA. However, the role of Hsp100 in chloroplast import has not been elucidated. It has been postulated that a crucial step in protein translocation into plastids requires the binding of an Hsp100 to an amino-terminal region of the protein called transit peptide (TP). However, evidences of Hsp100-TP interaction are lacking. The goal of this work is to demonstrate this interaction and gain further insight into its nature, using the TP of pea ferredoxin NADP+ reductase (FNR) as a model and two Hsp100 chaperons from *A. thaliana* (ClpC2 and ClpD). TP fused to the GST N-terminus interacts with both plant chaperones. On the contrary, fusions in which the TP was placed at the carboy-terminus of GST or between GST and FNR were not recognized by the chaperones. Moreover, evidences were obtained indicating that interaction with the full transit peptide was lower than a shorter version of this peptide. Our results show for the first time in vitro evidence for the interaction between a TP and an Hsp100 from *A. Thaliana*.

PL-P23

STRUCTURAL AND FUNCTIONAL ANALYSIS OF ADAPTOR PROTEINS OF THE PLANT PROTEOLYTIC Clp SYSTEM

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In chloroplasts, proteome homeostasis is regulated by a network of proteases, molecular chaperones and regulatory proteins. In particular, the tetradecameric Clp proteolytic complex, in assistance with Hsp100 chaperones, selects, disaggregates and unfolds proteins, which can then be re-folded or directed to proteolytic degradation. Little is known about the function of the Clp proteasome. In *Escherichia coli*, the protein ClpS associates with the bacterial Clp complex and modulates its proteolytic activity. In *Arabidopsis thaliana*, three proteins (ClpT1/2 and ClpS) were proposed to play this role. We characterized these proteins *in vitro* and studied their interaction with Hsp100 chaperones. All proteins were expressed in recombinant *E. coli* cells and purified to homogeneity. Since Clp proteins are usually oligomeric, we analyzed their assembly status by size exclusion chromatography. ClpT1 is a monomeric protein in the absence or presence of 5 mM ATP. Circular dichroism spectroscopy indicates that the protein is properly folded and that its thermal stability is rather low, with an unfolding transition at 45 ºC. ClpT1 stimulates the ATPase activity of the Hsp100 proteins ClpC2 and ClpD. Our results represent the first evidence of the regulatory function of ClpT1 on Hsp100 chaperones and will contribute to elucidate the poorly known regulatory system of the Clp complex from plants.

PL-P24

HETEROLOGOUS EXPRESSION OF A MULTIFUNCTIONAL WHEAT PROTEIN

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On the last years, increasing and detailed information about protein structure and function give place to the concept of multifunctionality on proteins. Germins and Germin Like Proteins (GLPs) constitute a large and highly diverse family of ubiquitous plant proteins. A wide range of activities is found among Germins and GLPs such as seed storage, sugar and hormone binding and structural protein. A remarkable biochemical attribute of GLPs is their resistance to desiccation, extreme pH, denaturalization and proteolysis. Previous studies at our lab showed an apoplastic GLP from wheat leaf, which has been characterized as SOD, AGPPase, and protease inhibitor (PI). The multifunctional protein was named after his later activity as GLPI and was also involved on plant pathogen defense mechanisms. Comparative genomic analysis of the GLPI sequence placed it on the subfamily 2 of GLPs, along barley GLPa2. Aiming to generate tools for the study of the structure and biochemical activities of GLPI, we expressed recombinant GLPI (rGLPI) fused to a N-terminal His tag. Bacterial rGLPI was affinity purified and showed PI activity over commercial trypsin on zymograms. These results indicated that active GLPI could be heterologously expressed and open the possibility of using it as molecular tool for structural and biochemical studies of protein multifunctionality.

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PL-P25
CHARACTERIZATION OF FRATAXIN HOMOLOGS IN PLANTS
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Frataxin is a highly conserved protein from bacteria to mammals that has been proposed to participate in iron-sulfur cluster assembly, mitochondrial iron homeostasis and defense against oxidative stress. In eukaryotes, frataxin is encoded in the nucleus but has mitochondrial localization. Recently, we reported the presence of a single gene that codify this protein in *Arabidopsis thaliana* (AtFH); while three different genes has been predicted to codify frataxin isoforms in maize (ZmFH EU968030, BT040175 y BT055005). We cloned and characterized two of these isoforms (EU968030 and BT055005). Our results indicate that these isoforms are functional and suggest that both have a similar role in maize. We also found that frataxin homologs interact with the mitochondrial cysteine desulfurase AtNfs1 using a pull-down assay. In addition, frataxin increases the catalytic efficiency of AtNfs1, suggesting a direct role of frataxin in the modulation of sulfide transfer into the scaffold proteins Isu and HscB.

PL-P26
REDOX REGULATION OF GLUCITOL DEHYDROGENASE FROM PEACH FRUITS
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Glucitol (Gol) is a major photosynthate in plants from the Rosaceae family. This polyol is synthesized in mature leaves and then translocated to heterotrophic tissues, such as fruits and young leaves, where it is converted into fructose in a reaction catalyzed by Gol dehydrogenase (GolDHase, EC 1.1.1.14). Despite the importance of this enzyme for carbon partitioning in these plants, little is known about its regulation. Therefore, we cloned the gene encoding for GolDHase from peach (*Prunus persica*) fruits (*Ppe*GolDHase) and expressed it in *Escherichia coli* cells. Kinetic parameters obtained with the recombinant protein were similar to those reported for the enzyme purified from apple and pear fruits. *Ppe*GolDHase was inhibited by Cu^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+}, with $I_{0.5}$ of 0.031, 0.096, 15.8 and 43.9 □M, respectively. Loss of activity was observed when *Ppe*GolDHase was incubated with diamide, H_2O_2 and oxidized glutathione (*k*'' values were 12.3, 6.93 and 0.086 $M^{-1}s^{-1}$, respectively). Interestingly, the activity of the oxidized enzyme was recovered by reduction with thioredoxin (from *E. coli* and wheat leaf) and reduced glutathione. Considering that Gol accumulation has been related with abiotic stress tolerance, we hypothesized that *Ppe*GolDHase could be inhibited under oxidative stress, thus maintaining levels of Gol high enough to exert a protective role as a hydroxyl-radical scavenger.

PL-P27
DISSECTING THE INTERACTION DETERMINANTS BETWEEN BSL PROTEIN PHOSPHATASES
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BSL Ser/Thr phosphatases belong to a novel family of PPP phosphatases found in all green algae, land plants and alveolates. BSL phosphatases are bimodular proteins with a beta-propeller domain at the N-terminus and a conserved catalytic domain at the C-terminus. We previously reported that these proteins are able to interact with themselves, and that this interaction is common to all the members of the family in Arabidopsis and broadly dissected its requirements. We have narrowed down the region necessary for the interaction to a stretch of about 60 aminoacids in a conserved sequence outside the catalytic domain. We are currently exploring the structural features of this region. We have also shown, through different approaches, that this interaction takes place in vivo and with similar requirements. Having found that the homologs from the moss *Physcomitrella patens* and the unicellular green alga *Chlamydomonas reinhardtii* behave in a similar way, we have now extended this analysis to a model alveolate, the unicellular apicomplexan *Toxoplasma gondii*. The ability to interact with themselves is an unusual and novel feature among Ser/Thr phosphatases. We are presently exploring the regulatory implications on the activity.

PL-P28
DETECTION OF A CYSTEINE PROENZYME IN THE *Vasconcellea quercifolia* LATEX
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The enzymes belonging to the C1A subfamily are synthesized as inactive precursors, with N-terminal propeptides. The propeptides contain characteristic elements which are highly conserved, as the ERFNIN motif and the GXNXFXD heptapeptide. From the latex of *Vasconcellea quercifolia* were isolated by ion exchange chromatography seven fractions containing cysteine peptidases, one of they showed an additional band of 31 kDa by SDS-PAGE suggesting the presence of the proenzyme. This fraction was incubated for 10 min at 60ºC in buffer pH 4.0, with and without pepsine and in buffer pH 8.5; finally the activity was determined using casein as substrate. The results showed that when the fraction was incubated at pH 8.5 or with pepsin, the caseinolytic activity was increased by 40% and 30% over the control. Additionally, the SDS-PAGE analysis showed an increase of the band corresponding to the mature fraction after such treatments. Both results revealed the presence in the latex of a propeptide that can be removed by proteolytic action. This fact was confirmed by the detection of a peptide of 21 amino acids: KNNSYWLGLNVFADMSNDEFK (containing the GXNXFXD heptapeptide), when the tryptic PMF (peptide mass fingerprinting) of the band was compared with the *in silico* tryptic PMF of papain. Proforms (proenzymes) of proteinases had not been purified from plant latex up to date.

PL-P29

A YEAST SYSTEM TO STUDY THE ROLE OF *Arabidopsis thaliana* MutSγ HETERODIMER

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The mismatch repair system (MMR) is a highly conserved DNA repair pathway essential for the correct maintenance of genetic information across many generations. The first step of the pathway involves recognition of the mismatch by MutS homodimers in bacteria or MutS homologs (MSH) heterodimers in eukaryotes (MutSα and MutSβ). Higher plants contain an additional heterodimer, named MutSγ (MSH2-MSH7). To analyze the specific function of this heterodimer from *A. thaliana*, we expressed MSH2 and MSH7 both individually and as a complex in *Saccharomyces cerevisiae*. Strains used include E134 and its mutant derivative lacking msh2 (DAG60). Both strains share the same genetic background and contain three reporter genes to estimate mutation rates. The loci lys2::InsE and his7-2 are frameshift reversion reporters, while the CAN1 locus scores a broad variety of forward mutations. Our results indicate that expression of the individual AtMSH2 or AtMSH7 in both strains did not affect the mutation rates of the examined reporter genes. However, the co-expression of both subunits in strain E134 led to 4-fold increase in the mutation rate of the CAN1 reporter. These data suggest that AtMSH2 cannot complement the yeast msh2 disruption, and that AtMutSγ negatively affects yeast MMR function, with the exception of frasmeshift lesions.

PL-P30

INACTIVATION OF YEAST DNA MISMATCH REPAIR BY EXPRESSION OF PLANT PMS1 PROTEIN

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Highly conserved post-replicative mismatch repair system is crucial for maintaining genomic stability in all organisms. The main proteins involved in this DNA repair pathway include MutS and MutL homologues. Among the last complexes, MutL□, a heterodimer of MLH1 and PMS1, represents the major MutL activity in *Arabidopsis thaliana*. To analyze the *in vivo* function of AtPMS1, we cloned its cDNA in the expression vector YEP112SPGAL, under the control of galactose-inducible promoter GAL1, to carry out the expression of this plant protein in *Saccharomyces cereviciae* strain E134. This strain allows the assessment of spontaneous mutation rates in specific loci (lys2::InsEA14 and his7-2). Galactose induced expression was corroborated by Western Blot analysis, using previously generated rabbit anti-AtPMS1 antibodies. Following this, rates of His+ and Lys+ reversion were measured by fluctuation analysis revealing that expression of plant PMS1 dramatically increases yeast mutation rates at both loci studied (approximately 13 times at his7-2 locus and close to 2,000 times at locus lys2::InsEA14). These results suggest the inactivation of yeast MMR by heterologous protein expression, probably due to the formation of non-functional complexes, either AtPMS1 homodimers or yMLH1-AtPMS1 heterodimers.

PL-P31

CHARACTERIZATION OF ARABIDOPSIS MUTANTS DEFICIENT IN ALKALINE-NEUTRAL INVERTASES

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Invertases (Invs) catalyze the irreversible hydrolysis of sucrose into hexoses and play an important role when a demand of carbon and energy occurs. Moreover, it has become evident that sucrose and their hydrolysis products are important metabolic signals that modulate gene expression and regulate plant development. Alkaline-neutral invertases (A/N-Invs) are one of the two classes of invertase activities with a characteristic optimum pH between 6.58.0. The physiological functions of A/N-Invs are not clear yet. A/N-Invs are distributed between the cytosol and different subcellular locations suggesting different physiological functions. In this work, we focus on two potentially mitochondrial A/N-Invs in Arabidopsis (At-INVA and At-INVC). To explore their possible biological roles we analyzed *At-a/n-inva* and *At-a/n-invc* knockout mutant plants. Lack of *At-A/N-INVA* and *At-A/N-INVC* expression in homozygous mutant plants was verified by RT-PCR assays. Phenotype characteristics were analyzed for both mutants. Moreover, knockout plants display a lower total A/N-Inv activity compared to wild-type plants. Possible functional roles of alkaline/neutral invertases in mitochondria will be discussed.

PL-P32

EFFECT OF TWO PROLINE ANALOGS ON SECONDARY METABOLITE PRODUCTION IN *Rubia tinctorum* CULTURES

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Anthraquinones (AQs) are the main secondary metabolites produced by *Rubia tinctorum* L. through different metabolic routes. Chorismic acid, the end-product of shikimate pathway, becomes a branch point for primary and secondary metabolic pathways, and is an essential substrate for AQs biosynthesis. It has been proposed that the proline cycle could be coupled with the pentose phosphate pathway (PPP), since the $NADP^+$ generated by proline reduction from glutamate could act as a cofactor of the first enzymes of the PPP. This pathway generates erithrose-4-phosphate, the substrate of the shikimate pathway. In order to study the effect of the addition of two proline analogs on the proline cycle, the PPP and AQs production, three different treatments (besides from control) were performed in *R. tinctorum* cell suspension cultures: azetidine-2-carboxylic acid (A2C, 25 µM) and thiazolidine-4-carboxylic acid (T4C, 100 and 200 µM). The addition of 200 µM of T4C resulted in an increase in AQs production and also in the stimulation of the proline cycle, since a higher accumulation of this aminoacid was obtained. No induction of the PPP was observed after the treatment with proline analogs, so it can be assumed that this route was not a limitant factor as a carbon donor to the shikimate pathway.

PL-P33

CARBOHYDRATE METABOLISM IN *Ostreococcus tauri*: TREHALOSE IN GREEN LINEAGE BASE ORGANISMS

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Non-reducing disaccharides provide a soluble energy source in the form of a stable molecule that can also function as a protectant compound. In the green lineage the most important soluble disaccharide is Sucrose, mainly limited to oxygenic photosynthetic organisms (land plants, algae and cyanobacteria). Trehalose (Tre), another important disaccharide distributed in nature, is present mainly in arthropods, fungi and bacteria. While sucrose plays a role in land plants, that of Tre is still controversial. *Ostreococcus tauri*, the smallest free-living photosynthetic eukaryote, has recently been sequenced. It belongs to *Prasinophiceae*, one of the most ancient groups within the green lineage. The aim of this study is to understand the basis of carbohydrate metabolism in photosynthetic picoeukaryotes. Using sucrose-metabolism related sequences as queries against the sequenced genomes from *O. tauri* and *O. lucimarinus*, no homologous sequences could be retrieved. Sugars extracted from *O. tauri* cultures were separated by chromatography, showing Tre as the main soluble sugar. Enzyme activity of Tre-Phosphate Synthase (TPS) paralleled the accumulation of Tre. The effect of light and different nitrogen concentrations on Tre accumulation were also analyzed. We conclude that Tre plays a physiological function in *O. tauri* similar to that of sucrose in land plants.

PIP 0134, PICT 38144 and UNMdP.

PL-P34

COMPARISON OF 1H-NMR AND GC-MS METABOLIC PROFILE OF TOMATO FRUITS FROM A BREEDING PROGRAM PROGENY

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Tomato (*Solanum lycopersicum* L.) is a world-wide horticultural crop and a model plant for research on fruit metabolism. Data are emerging about the use of robust analytical platforms such as nuclear magnetic resonance spectroscopy (NMR) and gas chromatography-mass spectrometry (GC-MS) for global metabolite analysis of biological systems. The aim of this work was to examine the metabolome of tomato fruits with different organoleptic qualities by coupling NMR and GC-MS data through multivariate analysis. These fruits were obtained by crossing the domesticated *S. lycopersicum* (cv Caimanta) with a wild relative *S. pimpinellifolium* species. This later one is appreciated for its sweetness and stress-tolerance. Correlative analyses between metabolite signals were performed by principal component analysis. Results suggested that a substantial number of significantly correlating metabolites were qualitative- and quantitatively similar in the fruits, suggesting that both platforms complement each other. Examples are primary metabolites involved in the taste such as hexoses (fructose and glucose), organic acids (citrate and malate), and amino acids (alanine, aspartate, glutamate, valine and threonine). Our strategy provides complementary information about the potential of NMR and GC-MS as global metabolite fingerprinting analytical technologies for the study of tomato fruit quality.

PL-P35

ANTIMICROBIAL SNAKIN-1 PEPTIDE IS ALSO INVOLVED IN GA SIGNALING PATHWAY

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Snakin-1 (StSN1) is an antimicrobial peptide isolated from *S.tuberosum* that was shown to be active against fungal and bacterial pathogens in vitro and in vivo. Recently, we suggested that StSN1 has a dual function in defense and development. Other members of its family (snakin/GASA) have already been implied in diverse processes such as cell division/elongation, transition to flowering and signaling pathways. In addition, it has been demonstrated that most GASA genes are transcriptionally up-regulated by gibberellic acid (GA) and upon the analysis of transgenic lines harboring SN1 promoter region fused to GUS reporter gene we demonstrated that in potato SN1 promoter was induced after GA treatments. We have previously obtained potato transgenic lines overexpressing or silencing StSN1 gene and in this work they were further characterized. Even when overexpressing lines did not show remarkable phenotypic differences from wild type, silenced plants exhibited altered leaf morphology, internode elongation, flowering timing, tuberization, plant growth and development. Since their dwarf phenotype was rescued by exogenous GA3, it suggests that they are defective in GA biosynthesis. qRTPCR confirmed that GA 20-oxidase is down-regulated and consequently the contents of bioactive GAs could be decreased. Our results suggest that StSN1 is involved in the GA signaling pathway.

PL-P36

ASR PROTEINS ARE INVOLVED IN THE ABA-GA CROSS-TALK ALTERING PHOTOSYNTHETIC METABOLISM

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Asr (ABA, stress, ripening) is a gene family widely distributed in higher plants, whose products form group 7 of LEA proteins. Transcription of *Asr* genes is induced by different types of abiotic stress and during fruit ripening. Grape and tomato ASR proteins have been proposed to regulate the transcription of sugar- and abiotic stress-regulated genes in fruit and vegetative tissues, respectively. In this work, overexpressing and silenced *Asr1* tobacco plants were characterized. Silenced plants show shorter internodes, loss of apical dominance, chlorosis, leaf necrosis and late-flowering. Photosynthetic rates of these plants are diminished under a wide range of irradiances, while conductance and transpiration are only diminished under saturation levels of irradiance. Photosynthetic carbohydrate metabolism measurements showed that glucose levels of silenced plants are reduced during the diurnal cycle in comparison to wild-type plants. Phloem sap of over-expressing plants contains lower levels of sugars than that of wild-type plants, while the opposite was observed in silenced plants. ABA levels in expanded leaves from silenced plants are reduced, though they preserve the sensitivity to exogenous GA3. In conclusion, all these observations support the proposed function of ASR proteins.

PL-P37

FUNCTIONAL CHARACTERIZATION OF A SEC14-LIKE PROTEIN INVOLVED WITH TOMATO FRUIT TOCOPHEROL CONTENT

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Tomato (*Solanum lycopersicum*) is an important food crop worldwide and represents an excellent model plant for genomics and fruit metabolism research. Numerous wild related species have demonstrated to be an untapped source of valuable genetic variability including nutritional and industrial quality traits. Based on metabolic QTL mapping data from a *S. pennellii* introgression lines population, our group has identified a SEC14-like protein co-localizing with a α-tocopherol QTL on chromosome 9 (segment 9BDE). SEC14-like proteins have been identified in all kingdoms and showed to be involved in membrane trafficking, regulation of phospholipid metabolism and phosphoinositide signaling pathways. Sequence analyses revealed amino acid polymorphisms between the *S. lycopersicum* and *S. pennellii* alleles. Moreover, expression profiles in different tomato tissues also showed differential expression levels in mature fruits. Sub-cellular localization experiments showed that this protein is targeted to chloroplasts. Analyses of iRNA plants suggest impairment in the photosynthetic performance in silenced plants. These results underpin the involvement of the SEC14-like protein in tomato fruit tocopherol content.

PL-P38

CLONING AND EXPRESSION OF *BoMYR* DURING SENESCENCE AND THE EFFECT OF PLANT REGULATORS ON BROCCOLI

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Broccoli (*Brassica oleracea*) is a vegetable belonging to the family of *Cruciferae*. It is harvested when their inflorescences are still immature, which causes high stress and triggers the senescence. Fresh broccoli contains glucosinolates as secondary metabolites. There is strong evidence that consumption of brassica vegetables is associated with a decreased risk for several cancers, probably to their glucosinolate content. Glucosinolates co-exist with myrosinases, which are responsible for glucosinolate turn-over. When tissue is damaged, glucosinolates are hydrolyzed by myrosinases into different degradation products which play roles in plant interaction with pathogens. In this work, we cloned a fragment of Myrosinase (*MYR*) from broccoli and analyzed its expression by qPCR during postharvest senescence and under the effect of different plant regulators. Broccolis were treated with cytokinin and ethylene, in order to delay or accelerate senescence, respectively. It was observed that, the expression of *BoMYR* was higher in the inflorescences compared with the stems and leaves. The expression decreased during the senescence and it was modulated by the hormone treatments. It was observed that the expression decreased with ethylene treatment, but the decrement was delayed in samples treated with cytokinin. In this last treatment, the expression after 24 h had similar values to initials.

PL-P39

L-CYSTEINE DESULFHYDRASE ACTIVITY IS INVOLVED IN ABA-DEPENDENT STOMATAL CLOSURE

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Hydrogen sulfide (H_2S) is a small gas that has emerged as the third gasotransmitter after nitric oxide (NO) and carbon monoxide (CO). Both in animals and plants H_2S is synthesized by enzymes with L-Cys desulfhydrase (DES) activity. In animals H_2S has been implicated in several physiological processes. In plants, H_2S was reported to protect plants against oxidative stress, and recently to participate in ABA-dependent stomatal closure. In the present work, we report that ABA-treated Arabidopsis wild type plants show increased levels of DES1 transcript. Interestingly, T-DNA Arabidopsis mutants *des1-1 and des1-2,* reported to have reduced DES activity, are impaired to close stomata in response to ABA. As expected, exogenous addition of both H_2S and NO donors rescued *des1* plants sensibility to ABA. In addition, the NO scavenger cPTIO blocks H_2S-dependent stomatal closure. These results correlate with a H_2S-dependent increase of endogenous NO production. Consequently, it is postulated that NO acts downstream of DES1/H_2S in the signaling cascade driving ABA-dependent stomatal closure.

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PL-P40

NITRIC OXIDE GENERATION BY THE UNICELLULAR MARINE GREEN ALGA

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The *Prasinophiceae* is one of the most ancient groups within the green lineage. *O. tauri* and *O. lucimarinus* belong to *Prasinophiceae* and their genomes have been completely sequenced. *O. tauri* is an eukaryotic unicellular photosynthetic microalgae. Because of its simple physiology, its small genome size and its early branching in the green lineage, *O. tauri* is an important model for functional studies. NO (nitric oxide) production by algae could be of great importance in view of their occurrence in all biotopes and their contribution of more than 50 % to global NO productivity. The search for a NO Synthase (NOS) sequence in the plant kingdom retrieved two sequences. These correspond to *O. tauri* and *O. lucimarinus*. The specific fluorescent NO probe DAF-FMDA detected a rapid increase of NO when the NOS substrate L-arg was added to *O. tauri* cell culture. NO production was higher in *O. Tauri* growing with 100 µmol $m^{-2}s^{-1}$ of light irradiance compared with 40 µmol $m^{-2}s^{-1}$. The addition of NOS inhibitors or a NO quencher abolished the signal. One hour of treatment with the photosynthetic electron transport inhibitor Paraquat (PQ) induced NO and ROS production. The nitrate reductase (NR) inhibitor was able to block the PQ-induced NO production. These results suggest the involvement of NR and NOS as NO generating enzymes under different physiological conditions in *O. tauri.*

CONICET, ANPCyT, UNMdP.

PL-P41

ROLE OF ACDI, A PUTATIVE DNA GLYCOSYLASE, IN THE *Arabidopsis thaliana* DEVELOPMENT

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The Arabidopsis genome suffers a massive DNA demethylation and centromeric chromatin deconsensation in response to *Pseudomonas* attack. We speculate that the first response involves the action of DNA glycosylases which mediate demethylation of cytosine residues acting together with Base Excition Repair system. In Arabidopsis, the DNA glycosylases belonging to the DEMETER (DME) family are the best characterized. This family includes DEMETER, ROS1, DEMETER-LIKE 2 and DEMETER_LIKE 3. We are studying a novel putative DNA glycosylase, named ACDI, which carries a Helix-harpin-Helix DNA glycosylase Domain, and differs from the DEMETER family members in its secondary structure with. We have previously reported that ACDI affects chromatin decondensation during infection with *Pseudomonas*. Here we evaluate the involvement of ACDI in vegetative and reproductive development of Arabidopsis, and analyze the transcript level of *ACDI* in DME family plant mutants. Our results suggest that ACDI may be involved in regulation of developmental processes. Moreover, we found an enhanced level of *ACDI* transcript in some DME family plant mutants suggesting some compensation effect between these transcripts.

PL-P42

INCREASED SIZE EXCLUSION LIMIT 2 (ISE2) DEVH-BOX RNA HELICASE LOCALIZES IN CHLOROPLASTS

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Intercellular communication in plants is mediated by plasmodesmata (PD), cytoplasmic channels that span cell walls connecting directly the cytoplasm of juxtaposed adjacent cells, and play key roles in coordinating development programs. *Arabidopsis thaliana ISE2* gene encodes a DEVH-box type RNA helicase that is described to regulate PD function and architecture. Our firsts evidences localized ISE2-GFP fusion protein in cytoplasmic granules resembling RNA containing stress granules. However, recent proteomic database localized the endogenous ISE2 in chloroplast. In order to elucidate this controversy; we produced specific antiserum to ISE2. The carboxy-terminal region of ISE2 fused to His tag was produced in *E. coli*. The protein purification was then performed in nickel-agarose resins, followed by a mouse immunization protocol. Finally, the antiserum specificity and titer were determined by western blot analysis. We also purified chloroplasts from transgenic *Arabidopsis thaliana* plant overexpressing ISE2-GFP and detected it using anti-GFP antibody in western blot. We detected ISE2-GFP in the organella preparation, suggesting that endogenous ISE2 localizes in chloroplast. Endogenous ISE2 subcellular localization was analyzed by our ISE2 antiserum. Our results suggest that lack of ISE2 might produce chloroplast alteration leading to deregulation of PD function.

PL-P43

FUNCTIONAL CHARACTERIZATION OF THE *Arabidopsis* AtHB12, AN HD-ZIP TYPE I TRANSCRIPTION FACTOR

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AtHB12 belongs to the plant HD-Zip I family of transcription factors (TFs). In order to analyze the function of this TF and the putative regulation role of its C-terminus domain (CT), several Arabidopsis transgenic plants were generated: overexpressors of AtHB12 (AT12), overexpressors of a truncated version lacking the CT (AT12wct) and knock-out plants (iAT12). Developmental parameters were measured in these plants and the observations indicated that AT12 and WT plants initiated flowering at the same time whereas AT12wct plants had a delayed flowering. In addition, AT12wct plants exhibited shorter shoots and shorter roots than AT12 and WT plants. Besides, iAT12 plants showed longer roots than WT and AT12 plants. Finally, upon abiotic and biotic stresses, the genotypes presented different phenotypes. Transcript levels of AtHB7, a close homologue of AtHB12, seemed to be affected by the overexpression and by the knock out of the latter one, indicating that AtHB12 might regulate the expression of AtHB7. Moreover, the results indicated that the CT of AtHB12 is playing a crucial role in this gene function, probably by interacting with other proteins. Future assays including a yeast two hybrid screening for the identification of interacting partners will be performed in order to corroborate this hypothesis.

PL-P44

OVEREXPRESSION OF RIBONUCLEASES IN *Nicotiana alata* PLANTS EXPOSED TO PHOSPHATE STARVATION

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In many plant species the expression of different T2 ribonucleases is induced by phosphate starvation, possibly to scavenge phosphate from RNA. We assayed the induction of S-RNases, a particular type of T2 ribonuclease involved in self-incompatibility. Plants of *Nicotiana alata* were hydroponically cultivated in Hoagland´s medium with and without inorganic phosphate (Pi). Plants subjected to Pi starvation showed less growth and exhibited several morphological changes in the root system with respect to controls. Semiquantitative allele-specific RT-PCR showed expression of *R6*, a novel S-RNase allele of *Nicotiana alata*, only in roots exposed to Pi deprivation. The induction of *R6* is detected after 14 days of Pi depletion in the culture medium. Moreover, the induction was also detected using degenerated oligonucleotides based on S-RNase C2 and C4 conserved domains. This suggests that over expression is not restricted only to *R6*, but rather that, it would involve other ribonucleases. In gel ribonuclease activity assay showed several bands induced by Pi deprivation, two of which were slightly higher than 31 kDa, a molecular weight close to S-RNase sizes typically ranging between 30-35 kDa. These results suggest that S-RNase functions are not restricted to the reproductive process only and indicate that they could participate in other biological roles requiring ribonucleases.

PL-P45

ROLE OF AtTCP15, A TCP TRANSCRIPTION FACTOR, IN THE REGULATION OF ORGAN DEVELOPMENT IN ARABIDOPSIS

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TCP-domain proteins constitute a family of transcription factors found only in plants. They can be divided into two classes and are generally involved in the regulation of cell growth and proliferation. The precise function and mechanism of action of many class I TCP proteins is not well understood. We transformed *Arabidopsis thaliana* with a construct that expresses a fusion of AtTCP15 (class I) to the EAR repressor domain under the control of its own promoter. These plants show hyponastic leaves with smaller epidermal and palisade parenchyma cells, reduced stem and pedicel elongation and, in the strongest cases, downward pointing flowers with organs of reduced size in the outer three whorls. Leaves and flowers of these plants show increased expression of the boundary specific genes *CUC1*, *CUC2* and *LOB*, and also of the abaxial identity promoting factor *YABBY3*. In addition, AtTCP15 expression is increased in plants with mutations in *AS2*, a gene involved in the specification of leaf primordia. The results suggest that AtTCP15 is a regulator of cell growth in different organs and may participate in limiting the action of boundary specific genes in a pathway that is regulated by *AS2* and/or meristem-specific genes.

PL-P46

MIR396 AND GROWTH REGULATING FACTORS TRANSCRIPTION FACTORS IN ARABIDOPSIS ROOT DEVELOPMENT

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The indeterminate growth of plants is sustained by the activity of two apical meristems: the root apical meristem (RAM) and the shoot apical meristem (SAM). Leaf primordia are initiated by recruiting cells from the peripheral zone of the SAM and extensive cell proliferation occurs even days after primordia initiation. We have previously shown that miR396 limits the expression of the transcription factors GROWTH REGULATING FACTORS (GRF) to proliferating regions in developing leaves. Besides, high levels of miR396 repress the expression of target GRFs and reduce the proliferative phase during leaf development, leading to smaller leaves with fewer cells. In roots, cell proliferation is confined to a meristematic zone (MZ) at the first 200 micrometers of the organ. Above the MZ, zones of elongation and maturation produce the final mature organ. Here, we describe the role of miR396 and GRFs in Arabidopsis root development. Root growth rate was affected in plants with altered levels of miR396 or GRFs. The cellular basis of these defects was analyzed using laser scanning confocal microscopy on propidium iodide counterstained roots of plants expressing a mitotic reporter gene. Finally, GFP reporters were used to determine GRF and miR396 expression and activity pattern.

PL-P47

ROLE OF THE MIR319/TCP REGULATORY NETWORK DURING THE DEVELOPMENT OF *Arabidopsis thaliana*

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MicroRNAs (miRNAs) are small RNA molecules of ~21 nt. that are key regulators of gene expression in multicellular organisms. In plants, miRNAs have been implicated in diverse biological processes such as development, hormone signaling, and pathogen and stress responses. They recognize target RNAs through base complementarity and guide them to cleavage or translational arrest. Around 200 miRNA-encoding genes have been identified in Arabidopsis which can be grouped into 86 families that give rise to identical or almost identical mature miRNAs. The conserved miR319 family is comprised of three members that regulate five TCP transcription factors (TCP2, 3, 4, 10 & 24) which have been involved in the control of cell proliferation and differentiation. In our present work we set on studying the role of the miR319/TCP regulatory network in Arabidopsis leaf and root development. For this, we analyzed cell and organ morphology of wild-type and mutant lines through DIC and laser scanning confocal microscopy, as well as, determining the expression profile of the system components. We found that plants with decreased levels of TCPs showed bigger leaves and this was due to a change in cell proliferation. Also, increased levels of TCPs were detrimental for proper root organogenesis. The role of the miR319 regulatory network in Arabidopsis development will be discussed.

PL-P48

REDOX REGULATION OF CLASS I TCP TRANSCRIPTION FACTORS FROM *Arabidopsis thaliana*

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The TCP domain is a conserved domain present only in plant transcription factors. It contains a basic region followed by a helix-loop-helix motif that resembles the bHLH domain. Phylogenetic studies showed that there are two major classes of TCP domains and a consensus sequence for each class has been derived. Class I proteins contain a highly conserved Cys residue in helix I. In this study we evaluated the effect of redox agents on the DNA binding capacity and quaternary structure of members of the class I TCP family from *A. thaliana*. Our assays show that DTT, GSH or thioredoxin treatments increase the DNA binding efficiency of three different class I proteins, while the oxidation with diamide, H_2O_2 or GSSG prevents the interaction with DNA. For TCP20 and TCP21, this is due to the formation of intermolecular disulfide bonds. TCP15 is inactivated by GSSG without a change in electrophoretic mobility, suggesting that it may be glutathionylated under these conditions. The DNA binding activity of TCP4, a class II protein, and of TCP16 (a class I protein that lacks the conserved Cys) is not affected by redox agents. The results presented in this work constitute the first report of a redox regulation of TCP transcription factors. These modifications may act to regulate the activity of class I factors according to the redox conditions of plant cells.

PL-P49

THE MOLECULAR BASIS FOR THE DIFFERENT DNA BINDING SPECIFICITIES OF CLASS I AND II PLANT TCP PROTEINS

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TCP genes encode plant-specific transcription factors with a noncanonical bHLH domain (the TCP domain) that allows DNA binding and proteinprotein interactions. Based on sequence homology, TCP proteins can be divided in two classes, I and II. SELEX experiments using rice proteins suggested that proteins from each class have distinct DNA binding specificities (GTGGGNCC versus GTGGNCCC). To gain insight into the DNA binding properties of class I TCP transcription factors, we performed a SELEX experiment with the Arabidopsis class I protein TCP16, a divergent member of this class. This assay indicated that TCP16 prefers the class II-like sequence GTGGNCCCGS. In an effort to understand this behavior, we constructed site-specific mutants of TCP16, TCP20 (class I) and TCP4 (class II) in residues conserved inside each class and chimeras between the TCP domains of TCP4 and TCP20 and analyzed their DNA binding properties. Altogether these assays demonstrated that the presence of Gly at position 11 of the TCP domain of class I proteins and Asp11 in TCP16 and class II proteins is responsible for the different DNA binding preferences of these proteins. The results also showed that the presence of the class I-HLH domain confers higher DNA binding selectivity

PL-P50

DNA BINDING PROPERTIES OF THE ARABIDOPSIS ATTCP11 TRANSCRIPTION FACTOR. THE ROLE OF THR15

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TCP transcription factors are regulators of plant cell growth and proliferation. This proteins contain the TCP domain that is predicted to form a non-canonical basic helix-loop-helix (bHLH) structure required for DNA binding and dimerization. TCP proteins are divided into two classes and bind DNA sequences that differ from the consensus binding sites of other bHLH. To gain insight into the DNA binding properties and functions of class I TCP transcription factors from Arabidopsis, we have examined the interaction of three of these factors with DNA. SELEX experiments performed with TCP15 and TCP20 indicated that these proteins have similar, though not identical, DNA binding preferences and are able to interact with non-palindromic binding sites of the type GTGGGNCCNN. TCP11 shows a different DNA binding specificity, with a preference for the sequence GTGGGCCNNN. The distinct DNA binding properties of TCP11 are due to the presence of Thr at position 15 of the TCP domain, a position that is occupied by Arg in other TCP proteins. In addition, TCP11 forms heterodimers with TCP15 that have increased DNA binding efficiency and are able to interact with DNA sequences bound only poorly by the respective homodimers. The results suggest that changes in DNA binding preferences may be one of the mechanisms through which class I TCP proteins achieve functional specificity.

PL-P51

DECIPHERING THE NATURE OF A RETROGRADE SIGNAL THAT REGULATES ALTERNATIVE SPLICING IN ARABIDOPSIS

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With the aim of understanding alternative splicing regulation in plants we analyzed about 100 alternative splicing events with a high-resolution RT-PCR panel and found that 40% of them respond to light/dark transitions. We chose the mRNA of the SR protein Rsp31 as a model to investigate the mechanisms involved. Light exposure, perceived by the chloroplast, increases the proportion of the functional mRNA splicing isoform of RSp31. The effect is only observed in roots that have not been dissected from shoots before light exposure, suggesting that the signal is able to travel from leaves to roots. Chloroplast signals include reactive oxygen species (ROS), sugars, the redox-state of the electronic transport chain, plastid gene expression and chlorophyll metabolism. Mutants of the chlorophyll biosynthesis pathway behave as wild type plants, ruling out the involvement of this pathway. Both H_2O_2 and sucrose mimic the effects of light on Rsp31 alternative splicing. However, flavodoxin-overexpressants do not inhibit the effect of light, and methylviologen treated plants behave as control plants, which favors a role for sugar signaling but does not completely rule out ROS. To get deeper insights into the nature of the signaling we are currently using Arabidopsis mutants and drugs that affect the redox-state of the plastoquinone pool.

PL-P52

ALTERNATIVE SPLICING MODULATION BY PRMT5 AND A POSSIBLE INTERACTION WITH miRNA PRODUCTION

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Circadian rhythms allow organisms to time biological processes to the most appropriate phases of the day/night cycle. PROTEIN ARGININE METHYL TRANSFERASE 5 (PRMT5), which transfers methyl groups to arginine residues present in histones and Sm spliceosomal proteins, links the circadian clock to the control of alternative splicing in plants. We found that mutations in *PRMT5* impair multiple circadian rhythms in *Arabidopsis thaliana* and this phenotype is caused, at least in part, by a strong alteration in alternative splicing of the core-clock gene *PSEUDO RESPONSE REGULATOR 9*. Furthermore, genome wide studies show that PRMT5 contributes to regulate many pre-mRNA splicing events most likely modulating 5´splice site recognition. Among the altered alternative splicing events, we detected an inhibition of splice variants accumulation of the *npcRNA78* gene that contains the miR162a sequence in an alternative intron. This miRNA targets and produces the cleavage of the Dicer Like-1 (DCL1) mRNA, which is the responsible enzyme of miRNA production in *Arabidopsis thaliana*. With the aim of understanding the interaction between the splicing process and the DCL1-catalyzed miRNA production, we will evaluate whether the *npcRNA78*'s splicing inhibition in the *prmt5* mutant leads to an over-accumulation of miR162a and whether this fact could cause an unbalance in global miRNA production.

PL-P53

TEMPERATURE EFFECTS ON POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION BY MICRORNAS

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Plants are sessile organisms that must adapt to changes in the environment to survive. One of the most common daily challenges they face are temperature shifts. The response of plants to temperature requires the coordinated action of many different genes and cellular components. Post transcriptional regulation of gene expression by microRNAs has proved to be involved in developmental pathways as well as stress responses. MicroRNAs identify target genes by base complementarity and guide them to cleavage or translational arrest, resulting in a decrease in its expression. In this work we aim to characterize alterations in microRNA regulation due to a switch in the growth temperature. *Arabidopsis thaliana* plants grown under normal conditions were subjected to a cold or heat treatment. The levels of different mature microRNAs were quantified by RT-qPCR or blots. We also scanned for modifications in their target gene expression levels. We analyzed several microRNA networks involved both in stress response and development, as well as key components of the microRNA regulatory pathway such as ARGONAUTE1 or DICER-LIKE1. The effects of temperature on the regulation of gene expression by microRNAs in Arabidopsis will be discussed.

PL-P54

CHARACTERIZATION OF THE LOOP-TO-BASE PROCESSING MECHANISM RESPONSIBLE FOR THE BIOGENESIS OF PLANT miRNAs

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MicroRNAs 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 secondary structure of the precursor seems to contain the necessary information that determinates the position of the miRNA within its sequence. The first step in microRNA biogenesis usually involves a cleavage at the base of its fold-back precursor. We have found a non-canonical processing mechanism for microRNAs miR319 and miR159 in plants. Their biogenesis begins with the cleavage of the loop, instead of the usual cut at the base of the stem-loop structure. DICER-LIKE1 proceeds then with three additional cuts until the mature microRNA is released. We characterized by site directed mutagenesis essential determinants in this non-canonical processing. The biogenesis of miR319 and miR159 was also analyzed in different Arabidopsis mutants impaired in the production of small RNAs. The characteristics of this unusual processing pathway will be discussed.

PL-P55

DIVERSIFICATION OF THE MIR396 REGULATORY NETWORK DURING EVOLUTION IN PLANTS

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MicroRNAs are ~21 nt small RNAs that negatively regulate gene expression. Many of them can be grouped into families encoding similar or identical microRNAs. More than twenty microRNA families are deeply conserved in seed plants and regulate key aspects of plant biology. We present a detailed evolutionary analysis of the conserved microRNA miR396 network in plants. This miRNA regulates transcription factors of the GROWTH REGULATING FACTORS (GRF) family, which have been shown to play important roles in the regulation of cell division in plants. Through a survey for variations in the miRNA sequences of family members in different species, we found species specific miR396 variants with differences in the 5' portion of the miRNA, a region known to be important for miRNA activity. Expression in *Arabidopsis thaliana* of these small RNAs revealed that some variants have specialized to regulate the GRFs with high efficiency. We also found that the miR396 regulatory network has expanded in the *Brassicaceae* family to regulate a basic-Helix-Loop-Helix (bHLH) transcription factor. The regulation of this novel target is important to Arabidopsis development. Expression studies have shown that miR396 coordinates the temporal expression of the GRFs and bHLH transcription factors. The emerging picture is that conserved microRNA regulatory networks could be more dynamic than previously thought.

PL-P56

AQUAPORIN EVOLUTION: A FRAMEWORK FOR ORTHOLOGY ASSIGNMENT AND ANIMAL-PLANT INTEGRATION

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Aquaporins (AQPs) comprise a diverse family of channel protein require to transport water and small solutes across cell membranes in the three domains of life. When we restrained the analysis to proteins with high aminoacid identity, we showed for the first time a complete congruence between AQPs and organismal trees. On the basis of this analysis, we defined twenty orthologous gene clusters in flowering species. We described specific conserved motifs for each subfamily and each cluster. The simultaneous assessment of motifs and phylogenetic trees for AQPs protein taken at random showed that the use of motifs is a powerful method for the automatic classification of plant AQPs. In animals, we defined four AQP subfamilies: AQP1-like, AQP8-like, AQP3-like and AQP12-like. These groups were related with plant AQPs: PIPs, TIPs, NIPs and SIP, respectively. This result suggests that the common ancestor of plants and animals had at least four AQP subfamilies. Finally, a new nomenclature based on evolution relationships is proposed.

PL-P57

GENETIC DIFFERENTIATION AND DIVERSITY OF *Rosa rubiginosa L* IN DIFFERENT ARGENTINEAN ECOREGIONS

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The aim of the study was to evaluate the genetic differentiation and genetic diversity of *Rosa rubiginosa* by RAPDs from populations growing in different experimental sites from San Luis (Potrero de los Funes, Estancia Grande) and Neuquén (Andacollo-La Primavera y La Tricahuera). Leaf material was collected during the spring-summer of 2006 and 2010. Genetic differentiation between populations was estimated by AMOVA . UPGMA dendrogram and principal coordinate analysis (PCoA)- clearly suggest a geographical differentiation of the provenances of *R. rubiginosa* populations. AMOVA analyses revealed high genetic variation within populations and low variation between populations, in agreement with values estimated by Shannon-Weaver's index. The low interpopulation value obtained suggests genetic homogeneity between the populations. The presence of specific monomorphic bands accounts for the genetic differentiation between populations. The high percentage of within-population genetic diversity suggests the introduction of genetic variation into both ecoregions. From the present results we can conclude that we observed two independently established populations with high similarity between them and a strong intrapopulation differentiation. The empty niche hypothesis for explaining invasion success might explain the invasiveness of *R. rubiginosa* in Argentinean ecoregions.

PL-P58

EVALUATION OF THE IMMUNOSTIMULATORY PROPERTIES OF PLANT HSP90 TO USE AS NOVEL ADJUVANT CARRIERS

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In general terms, the production of high amounts of antigen in plant is hard, having to develop different strategies to increase their expression. An interesting option is to express the polypeptide of interest in the plant with a carrier that could provide stability and therefore increase the polypeptide production. We consider that plant Hsp90 could be such a carrier. In addition, if plant Hsp90 presents adjuvant properties, it could arise as a novel and interesting carrier for proteins or peptides with immumoprotective value, improving the immunogenicity property of the transgenic plant extract. We have previously demonstrated that recombinant plant Hsp90 proteins (rpHsp90) induced prominent proliferative responses in spleen cells form BALB/c mice. Here, it was found that *in vitro* incubation of spleen cells with rpHsp90 leads to the expansion of CD19-bearing populations, suggesting a direct effect of these proteins on B lymphocytes. Furthermore, we examined the involvement of TLR4 in the rpHsp90s induction of B cell proliferation. Spleen cells of C3H/HeJ mice, which are hyporesponsive to LPS, also responded poorly to rpHsp90 indicating that rpHsp90 B-cell mitogenic properties are through the TLR4 receptor. The immunostimulatory properties of rpHsp90 observed here support the idea that these proteins could be excellent carriers for antigens and peptides expressed in plant.

PL-P59

EXPRESSION OF *T. Gondii* SAG1 ANTIGEN FUSED TO *L. Infantum* HSP83 IN TOBACCO TRANSPLASTOMIC PLANTS

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We have previously determined that it was feasible to produce a *T. gondii* SAG1 vaccine in plant by transient protein expression. This vaccine could be used in oral and subcutaneous immunization protocols. Chloroplast transformation has the capacity to accumulate high levels of recombinant proteins and increased biosafety due to maternal plastid inheritance. Here, we evaluate tobacco chloroplast transformation for the production of *T. gondii* SAG1 antigen to increase accumulation levels. We evaluated the expression levels of two constructs. In one of them, the SAG1 peptide was expressed alone and in the other, the antigen was expressed as a fusion protein with *L. infantum* Hsp83, which showed a considerable efficacy to induce a strong humoral and cellular immunoresponse. Our results showed that the expression levels of SAG1 were significantly increased when the protein was fused to LiHsp83. The recombinant protein represented more than 5% of the total soluble proteins in mature leaves. Despite the fact that the transplastomic plants expressing the LiHsp83-SAG1 showed a clorotic color in their leaves, they could give flowers and fructify normally. Here we demonstrated that LiHsp83 is a good candidate to carry antigens by increasing the polypeptide production. In addition, the LiHsp83 adjuvant properties could improve the immunogenicity property of the transgenic plant extract.

PL-P60

MECHANISMS OF LIPID ACCUMULATION IN NONTRADITIONAL OILSEED SPECIES

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The increasing demand of energy has driven the development of biofuels. Nonedible crops like castor bean plant (*Ricinus communis*) emerge as a viable possibility for their production. In this work the mechanisms of accumulation of lipid in castor bean and in seeds of the model species *A. thaliana* were investigated. Determinations performed on extracts from castor bean endosperm showed that the activity of NADP-dependent malic enzyme (NADP-ME) reaches its maximum value at the stage of seed development when the fatty acid biosynthesis occurs. NADP-ME produces pyruvate and NADPH, which would be used as a source of carbon and reducing power to support such metabolic pathway. Three sequences homologous to NADP-ME were identified in databases of castor oil plant. Analysis of real-time PCR indicated that all genes are expressed in all the stages of seed development assayed, although at different levels, and no correlation between a single isoform to the activity profiles was observed. Finally, single mutant lines of *A. thaliana* were used as a system for studying the relationship between each enzyme and lipid accumulation. Either the absence of AtME1 or AtME4 produced a significant decrease in the content of glycerolipids in seeds, while the absence of AtME2 or AtME3 did not have any effect, indicating a specific role of certain NADP-ME isoforms in the synthesis of these compounds.

PL-P61

BIOLOGICAL ACTIVITIES OF YACON LEAVES IN DIABETIC

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Medicinal plants have long been an excellent source of pharmaceutical agents. Accordingly, the objectives of many investigations are to discover and isolate new therapeutic agents based on plant-derived compounds. *Smallanthus sonchifolius* (yacón) is an Andean crop used for centuries by prehispanic population in traditional medicine. We demonstrated that a 10% yacón leaves decoction (140mg dry extract/kg bw) produced a fast and significant decrease in plasma glucose levels of diabetic rats. The bioactivity screening of organic extracts of yacón leaves revealed that butanol and ethyl acetate are the most effective fractions to reduce postprandial glucose levels. Phytochemical analysis of butanol extract showed the presence of caffeic, chlorogenic and dicaffeoilquinic. Enhydrin was the major sesquiterpene lactone in the ethyl acetate fraction and had significant hypoglycemic action at dose of 0,8mg dry extract/kg bw. In order to clarify the mechanism of hypogycemic action of enhydrin and yacón decoction we tested the inhibitory activity on α-glucosidase *in vitro* and *in vivo*. The results showed that both samples suppressed the carbohydrate absorption from intestine reducing the postprandial increase of blood glucose. Toxicity study of yacón decoction and enhydrina using normal Wistar rats demonstrated that yacón decoction and enhydrina, are safe in the therapeutic dosage range.

PL-P62

INCREASE OF GLUCAGON-LIKE PEPTIDE-1 BY YACON FOS AND ITS EFFECTS ON DIABETIC RATS

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Smallanthus sonchifolius [Poepp. & Ende] H. Robinson (yacón) is a plant originating from South America. Its roots contain low polymerization degree oligosaccharides (FOS) as the main storage saccharides. We have evaluated the influence of yacón roots on glucose homeostasis, insulin production, intestinal and plasmatic glucagon-like peptide-1 (GLP-1) content in streptozotocin (STZ)-induced diabetic rats. Male Wistar rats received either i.p. streptozotocin (45mg/kg) (STZ) or vehicle (CT); one week later, they were fed for 8 weeks with either the standard diet (STZ), or with a diet containing yacón roots in a dose equivalent to 340 mg FOS/Kg/day (STZ-Y340). Yacón roots improve glucose tolerance in the post-prandial state and reduced slightly food intake as compared with STZ rats. After 8 weeks, plasmatic insulin levels were doubled in STZ-Y340 rats. We have shown that yacón treatment increased GLP-1 levels in blood and *caecum*. This result is accompanied with higher levels of GLP-1 receptor mRNA in pancreatic tissue. We propose that yacón FOS, through its fermentation in the colon, promoted the secretion of colonic GLP-1, with beneficial consequences on post-prandial glycaemia and insulin secretion in diabetic rats.

PL-P63

ENDOCYTOSIS IS REQUIRED DURING EARLY GERMINATION IN *Arabidopsis thaliana*

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Endocytosis is required to overcome a variety of cellular functions, including the uptake of extracellular molecules, intercellular signal transduction, and regulation of receptors on the plasma membrane and establishment of cell polarity. During germination, the rapid change from a metabolically resting state to the active condition requires not only the synthesis-a comparative slow process- but the recruitment of several molecules that has been strategically separated from its target localization during seed maturation. We have previously demonstrated that an extracellular lipid transfer protein from sunflower seeds is internalized by endocytosis upon germination. This process probed to be conserved between species, since Arabidopsis seeds also internalized an endocytosis marker during imbibition. In this work we demonstrate the internalization of rhamnogalacturonan-II upon early imbibition in Arabidopsis seeds. To determine the effect of inhibitors of vesicular trafficking in the germination capabilities of Arabidopsis seeds, germination kinetics were analyzed in the presence of 30 to 50 μM of BFA, Wortmannin, Ly294002 and Tyrphostin A23. The results show that the rate of seed germination is clearly affected when endocytosis is restricted. These findings suggest that recycling of membrane components and&or protein internalization could be important cues during early germination.