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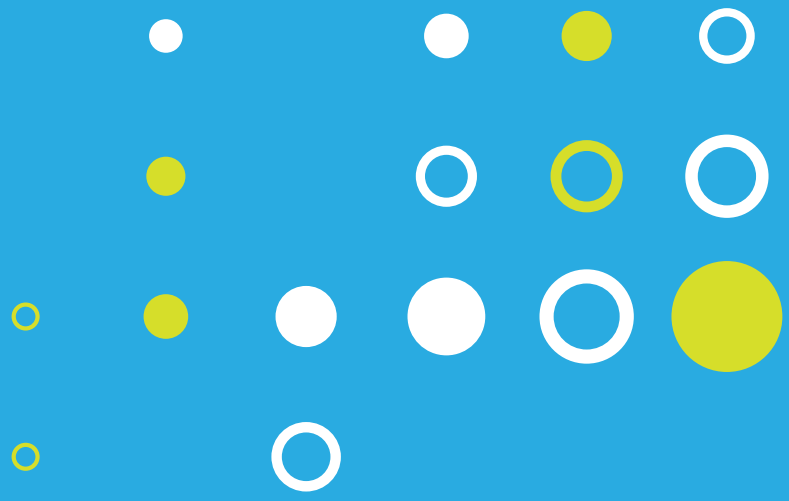

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y Biología Molecular

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- SAIB -

47th Annual Meeting
Argentine Society for Biochemistry and Molecular Biology

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

October 30 - November 2, 2011

Potrero de los Funes, San Luis
República Argentina

MEMBERS OF THE SAIB BOARD

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-Plant biochemistry and molecular biology-
Diego Gómez-Casati
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Universidad Nacional de Rosario

-Signal Transduction-
Silvia Moreno de Colonna
Dpto. de Química Biológica, Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires

ABSTRACT EVALUATION COMMITTEE

The abstracts were evaluated by the members of the SAIB Board with the help of José Luis Daniotti, Gabriela Salvador, Ana Virginia Rodriguez, Claudia Banchio, Eleonora García Véscovi and Eduardo Zabaleta

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María Sofía Giménez and **Gladys Ciuffo**
Universidad Nacional de San Luis

ACKNOWLEDGMENTS

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Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT)

European Molecular Biology Organization (EMBO)

Panamerican Association for Biochemistry and Molecular Biology (PABMB)

Biochemical Journal

Gobierno de la Provincia de San Luis, Argentina

SAIB 2011 CONGRESS

Sunday, October 30th	Monday, October 31st	Tuesday, November 1st	Wednesday, November 2nd
	8:30 - 10:30 **Symposia** *Room A:* Microbiology* *Room B:* Neuroscience	8:30 - 10:30 **Symposia*** *Room A:* Lipids *Room B:* Cell Biology	8:30 – 10:30 **Symposia*** *Room A:* Plant Biochem. & Mol Biol. *Room B:* Signal Transduction
	10:30-11:00 Coffee break	10:30-11:00 Coffee break	10:30 - 11:00 Coffee break
	11:00-13:00 **Oral Communications** *Room A:* CB (C01/08) *Room B:* MI (C01/08) *Room C:* SB (C01/02) and ST (C01/06)	11:00-13:00 **Oral Communications** *Room A:* CB (C09/16) *Room B:* MI (C09/16) *Room C* PL (C01/06) and BT (C01/03)	11:00-13:00 **Oral Communications** *Room A:* CB (C17/22) and NS (C01) *Room B:* MI (C17/18) and LI (C01/06) *Room C* PL (C07/12) and BT (C04/06)
14:00-18:00 Registration	13:00-15:30 Time for Lunch	13:00-15:30 Time for Lunch	13:00-15:30 Time for Lunch
	15:30-16:30 **Plenary Lecture*** Gerhard Braus	15:30-16:30 **Short Lectures (2)** Junior Faculty	15:30-16:30 **Plenary Lecture*** Jean Philippe Vielle Calzada
18:30-18:45 (*Room A*) **Opening Ceremony**	16:30 – 19:00 **Posters & coffee** CB (P01/20) MI (P01/20) EN (P01/05) BT (P01/09) LI (P01/10) PL (P01/19) NS (P01/07) ST (P01/06)	16:30 – 19:00 **Posters & coffee** CB (P21/40) MI (P21/40) EN (P06/10) BT (P10/19) LI (P11/20) PL (P20/41) NS (P08/13) ST (P07/12)	16:30 – 19:00 **Posters & coffee** CB (P41/59) MI (P41/53) ST (P13/19) BT (P20/26) LI (P21/31) PL (P42/66) SB (P01/04)
18:45 – 19:00 **Tribute to Dr. H. Torres** Alberto R. Kornblihtt			
19:00-20:00 **PABMB Lecture*** Suzanne Pfeffer	19:00-20:00 **Plenary Lecture*** William Wickner	19:00-20:00 **Plenary Lecture*** Sarah Spiegel	19:00 - 20:00 **EMBO Lecture*** Francisco Baralle
20:00-21:00 **Sols Lecture** Juan Pedro García Ballesta	20:00 – 21:00 ***Round Table:*** Grant reviewing at the ANPCYT	20:00 – 21:00 **Plenary Lecture*** Alexandra Newton	
21:30 Cocktail		21:30 SAIB Assembly	21:30 Closing Dinner

*Activities in English. Plenary Lectures, Round Table, and SAIB Assembly will be at *Room A*

PROGRAM

SUNDAY, October 30th, 2011

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

18:30-18:45 ***OPENING CEREMONY (Room A)***

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

18:45-19:00 ***TRIBUTE TO Dr. HÉCTOR TORRES***

Alberto R. Kornblihtt, María Teresa Tellez and Guillermo Alonso

19:00-20:00 ***PABMB LECTURE***

Suzanne Pfeffer
Stanford University School of Medicine-Biochemistry, USA
"Transport of endocytosed cholesterol from lysosomes into the cytoplasm"
Chairperson: Luis Mayorga

20:00-21:00 *"**ALBERTO SOLS" LECTURE***

Juan Pedro García Ballesta
Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Spain
"The ribosome is engaged in eukaryotic specific translation regulatory mechanisms"
Chairperson: Eduardo Cánepa

21:30 **COCKTAIL**

MONDAY, October 31st, 2011

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

Room A

MICROBIOLOGY SYMPOSIUM

Chairpersons: Carlos Argaraña and Ricardo Morbidoni

08:30-09:00 ***Mark Buttner***
Department of Molecular Microbiology, John Innes Centre, Norwich - United Kingdom
"*Streptomyces venezuelae* as a Genetic and Developmental System"

09:00-09:30 ***Josef Deutscher***
Laboratoire de Microbiologie et Génétique Moléculaire, INRA-CNRS, Thiverval-Grignon - France
"Similarities of the mechanisms controlling carbon metabolism and virulence in bacteria"

09:30-10:00 ***Claudia Studdert***
Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina
"Chemoreceptors: conserved design for the detection of multiple signals"

10:00-10:30 ***Fernando Goldbaum***
Fundación Instituto Leloir, Ciudad Autónoma de Buenos Aires, Argentina
"Blue Light Activated Histidine Kinase: a novel two-Component sensor in *Brucella spp.*"

Room B

NEUROSCIENCE SYMPOSIUM

Chairpersons: Norma M. Giusto and Eduardo Garbarino Pico

08:30-09:00 ***Ruth E. Rosenstein***
Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, School of Medicine CEFyBO, UBA, CONICET
"New perspectives in glaucoma: unrecognized symptoms and looking for new therapeutic strategies"

09:00-09:30 ***Ana Belén Elgoyhen***
INGEBI CONICET, Ciudad Autónoma de Buenos Aires, Argentina
"The medial olivocochlear system and protection from acoustic trauma"

09:30-10:00 ***Martín Cammarota***
Laboratory of Neurochemistry & Neurophysiology, Memory Center for Memory Research Brain Research Institute, Porto Alegre, Brazil
"How to make it last longer? On the role of the VTA-hippocampus loop in memory persistence"

10:00-10:30 ***Arturo Romano***

Dpto. Fisiología, Biología Molecular y Celular
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Argentina
"Transcription factors and the memory fate after retrieval"

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

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

Room A

CELL BIOLOGY (C01/08)
Chairpersons: José Luis Daniotti and Marcela Michaut

11:00-11:15
CB-C01
A NOVEL MURINE XENOGRAFT MODEL FOR THE STUDY OF HUMAN BREAST CANCER
Orlando U., Garona J., Ripoll G., Alonso D., Gómez D., Duarte A., Maloberti P., Solano A., Podesta E.J.

11:15-11:30
CB-C02
HORMONE-DEPENDENT MITOCHONDRIAL FUSION REGULATES THE TRANSLOCATION OF ERK TO THE MITOCHONDRIA
Poderoso C., Duarte A., Cooke M., Orlando U., Cornejo Maciel F., Soria G.,Gottifredi V., Podesta E.J.

11:30-11:45
CB-C03
REGULATION OF CHKA EXPRESSION DURING RA-INDUCED NEURONAL DIFFERENTIATION. A MAJOR ROLE FOR C/EBP
Domizi P., Banchio C.

11:45-12:00
CB-C04
DECIPHERING THE ROLE OF CNBP DURING ZEBRAFISH CRANIOFACIAL DEVELOPMENT
Sdrigotti M.A.,Weiner A.M., Calcaterra N.B.

12:00-12:15
CB-C05
ANALYSIS AND VALIDATION OF CNBP TARGET GENES IN *Danio rerio*
Margarit E., Calcaterra N.B., Armas P.

12:15-12:30
CB-C06
E2F1 AND E2F2 CONTRIBUTE TO THE DNA DAMAGE RESPONSE IN NEURONAL CELLS
Castillo DS, Ogara MF, Cánepa ET, Pregi N.

12:30-12:45
CB-C07
ENDOCYTIC RECYCLING OF LRP1 IN ALPHA 2-MACROGLOBULIN-STIMULATED CELLS
Jaldin Fincati J.R., Barcelona P.F., Sánchez M.C., Chiabrando G.A.

12:45-13:00
CB-C08
VAMP7 IS INVOLVED IN HOMOTYPIC AND HETEROTYPIC FUSION OF *C. burnetii* PHAGOSOMES
Campoy E.M., Mansilla M.E., Colombo M.I.

Room B

MICROBIOLOGY (C01/08)

Chairpersons: Andrea Smania and Mónica Delgado

11:00-11:15
MI-C01
TRANSCRIPTIONAL CONTROL OF THE COPPER RESISTANCE REGULON IN *Salmonella enterica*
Pezza A, Pontel LB, Soncini FC

11:15-11:30
MI-C02
ABAR-TYPE RESISTANCE ISLANDS IN MULTIDRUG RESISTANCE *Acinetobacter baumannii* (MDRAB) ISOLATES
Ramirez MS, Vilacoba E, Stietz MS, Limansky AS, Márquez C, Cerquetti C, Catalano M, Centrón D.

11:30-11:45
MI-C03
***Helicobacter pylori* PLASTICITY ZONE: VARIABLE PUTATIVE PATHOLOGY-SPECIFIC-GENES CONTENT**
Armitano RI, Matteo M, Wonaga A, Cerquetti C, Viola L, Catalano M.

11:45-12:00
MI-C04
MULTILOCUS SEQUENCING TYPING (MLST) SCHEME OF *Acinetobacter baumannii* ISOLATED IN THE LAST THREE DECADES
Stietz MS, Ramírez MS, Cerquetti C, Jeric P, Centrón D, Catalano M.

12:00-12:15
MI-C05
INSIGHTS INTO THE CO-EVOLUTION OF REGULATOR/OPERATOR SELECTIVITY AMONG MERR TRANSCRIPTION FACTORS
Humbert MV, Checa SK, Soncini FC.

12:15-12:30
MI-C06
PBP2B CONTROLS THE SHAPE DETERMINATION AND CELL DIVISION MECHANISMS OF *Streptococcus pneumoniae*
Albarracin Orio A.G., Piñas G.E., Cortes P.R., Cian M.B., Echenique J.

12:30-12:45
MI-C07
ANALYSIS OF A *Bordetella bronchiseptica* CYCLIC-DI-GMP-BINDING PROTEIN REVEALS A ROLE IN MOTILITY
Sisti F, Fernandez J.

12:45-13:00
MI-C08
FASR, A NOVEL TRANSCRIPTIONAL ACTIVATOR OF THE *FAS* GENE OF *Mycobacterium tuberculosis*
Mondino SS, Gramajo H, Gago G.

Room C

STRUCTURAL BIOLOGY (C01/02) and SIGNAL TRANSDUCTION (C01/06)

Chairpersons: Alejandro Colman Lerner and Silvia Rossi

11:00-11:15
SB-C01
ANALYSIS OF PROTEIN-PROTEIN/PROTEIN-DNA INTERFACES OF *P. aeruginosa* MUTL N-TERMINAL DOMAIN
Miguel V, Correa ME, De Tullio L, Barra JL, Argaraña CE, Villarreal MA

11:15-11:30
SB-C02
SET-UP OF A REPRODUCIBLE PROCEDURE TO PURIFY CNBP SUITABLE FOR STRUCTURAL AND BIOCHEMICAL STUDIES
Challier E, Lisa MN, Nerli B, Calcaterra NB, Armas P

11:30-11:45
ST-C01
WHAT IS MORE IMPORTANT FOR DESK ACTIVITY REGULATION: HEADGROUPS OR ACYL CHAINS OF LIPIDS?
Martín M, Cirulli BA, de Mendoza D

11.45-12:00
ST-C02
HIV-1 TAT PROTEIN MAY DIRECTLY CONTRIBUTE TO NEUROAIDS : INHIBITORY EFFECT OF TAT ON NEUROSECRETION MECHANISM
Tryoen-Tóth P, Bader M-F, Beaumelle B, Vitale N

12:00-12:15
ST-C03
ANG II AT_2 RECEPTORS INDUCE APOPTOSIS IN HeLa CELLS
Manzur MJ, Beron W, Kotler ML, Ciuffo GM

12:15-12:30
ST-C04
ENDODERM GENERATION FROM EMBRYONIC STEM CELLS IS REGULATED BY EXTRA-CELLULAR MATRIX REMODELING
Villegas NH, Barrios-Llerena M, Brickman JM.

12:30-12:45
ST-C05
MARCKS REGULATES CALCIUM MOBILIZATION IN INTACT HUMAN SPERM
Rodríguez Peña MJ, Mayorga LS, Michaut MA

12:45-13:00
ST-C06
METABOLITE-IONIC FEEDBACK FOR SYNCHRONIZATION OF O_2 RELEASE AND SIGNAL TRANSDUCTION RESPONSIVENESS
Bennun A

13:00-15:30 ***TIME FOR LUNCH***

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

Gerhard Braus
Institute of Microbiology and Genetics, University of Göttingen, Germany
"Coordination of Fungal Secondary Metabolism and Development"
Chairperson: Hugo C. Gramajo

16:30-19:00 ***POSTERS & COFFEE***

Cell Biology (CB P01/20)	Microbiology (MI P01/20)
Enzymology (EN P01/05)	Biotechnology (BT P01/09)
Lipids (LI P01/10)	Plants Bioch. & Mol. Biol. (PL P01/19)
Neuroscience (NS P01/07)	Signal Transduction (ST P01/06)

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

William Wickner
Dartmouth Medical School, Hanover, USA
"Membrane fusion: 5 lipids, 4 SNARES, 3 chaperones, 2 nucleotides, and a GTPase, dancing in a ring"
Chairperson: Claudia Tomes

20:00-21:00 **Science policy in Argentine (*Room A*)**

Round Table: **"Mecanismos y criterios de evaluación de PICTs en la ANPCyT"** (en castellano)
Speakers: Carlos Cassanello, Pablo Wappner y María Isabel Colombo
Chairperson: Nora Calcaterra

TUESDAY, November 1st, 2011

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

Room A

LIPIDS SYMPOSIUM

Chairpersons: Mario Guido and Gabriela Salvador

08:30-09:00 ***Juan Carlos Lacal Sanjuan***
Instituto de Investigaciones Biomédicas, CSIC, Madrid, Spain
"Choline kinase α, a novel molecular target in cancer therapy"

09:00-09:30 ***Beatriz L. Caputto***
CIQUIBIC-CONICET. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
"c-Fos physically associates and activates specific enzymes of the pathway of phospholipid synthesis"

09:30-10:00 ***Nora P. Rotstein***
Instituto de Investigaciones Bioquímicas (INIBIBB), UNS-CONICET, Bahía Blanca, Buenos Aires, Argentina.
"Staying alive in the retina: crucial roles for simple sphingolipids"

10:00-10:30 ***Robin Irvine***
Department of Pharmacology, University of Cambridge, Cambridge United Kingdom
"Functions of plasma membrane phosphatidylinositol 4-phosphate"

Room B

CELL BIOLOGY SYMPOSIUM

Chairpersons: José Luis Bocco and Claudia E. Banchio

08:30-09:00 ***Marcelo G. Kazanietz***
Department of Pharmacology, School of Medicine - University of Pennsylvania USA
"ErbB receptor signaling in cancer: novel roles for PKCε; and the Rac-GEF P-Rex1"

09:00-09:30 ***Mario Rossi***
Department of Pathology, School of Medicine - New York University USA
"FBH1 is required to inactivate stalled DNA replication forks upon replication stress"

09:30-10:00 ***Ramiro E. Verdun***
Miller School of Medicine, University of Miami, Miami USA
"Telomere maintenance during the cell cycle"

10:00-10:30 ***Vanesa Gottifredi***
Fundación Instituto Leloir-IBBA (CONICET), Buenos Aires, Argentina
"Novel regulators of the replication of damaged DNA"

10:30-11:00 ***COFFEE BREAK***

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

Room A

CELL BIOLOGY (CB C09/16)
Chairpersons: Cecilia Alvarez and Carolina Touz

11:00-11:15
CB-C09
TRANS-ACTIVITY OF PLASMA MEMBRANE ASSOCIATED GANGLIOSIDE SIALYLTRANSFERASE IN MAMMALIAN CELLS
Vilcaes AA, Torres DemichelisV, Daniotti JL.

11:15-11:30
CB-C10
STARVATION-INDUCED AUTOPHAGY CAUSES A REDISTRIBUTION OF VAMP7 VESICLES TO FOCAL ADHESIONS IN HELA
Fader CM, Colombo MI

11:30-11:45
CB-C11
RAB5 AND RAB11 ARE INVOLVED IN THE TRAFFICKING OF VINCULIN IN RENAL PAPILLARY COLLECTING DUCT CELLS
Márquez MG, Guaytima EV, Brandan YR, Favale NO, Pescio LG, Sterin-Speziale NB

11:45-12:00
CB-C12
ROLE OF 2-OXOGLUTARATE-DEPENDENT DI-OXIGENASES IN *Drosophila* DEVELOPMENT
Acevedo J, Katz M, Wappner P

12:00-12:15
CB-C13
AIR POLLUTION AND OBESITY ASSOCIATED METABOLIC COMPLICATIONS
Gomez-Mejiba SE, Jiménez MS, Ramirez DC

12:15-12:30
CB-C14
INFLAMMATORY MACROPHAGES, MYELOPEROXIDASE AND ADIPOSE TISSUE DYSFUNCTION
Gomez-Mejiba SE, Zhai Z, Ramirez DC

12:30-12:45
CB-C15
ROLE OF SPHINGOLIPID METABOLISM IN MDCK CELLS TRANSITION FROM POLARIZED TO DIFFERENTIATED PHENOTYPE
Favale NO, Santacreu B, MarquezMG, Sterin-Speziale NB

12:45-13:00
CB-C16
IDENTIFICATION OF POTENTIAL TRANSCRIPTIONAL REGULATORY SEQUENCES IN INTRON5 OF THE RUNX1 GENE
Alarcón R, Rebolledo B, Martínez M, Stuardo M, Gutiérrez S

Room B

MICROBIOLOGY (MI-C09/16)

Chairpersons: José Luis Barra and Claudia Studdert

11:00-11:15
MI-C09
IDENTIFICATION OF A PLANT EXTRACT WITH INHIBITORY ACTIVITY AGAINST *S. enterica* PHOPQ SYSTEM
Viarengo G, Salazar M , Furlán RL, García Véscovi E

11:15-11:30
MI-C10
TRANSCRIPTIONAL REGULATION OF AGMATINE DEIMINASE PATHWAY IN *Enterococcus faecalis*
Suárez CA, Espariz M, Blancato VS, Magni C

11:30-11:45
MI-C11
ENTEROBACTIN PROTECTS *E. coli* FROM THE ROS-MEDIATED TOXIC EFFECTS OF PYOCHELIN
Adler C, Peralta D, Michavilla G, Pomares MF, Corbalan NS, De Cristóbal RE, Vincent PA

11.45-12:00
MI-C12
ROLE OF THE REPLICATION PROCESSIVITY FACTORß CLAMP IN THE MISMATCH REPAIR OF *Pseudomonas aeruginosa*
Monti MR, Miguel V, Borgogno MV, Argaraña CE

12:00-12:15
MI-C13
BIOFILM DEVELOPMENT IS INFLUENCED BY THE GLOBAL REGULATOR ANR IN *Pseudomonas extremaustralis*
Tribelli PM, Raiger Iustman LJ, Hay AG, Lopez NI

12:15-12:30
MI-C14
POLYPHOSPHATE DEGRADATION IN STATIONARY PHASE TRIGGERS BIOFILM FORMATION VIA AI-2 IN *E. coli*
Grillo-Puertas M, Rintoul MR, Rapisarda VA

12:30-12:45
MI-C15
FIRST GENETIC EVIDENCE OF OXALOACETATE DECARBOXYLASE INVOLVEMENT IN CITRATE METABOLISM IN FIRMICUTES
Repizo GD, Blancato VS, Magni C

12:45-13:00
MI-C16
PURINE METABOLISM AND POLYKETIDE PRODUCTION IN *Streptomyces coelicolor*
Navone L, Gramajo H, Rodríguez E

Room C

PLANTS BIOCHEMISTRY & MOLECULAR BIOLOGY (PT C01/06) and BIOTECHNOLOGY (C01/03)

Chairpersons: María Fabiana Drincovich and Mariana Del Vas

11:00-11:15
PT-C01
EXPRESSION AND REGULATION OF FLAVONOL SYNTHASES FROM MAIZE
Falcone Ferreyra ML, Questa J, Casas MI, Herrera AL, Grotewold E, Casati P

11:15-11:30
PT-C02
CHARACTERIZATION AND CDNA SEQUENCING OF A NOVEL FUNGAL SUBTILISIN WITH DEFENSE ELICITING ACTIVITY
Chalfoun NR, Grellet Bournonville CF, Martínez Zamora MG, Castagnaro AP, Díaz Ricci JC

11:30-11:45
PT-C03
DC1 DOMAIN PROTEINS INVOLVED IN DEFENSE RESPONSE AND FEMALE GAMETOPHYTE DEVELOPMENT IN *A. thaliana*
Pagnussat GC, Casalongue CA, Fiol DF

11:45-12:00
PT-C04
A NEW RETROGRADE PATHWAY THAT FUNCTIONS IN DROUGHT AND HIGH LIGHT SIGNALLING IN *Arabidopsis*
Estavillo G, Crisp PA, Pornsiriwong W, Wirtz M, Collinge D, Carrie C, Giraud E, Whelan J et al.

12:00-12:15
PT-C05
NON RACE-SPECIFIC RESISTANCE OF POTATO TO *Phytophthora infestans* INVOLVES DEVDASE ACTIVITY
Fernández MB, Daleo GR, Guevara MG

12:15-12:30
PT-C06
EXPLORING THE LINK BETWEEN EPIGENETICS AND ADAPTIVE STRESS RESPONSES IN PLANTS
González RM, Ricardo MM, Estévez JM, Iusem ND

12:30-12:45
BT-C01
PLANT HSP90 PROTEINS INTERACT WITH B-CELLS AND STIMULATE THEIR PROLIFERATION
Corigliano MG, Maglioco A, Laguia Becher M, Goldman A, Martin V, Angel SO, Clemente M

12:45-13:00
BT-C02
SELF-ASSEMBLED GANGLIOSIDE MICELLES AS NANODELIVERY VEHICLES OF TAXANES
Leonhard V, Alasino RV, Bianco ID, Beltramo DM

13:00-13:15
BT-C03
SEQUENTIAL LOADING OF PACLITAXEL (PTX) AND DOXORUBICIN(D) INTO GANGLIOSIDE MICELLES
Alasino RV, Leonhard V, Bianco ID, Beltramo DM

13:00-15:30 ***TIME FOR LUNCH***

15:30-16:30 ***SHORT LECTURES (Room B)***

"JUNIOR FACULTY"

Chairperson: Beatriz Caputto

José Manuel Estevez
IFIByNE-CONICET, Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires, Argentina
"The sweet growth of plant cells"

Javier E. Girardini
Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas Universidad Nacional de Rosario - Argentina
"Molecular bases of tumor aggressiveness: mutant p53 and the gateway to altered states"

16:30-19:00 ***POSTERS & COFFEE***

Cell Biology (CB P21/40)	Microbiology (MI P21/40)
Enzymology (EN P06/10)	Biotechnology (BT P10/19)
Lipids (LI P11/20)	Plants Bioch. & Mol. Biol. (PL P20/41)
Neuroscience (NS P08/13)	Signal Transduction (ST P07/12)

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

Sarah Spiegel
Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, School of Medicine, Richmond, Virginia, USA
"Sphingosine-1-phosphate: a multi-functional lipid mediator"
Chairperson: Hugo Maccioni

20:00-21:00 ***PLENARY LECTURE* (*Room A*)**

Alexandra Newton
Department of Pharmacology, University of California San Diego, USA
"Protein Kinase C signaling"
Chairperson: Silvia Moreno de Colonna

21:30 **SAIB GENERAL ASSEMBLY**

WEDNESDAY, November 2nd, 2011

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

Room A

PLANTS BIOCHEMISTRY & MOLECULAR BIOLOGY SYMPOSIUM
Chairpersons: Guillermo Santa María and Norberto Iusem

08:30-09:00 ***Daniel H. González***
Instituto de Agrobiotecnologia del Litoral - Univ Nac del Litoral, Santa Fe Argentina
"Homologues of COX assembly factors modulate copper homeostasis and stress responses in plants"

09:00-09:30 ***Jorge J. Casal***
IFEVA-CONICET, Facultad de Agronomía, Universidad de Buenos Aires; and Fundación Instituto Leloir, Ciudad Autónoma de Buenos Aires Argentina
"Light signaling networks in plants"

09:30-10:00 ***Alison Smith***
Department of Metabolic Biology, John Innes Centre, Norwich - United Kingdom
"How plants survive the night"

10:00-10:30 ***Birte Svensson***
Department of Systems Biology, Technical University of Denmark, Lyngby Denmark
"Starch degrading enzymes, inhibitors and the thioredoxin system in germinating barley seeds"

Room B

SIGNAL TRANSDUCTION SYMPOSIUM

Chairpersons: Norma Sterin and Cristina Paz

08:30-09:00 ***Silvia Rossi***
Laboratorio de Biología Molecular. Dpto. Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Argentina
"Proteins interacting with Protein Kinase A from *Saccharomyces cerevisiae*"

09:00-09:30 ***Claudia Tomes***
Laboratorio de Biología Celular y Molecular-IHEM-CONICET
Universidad Nacional de Cuyo, Mendoza Argentina
"Sperm exocytosis requires cAMP, Epac, intracellular calcium and multiple protein-protein interactions"

09:30-10:00 ***Alejandro Colman-Lerner***
Laboratorio de Fisiología y Biología Molecular. Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires Argentina
"Information transmission and gradient sensing in the *S. cerevisiae* pheromone response pathway"

10:00-10:30 ***Jin Zhang***
Department of Pharmacology and Molecular Sciences
The Johns Hopkins University School of Medicine USA
"Spatiotemporal regulation of protein kinases in living cells"

10:30-11:00 ***COFFEE BREAK***

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

Room A

CELL BIOLOGY (CB-C17/22) and NEUROSCIENCE (C01)

Chairpersons: Gladys Ciuffo and Carlos Arregui

11:00-11:15
CB-C17
HISTONE ACETYLATION ANALYSIS OF ETO GENE BREAKPOINT REGIONS INVOLVED IN T(8;21) FORMATION
Stuardo M, Gutiérrez S

11:15-11:30
CB-C18
CROSSTALK BETWEEN α AND δ PKC ISOFORMS AND RETINOIC ACID SYSTEM IN MALIGNANT PHENOTYPE REVERSION
Berardi DE, Díaz Bessone MI, Campodónico PB, Flumian C, Bal de Kier Joffé ED, Urtreger AJ, Todaro LB

11:30-11:45
CB-C19
UNDERSTANDING POSTTRANSLATIONAL MODIFICATIONS IN *G. lamblia*, ONE OF EARLIEST DIVERGENT EUKARYOTES
Vranych CV, Merino C, Touz MC, Rópolo AS

11:45-12:00
CB-C20
ALTERATION OF POLYAMINE METABOLISM IN THE EFFECT OF AZINPHOS-METHYL ON *Rhinella arenarum* DEVELOPMENT
Lascano CI, Ferrari A, Maiale S, Ruiz OA,Venturino A

12:00-12:15
CB-C21
***Chlamydia trachomatis* USES HOST AKT/AS160 PATHWAY TO ENSURE ITS DEVELOPMENT**
Capmany A, Leiva N, Gambarte J, Damiani MT

12:15-12:30
CB-C22
SEPARATION OF ACROSOME REACTED LIVING HUMAN SPERM BASED ON THE USE OF PSA-FITC AND FACS
Zoppino FCM, Halón ND, Mayorga LS

12:30-12:45
NS-C01
THE ROLE OF ALPHA-2M/LRP1 SYSTEM IN THE REGULATION OF MATRIX METALLOPROTEINASES IN MÜLLER CELLS
Barcelona PF, Jaldin Fincati JR, Chiabrando GA, Sanchez MC

Room B

MICROBIOLOGY (MI-C17/18) and LIPIDS (LI C01/06)

Chairpersons: Eleonora García Véscovi and María Sofía Gimenez

11:00-11:15
MI-C17
THE MCE1 PROTEINS OF *Mycobacterium tuberculosis* ARE INVOLVED IN THE UPTAKE AND METABOLISM OF LIPIDS
Forrellad MA, Klepp LI, Blanco FC, Santangelo MP, Bianco MV, Gutierrez M, Jackson M, Bigi F

11:15-11:30
MI-C18
INHIBITION OF DES TRANSCRIPTION BY CERULENIN IS MEDIATED BY THE LENGTH OF PHOSPHOLIPID ACYL CHAINS
Porrini L, Mansilla MC, de Mendoza D

11:30-11:45
LI-C01
THE TYROSINE PHOSPHATASE SHP2 REGULATES THE EXPRESSION OF THE ACYL-COASYNTHETASE ACSL4
Cooke M, Orlando U, Maloberti P, Podesta EJ, Cornejo Maciel F

11.45-12:00
LI-C02
PHOSPHATIDYLCHOLINE: STRUCTURAL AND SIGNALING ROLE IN NEURONAL DIFFERENTIATION
Paoletti L, Elena C, Banchio C

12:00-12:15
LI-C03
BIOSYNTHESIS OF VERY LONG CHAIN POLYENOIC FATTY ACID-CONTAINING SPHINGOLIPIDS IN GERMINAL CELLS
Furland NE, Oresti GM, Aveldaño MI

12:15-12:30
LI-C04
THE LIPID PROFILE IS A ROBUST INDICATOR OF FUNCTIONAL SENESCENCE IN THE MEDFLY *Ceratitis capitata*
Pujol-Lereis LM, Rabossi A, Quesada-Allué LA

12:30-12:45
LI-C05
NUCLEAR LIPID DROPLETS HAVE AN ACTIVE LIPID METABOLISM
Layerenza JP, Lagrutta LC, Sisti MS, Ves-Losada A

12:45-13:00
LI-C06
A COMPREHENSIVE STUDY OF PHOSPHOLIPID METABOLISM IN BREAST CANCER
Gómez del Pulgar T, Álvarez N, Cebrián A, Lacal JC

Room C

PLANT BIOCHEMISTRY & MOLECULAR BIOLOGY (PL C07/12) and BIOTECHNOLOGY (BT C04/06)

Chairpersons: Estela Valle and Juan Carlos Díaz Ricci

11:00-11:15
PL-C07
GENOMIC ANALYSIS OF THE STILBENE SYNTHASE GENE FAMILY THROUGH THE ELICITATION OF GRAPE CELL CULTURES
Lijavetzky D, Almagro L , Chialva CS , Belchi-Navarro S , Bru R, Martinez-Zapater JM , Pedreño MA

11:15-11:30
PL-C08
INCREASED TOLERANCE TO OXIDATIVE STRESS IN PLANTS EXPRESSING *IN VIVO* SUBSTRATE AMPLIFICATION SYSTEM
Giró M, Ceccoli R, Carrillo N, Lodeyro A

11:30-11:45
PL-C09
METAL ACCUMULATION IN SOYBEAN: METALLOTHIONE IN FAMILY CHARACTERIZATION
Pagani MA, Carrillo J , Reggiardo M , Tomas M , Capdevila M, Bofia R , Atrian S, Andreo CS

11.45-12:00
PL-C10
BASAL GENE TRANSCRIPTION CONTRIBUTES TO DISEASE TOLERANCE IN *Lotus japonicus* ECOTYPE GIFU
Bordenave CD, Babuin MF, Escaray FJ, Campestre MF, Rocco RA, Antonelli C, Serna E, Ruiz OA

12:00-12:15
PL-C11
RECENT HORIZONTAL TRANSFERS OF THE *COXI* INTRON IN *Solanaceae* DUE TO A FUNCTIONAL HOMING ENDONUCLEASE
Abbona CC, Kushnir S, Geelen D, Palmer JD, Sanchez Puerta MV

12:15-12:30
PL-C12
GREAT EXPANSION OF THE *MI-1* GENE CLUSTER IN *Solanum tuberosum*
Segura D, Sanchez Puerta MV, Masuelli RW

12:30-12:45
BT-C04
CRITICAL ASPECTS ON SCALING UP PHYTOREMEDIATION PROCESSES
Merini LJ, Giulietti AM

12:45-13:00
BT-C05
THERMOPHILES BIOCATALYSTS FOR ANTIVIRAL COMPOUNDS SYNTHESIS
Rivero C, De Benedetti E, Lozano M, Trelles J

13:00-13:15
BT-C06
IMMOBILIZATION OF LACTIC ACID BACTERIA AND THEIR APPLICATION IN GREEN BIOCATALYSIS
Britos C, Cappa V, Sambeth J, Lozano M, Trelles J

13:00-15:30 ***TIME FOR LUNCH***

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

Jean Philippe Vielle Calzada
LANGEBIO - Cinvestav, Unidad Irapuato, Mexico
"From sexuality to apomixis in plants: epigenetic regulation of gametogenesis in *Arabidopsis thaliana*"
Chairperson: Diego Gomez Casati

16:30 19:00 ***POSTERS & COFFEE***

Cell Biology (CB P41/59)
Signal Transduction (ST P13/19)
Lipids (LI P21/31)
Structural Biology (SB P01/04)
Microbiology (MI P41/53)
Biotechnology (BT P20/26)
Plant Bioch. & Mol. Biol. (PL P42/66)

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

Francisco Baralle
International Centre for Genetic Engineering and Biotechnology -Trieste, Italy
"New aspects of TDP 43 self-regulation mechanisms and their connection with neurological disease"
Chairperson: Alberto R. Kornblihtt

21:30 ***CLOSING DINNER***

L01.
PABMB Lecture
TRANSPORT OF ENDOCYTOSED CHOLESTEROL FROM LYSOSOMES INTO THE CYTOPLASM
Deffieu M, Pfeffer SR.
Department of Biochemistry, Stanford University School of Medicine, Stanford, California USA 94305. E-mail: pfeffer@stanford.edu

NPC1 protein is needed for cellular utilization of low density lipoprotein-derived cholesterol that has been delivered to lysosomes. The protein has 13 transmembrane domains, three lumenal domains, and a cytoplasmic tail. NPC1's lumenal, N-terminal domain binds cholesterol and is thought to receive cholesterol from NPC2 as part of the process by which cholesterol is exported from lysosomes into the cytosol. Using surface plasmon resonance and affinity chromatography, we have shown that the second lumenal domain of NPC1 binds directly to NPC2 protein. For these experiments, a soluble NPC1 lumenal domain 2 was engineered by replacing adjacent transmembrane domains with anti-parallel coiled coil sequences. Interaction of NPC2 with NPC1 lumenal domain 2 is only detected at acidic pH, consistent with the acidic environment where binding would occur. Binding to NPC1 domain 2 requires the presence of cholesterol on NPC2 protein, a finding that supports directional transfer of cholesterol from NPC2 onto NPC1's N-terminal domain. Finally, human disease-causing mutations in NPC1 domain 2 decrease NPC2 binding, suggesting that NPC2 binding is necessary for NPC1 function in humans. These data support a model in which NPC1 domain 2 holds NPC2 in position to facilitate directional cholesterol transfer from NPC2 onto NPC1 protein for export from lysosomes.

L02.
SOLS Lecture
THE RIBOSOME IS ENGAGED IN EUKARYOTIC SPECIFIC TRANSLATION REGULATORY MECHANISMS
Ballesta JPG.
Centro de Biología Molecular Severo Ochoa, CSIC and UAM, Cantoblanco, 28049 Madrid, España. E-mail: jpgballesta@cbm.uam.es

The eukaryotic ribosome is more complex than its prokaryotic counterpart. The function of the eukaryotic specific elements is largely unknown. The higher complexity of the eukaryotic ribosomal stalk, a 60S subunit domain involved in the soluble factor activity, has been shown to be involved in modulating the expression of specific proteins. These results led us to propose the existence of a eukaryotic specific translation regulatory mechanism based on the stalk ribosome heterogeneity. Ribosome subpopulations carrying diverse stalk compositions translate mRNAs with different efficiency. Data will be presented showing how evolution has generated a higher dynamism of the ribosomal stalk, essential for the new ribosome regulatory capacities. It will be also shown that the assembly process seems designed to generate a certain level of stalk heterogeneity, which can be regulated by controlling of the accumulation of the stalk components. Moreover, our stalk assembly analysis opens the possibility that heterogeneity can be present in other ribosomal domains, which are susceptible to be also involved in translation regulatory functions. Finally, recent results showing that free stalk ribosomal components can also participate in translation control mechanism will be presented.
All together, the data suggest that ribosome evolution has generated new translation regulatory possibilities.

L03.
EMBO Lecture
NEW ASPECTS OF TDP-43 SELF-REGULATION MECHANISMS AND THEIR CONNECTION WITH NEUROLOGICAL DISEASE
Baralle FE.
International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34149 Trieste, Italy.

Pre-mRNA splicing accounts for 20% of genomic changes that lead to inherited human diseases. We have studied pre-mRNA splicing defects on a variety of genes including CFTR, NF-1, and ATM. Recently, we have looked at a more complex system of mutually exclusive exons in the SCN8A and SCN9A genes. SCN8A encodes a sodium channel that may have a role in Multiple Sclerosis. Mutations in SCN9A cause pain-related diseases such as inherited erythromelalgia, paroxysmal extreme pain disorder, and complete insensitivity to pain. We have characterized various cis-acting sequences and trans-acting factors involved in SCN9A splicing.
Additionally we have studied the molecular mechanisms that control TDP-43 expression. This hnRNP was initially identified by us in 2001 as a regulator of CFTR exon 9 splicing. In 2006, TDP-43 was found as the major protein of intracellular inclusions of neurons of patients with Amyotrophic Lateral Sclerosis and Fronto Temporal Dementia. In the neurons of affected individuals, TDP-43 is mislocalized in the cytoplasm, ubiquitinated, hyperphosphorylated and cleaved. This may play a role in neurodegeneration through the loss of TDP-43 nuclear functions. We have observed that TDP-43 can regulate its own pre-mRNA levels by binding to specific regions in its 3'UTR, indicating that disease-associated TDP-43 aggregates may disrupt TDP-43 self-regulation.

L04.
Junior Faculty
THE SWEET GROWTH OF PLANT CELLS
Estevez JM.
IFIByNE, CONICET, Buenos Aires, Argentina. E-mail: jestevez@fbmc.fcen.uba.ar

The structural plasticity of carbohydrates is greater than that of amino acids but our understanding of the implications of such plasticity and how it relates to a potential biological function is still limited. We have examined the fundamental role that carbohydrates play in the growth of root hairs cells of *Arabidopsis thaliana*. Growing root hairs require intensive cell-wall changes to accommodate cell expansion at the apical end by a process known as **tip growth**. The plant cell wall is a very complex structure comprised of polysaccharides and hydroxyproline-rich glycoproteins (HRGPs) that include extensins (**EXTs**). Proline hydroxylation into hydroxyproline (Hyp), an early posttranslational modification of HRGPs that is catalyzed by prolyl 4- hydroxylases (**P4Hs**), defines the subsequent *O*-glycosylation sites in **EXTs. EXTs** are glycosylated on Hyp with 1-5 units of arabinose by specific arabinosyltransferases (**AraTs**). Biochemical inhibition or genetic disruption of **P4Hs**, **AraTs**, and **EXTs** resulted in the blockage of polarized growth in root hairs. Our results demonstrate that correct ***O*-glycosylation** on **EXTs** is essential for cell-wall self-assembly and, hence, root hair elongation. The changes that *O*-glycosylated cell-wall proteins undergo during growth represent a starting point to unravel the entire biochemical pathway involved in plant growth and development.

L05.
Junior Faculty
MOLECULAR BASES OF TUMOR AGGRESSIVENESS: MUTANT P53 AND THE GATEWAY TO ALTERED STATES
Girardini JE.
Instituto de Biología Molecular y Celular de Rosario. IBR-CONICET. E-mail: girardini@ibr.gov.ar

Cancer is a complex disease originated from alterations in the mechanisms that regulate cell behavior and tissue homeostasis. It is commonly accepted that such perturbations are the consequence of accumulating genetic and epigenetic alterations. The amazing heterogeneity of alterations found in tumors has obscured our understanding of the etiology of cancer, making elusive the identification of common mechanisms that may be targeted for clinical therapy. 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. P53 mutants acquire new oncogenic functions and concur to the development of a metastatic phenotype. We showed that mutant p53 is activated by an oncogenic circuitry transmitted by proline-directed phosphorylation. A critical component of this mechanism is the prolil isomerase Pin1, which binds mutant p53 and amplifies its function. The concerted action of Pin1 and mutant p53 leads to a global alteration of gene expression, inducing a transcriptional program that promotes cell migration and invasion. Since Pin1 overexpression is also a frequent alteration in human tumors, we believe that p53 mutation and Pin1 overexpression may act as molecular switches that promote the progression to aggressive tumor phenotypes.

L06.
Plenary Lecture
COORDINATION OF FUNGAL SECONDARY METABOLISM AND DEVELOPMENT
Braus GH.
Molecular Microbiology and Genetics, Georg-August-Universität, Göttingen, Germany.

Antimicrobial resistance is spreading but the number of newly discovered antibiotics is declining. The genomes of filamentous fungi comprise numerous putative genes and gene clusters for chemically and structurally diverse secondary metabolites which are never expressed under laboratory conditions. Secondary metabolism can be linked to fungal developmental programs in response to various abiotic or biotic external triggers. The velvet family of regulatory proteins (1,2) and the eight subunit COP9 signalosome complex (3,4) play key roles in coordinating secondary metabolism and differentiation processes as asexual or sexual sporulation and sclerotia or fruiting body formation. The velvet family shares a protein domain that is present in most parts of the fungal kingdom from chytrids to basidiomycetes. Most of the current knowledge derives from the model *A. nidulans* where VeA, the founding member of the protein family was discovered almost half a century ago. Knowledge about the coordinated control of secondary metabolism opens new opportunities to find novel bioactive molecules. The current state of the work in the laboratory will be presented.

1. Bayram *et al*., Science. 320, 1504-1506 (2008).
2. Sarikaya *et al*., PLoS Genet. 6, e1001226 (2010)
3. Braus *et al*., Curr. Opin. Microbiol. 13, 1-5 (2010).
4. Nahlik *et al*., Mol. Microb. 78, 962-979 (2010).

L07.
Plenary Lecture
MEMBRANE FUSION: 5 LIPIDS, 4 SNARES, 3 CHAPERONES, 2 NUCLEOTIDES, AND A GTPase, DANCING IN A RING
Wickner WT.
Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire, U.S.A.
E-mail: William.T.Wickner@Dartmouth.EDU

Proteins move between organelles by vesicular traffic, the budding of vesicles, traveling to target organelles, and selective fusion. Membrane fusion between vesicles and the plasma membrane underlies cell growth, hormone secretion, neurotransmission, and organelle biogenesis. Biological fusion occurs without lysis and preserves lumenal compartment identity. Fusion requires conserved proteins: SNAREs, which can bind to each other and stress the bilayer, chaperone proteins, GTPases (which regulate the process), and "effector proteins" for the GTPases. Specific lipids are vital for fusion, often by binding clusters of fusion-related proteins. Finally, fusion requires lipid bilayer remodeling. Molecular mechanisms will be reviewed, with particular emphasis on the neuron and yeast. The methodologies of this field will also be explored: 1. Enzymology, in which biochemical reactions which recapitulate the essential features of vesicle trafficking are created in a test tube and the relevant factors and reaction intermediates explored, 2. Structural biology, from visualization by fluorescence microscopy or electron microscopy to the structure of relevant proteins, and 3. Genetics, in which reaction catalysts are identified and functional relationships determined. How to find such a field when it is new, and how to attempt creative approaches, will be explored in the discussion.

L08.
Plenary Lecture
FROM SEXUALITY TO APOMIXIS IN PLANTS: EPIGENETIC REGULATION OF GAMETOGENESIS IN *Arabidopsis thaliana*
Vielle Calzada JP.
Langebio CINVESTAV Irapuato, México. E-mail: vielle@ira.cinvestav.mx

In flowering plants, gametogenesis presents an opportunity for natural selection to act on the haploid genome as an evolutionary force that is at the origin of mechanisms that ensure a tight regulation of reproductive development. Despite this selective pressure, there are numerous examples of naturally occurring developmental alternatives that suggest a flexible control of cell specification and subsequent gamete formation, often resulting in the generation of clonal seeds (apomixis). Our findings indicate that epigenetic mechanisms related to the activity of small RNA pathways play an essential role in cell specification and genome integrity. In the ovule of Arabidopsis, gametogenesis initiates from a single haploid product following meiosis. ARGONAUTE9 (AGO9) is a PIWI/PAZ protein that controls female gametogenesis by restricting the specification of gamete precursors in a non-cell autonomous manner. AGO9 preferentially interacts with 24 nt small RNAs (sRNAs) derived from transposable elements (TEs), and its somatic activity is required to silence TEs in the female gametophyte. These repetitive elements are located in the pericentromeric regions of all 5 chromosomes, suggesting a link between the AGO9-dependent sRNA pathway and heterochromatin formation. Our findings open new venues to explore the origins of the reproductive versatility that prevails in flowering plants

L09.
Plenary Lecture
PROTEIN KINASE C SIGNALING
Newton AC.
Department of Pharmacology, University of California at San Diego, La Jolla, California 92093-0721. E-mail: anewton@ucsd.edu

Precise control of the amplitude of protein kinase C signaling is essential to maintain cellular homeostasis and disruption of this control leads to pathophysiological states such as cancer. Two mechanisms critically control the amount and activity of protein kinase C in cells: 1. phosphorylation/dephosphorylation and 2. binding to lipid second messengers. First, a series of phosphorylation events prime conventional and novel protein kinase C isozymes into stable, signaling-competent species. Dephosphorylation is triggered by the PH domain Leucine Rich Repeat Protein Phosphatase, PHLPP, an event that destabilizes activated protein kinase C and initiates its down-regulation. Second, signals that cause phospholipid hydrolysis cause protein kinase C to bind to membrane lipids, an interaction that allosterically activates the kinase. Deregulation of either phosphorylation or lipid bindings alters the amplitude of protein kinase C signaling in the cell, resulting in pathophysiological states. This contribution focuses on the molecular mechanisms by which phosphorylation/dephosphorylation and lipid binding control protein kinase C.

L10.
Plenary Lecture
SPHINGOSINE-1-PHOSPHATE: A MULTI-FUNCTIONAL LIPID MEDIATOR

Spiegel S, Milstien S.
Department of Biochemistry and Molecular Biology and the Massey Cancer Center, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298, USA. E-mail: sspiegel@vcu.edu

Sphingosine-1-phosphate (S1P) is a potent sphingolipid mediator formed inside cells by phosphorylation of sphingosine (Sph) catalyzed by 2 Sph kinases (SphK), SphK1 and SphK2. The most well known actions of S1P are mediated by binding to a family of 5 G protein-coupled receptors ($S1P_{1-5}$). We have identified several novel S1P intracellular targets and shown that SphK1 and production of S1P is necessary for Lys 63-linked polyubiquitination of RIP1, phosphorylation of IKK and IkB , and IkB degradation, leading to NF- B activation, independently of S1P receptors. S1P specifically binds to TNF receptor-associated factor 2 (TRAF2), a key component in NF- B signaling triggered by TNF- , at the N-terminal RING domain and stimulates its E3 ligase activity (Alvarez *et al.*, *Nature* 2010). Thus, TRAF2 is another intracellular S1P target that is the missing co-factor for TRAF2 E3 ubiquitin ligase activity. This study highlights the key role of SphK1 and its product S1P in TNF- signaling and the canonical NF- B activation pathway and provides a mechanistic explanation for the importance of SphK1 in inflammatory, anti-apoptotic, and immune processes. We have found that S1P is also a cofactor for some other ubiquitin ligases that are critical for signaling by the pro-inflammatory cytokine IL-1. Our results suggest a new paradigm for regulation of Lys 63-linked polyubiquitination signaling by S1P.

This work was supported by grants from the NIH R37GM043880 and R01CA61774, NIH RO1AI500941 and U19AI077435.

NS-S01.

NEW PERSPECTIVES IN GLAUCOMA: UNRECOGNIZED SYMPTOMS AND LOOKING FOR NEW THERAPEUTIC STRATEGIES

Rosenstein RE, De Zavalía N, Keller Sarmiento MI, Lanzani F, Belforte N.
Laboratory of Retinal Neurochemistry & Exp. Ophthalmology, School of Medicine, CEFyBO, UBA, CONICET. E-mail: ruthr@fmed.uba.ar

Glaucoma is a leading cause of blindness, due to retinal ganglion cells (RGC) death and optic nerve damage. Retinal ischemia participates in glaucomatous damage. Recent evidences indicate that a population of RGC is intrinsically photosensitive (through the expression of a fotopigment, melanopsin), and transmits light information to the suprachiasmatic nuclei (SCN), the principal pacemaker for circadian rhythms. We analyzed: 1) the effect of ischemic conditioning on retinal damage induced by experimental glaucoma, and 2) the non-image forming visual system in experimental and human glaucoma. Weekly injections of vehicle or chondroitin sulfate were performed in the rat eye anterior chamber for 10 weeks. Ischemic conditioning was induced by weekly increasing intraocular pressure to 120 mmHg for 5 min. Brief ischemia pulses reversed the effect of glaucoma on retinal function and histology. Experimental glaucoma induced alterations in melanopsin levels, light suppression of nocturnal pineal melatonin, and light-induced c-Fos expression in the SCN. Glaucomatous animals exhibited an increase in the diurnal activity, and glaucomatous patients showed a significant decrease in the sleep quality. Conclusion: The induction of ischemic tolerance could constitute a new therapeutic strategy for glaucoma treatment. Glaucoma induced significant alterations in the non-image forming visual system.

NS-S02.

THE MEDIAL OLIVOCOCHLEAR SYSTEM AND PROTECTION FROM ACOUSTIC TRAUMA

Elgoyhen AB.
Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET), Argentina. E-mail: elgoyhen@dna.uba.ar

Sound-induced acoustic injury is one of the most common causes of hearing loss and tinnitus. Finding approaches to increase resistance to damage is a research field of great interest. The medial olivocochlear (MOC) pathway provides inhibitory feedback, through the release of acetylcholine (ACh) onto outer hair cells (OHCs) of the cochlea, thus reducing cochlear sensitivity. We have explored the MOC pathway's function by generating genetically modified mice carrying a mutation in the nicotinic acetylcholine receptor (nAChR) subunit expressed by OHCs. Mutant cells exhibited greater sensitivity to exogenous ACh and prolonged synaptic currents, indicating that the mutation enhanced nAChR function. To determine the consequences of this enhanced receptor function for cochlear responses, we measured auditory brainstem responses and distortion product otoacoustic emissions. The suppression of OHC-mediated amplification produced by stimulating the MOC pathway was enhanced and dramatically prolonged in mutant mice. Moreover, mutant mice had a greater resistance to permanent acoustic injury, indicating that activation of the MOC feedback can protect the inner ear from noise-induced damage. Thus, the efferent pathway provides a promising target for pharmacological prevention of inner ear pathologies derived from acoustic injury, such as hearing loss and tinnitus.

NS-S03.

TRANSCRIPTION FACTORS AND THE MEMORY FATE AFTER RETRIEVAL

De la Fuente V, Romano A.
Lab. Neurobiol. Memoria, Dpto. Fisiol., Biol. Mol. y Cel., FCEN-UBA. IIFIBYNE-CONICET. Buenos Aires. Argentina. E-mail: aromano@fbmc.fcen.uba.ar

In fear conditioning, aversive stimuli are readily associated with contextual features. Ones fear memory becomes consolidated, a brief reexposure to the training context causes reconsolidation, whereas a prolonged reexposure induces extinction. The regulation of hippocampal gene expression plays a key role in contextual memory consolidation and reconsolidation. However, the mechanisms that determine whether memory will reconsolidate or extinguish are not known. Recently, we demonstrate opposing roles for two evolutionarily related transcription factors in the mouse hippocampus. We found that nuclear factor-kB (NF-kB) is required for fear memory reconsolidation. Conversely, calcineurin phosphatase inhibited NF-kB and induced nuclear factor of activated T-cells (NFAT) nuclear translocation in the transition between reconsolidation and extinction. Accordingly, the hippocampal inhibition of both calcineurin and NFAT independently impaired memory extinction, whereas inhibition of NF-kB enhanced extinction. These findings represent the first insight into the molecular mechanisms that determine memory reprocessing after retrieval, supporting a transcriptional switch that directs memory toward reconsolidation or extinction. The precise molecular characterization of postretrieval processes has potential importance to the development of therapeutic strategies for fear memory disorders.

NS-S04.

HOW TO MAKE IT LAST LONGER? ON THE ROLE OF THE VTA-HIPPOCAMPUS LOOP IN MEMORY PERSISTENCE

Cammarota M.
Laboratory of Neurochemistry & Neurophysiology of Memory – CeMeM, PUCRS, Porto Alegre, Brazil. E-mail: mcammaro@terra.com.br

The hypothesis that entrance of recently acquired information into long-term memory (LTM) depends on dopamine mechanisms mediated by VTA/hippocampus interactions has been postulated on the basis of theoretical considerations. In my talk I will present data showing that the role of these interactions is broader than previously thought and that, indeed, persistence of LTM depends on the activation of a VTA/hippocampus dopaminergic loop and can be specifically modulated by manipulating it, or its afferents, at definite post-learning time points. These findings are essential for understanding the behavioral and neurochemical basis of persistent behaviors, as well as for the development of drugs and therapies able to modulate not just the formation but, importantly, the persistent storage of the engram.

MI-S01.
Streptomyces venezuelae AS A GENETIC AND DEVELOPMENTAL SYSTEM
Buttner MJ, Bibb MJ, den Hengst CD, Bassam MA, Chandra G, Domonkos A.
Department of Molecular Microbiology, John Innes Centre, Colney, Norwich, NR4 7UHUK.

Very significant progress is being made in understanding the cell biological processes underlying morphogenesis in the sporulating, filamentous bacteria *Streptomyces*, and all of the *bld* and *whi* developmental master regulators defined by classical mutant hunts have been cloned and characterised. A major challenge now is to connect the developmental cell biological processes to these master regulators by dissecting the regulatory networks that link the two. In the classical model species, *S. Coelicolor*, differentiation occurs on solid medium but not in liquid. Further, the differentiating part of the colony (the aerial mycelium) constitutes only ~5% of the total biomass. This makes the detection of subtle changes in the transcriptome associated with sporulation problematic, and makes the application on ChlP-seq to sporulation-specific transcription factors impractical in *S. coelicolor* in most cases. In contrast, *S.venezuelae* sporulates to completion in liquid culture (>90% biomass differentiates into spores), and thus provides an unusual opportunity to bring the full power of post-genomic technologies to the analysis of synchronously developing submerged *Streptomyces* cultures. We have extensively developed *S. venezuelae* as a new model species for the genus and used this system to begin to charactirize the regulatory networks that underpin morphological differentiation in *Streptomyces.*

MI-S02.
SIMILITARITIES OF THE MECHANISMS CONTROLLING CARBON METABOLISM AND VIRULENCE IN BACTERIA
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The phosphoenolpyruvate: carbohydrate phosphotransferase system (PTS) catalyzes the uptake and concomitant phosphorylation of sugars in bacteria. It is usually composed of five proteins or protein domains, which also carry out numerous regulatory functions. They are involved in carbon catabolite repression, inducer exclusion and other regulatory processes related to carbon metabolism. In addition, our recent results established that mechanisms similar to those regulating carbon metabolism also control the virulence of certain pathogens. For example, we observed that deletion of the general PTS components EI or Hpr prevents the expression of the *Brucella melitensis virB* genes, which encode a type IV secretion system essential for the virulence of this pathogen. In *Listeria monocytogenes* the repressive effect of efficiently metabolizable carbon sources, such as glucose, fructose, cellobiose, etc., on the expression of PrfA-controlled virulence genes seems to be mediated via PTS components. We identified the ManL protein as potential interaction partner for the transcription activator PrfA. Finally, Hpr of *Neisseria meningitidis* was found to interact withthe transcription regulator CrgA, which controls the expression of the capsule and pili genes of this organism. Indeed, deletion of Hpr lowers capsule synthesis and increases the adhesion of *N. meningitidis* to its host cells.

MI-S03.
CHEMORECEPTORS: CONSERVED DESIGN FOR THE DETECTION OF MULTIPLE SIGNALS
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Bacteria seek for favorable conditions by modulating their swimming mode in response to environmental signals. Chemoreceptors detect changes in the concentration of a great variety of chemicals and transmit the information to the flagellar motors through a phosphorylation cascade.
We are interested in the architecture of the large signaling arrays composed by chemoreceptors of different specificities and their associated signaling proteins. These arrays are typically located at the poles of the cell and are very well conserved in prokaryotes. With a relatively simple design, they are capable of remarkable sensitivity along a broad operation range.
The cytoplasmic domain of chemoreceptors consists in a long alpha-helical hairpin that forms, in the dimer, a coiled-coil four-helix bundle. Chemoreceptors from different microorganisms share the basic structure but differ in the length of the cytoplasmic domain. In our laboratory, *in vivo* crosslinking approaches have been used to elucidate the precise arrangement of the signaling complexes. In *E.coli* cells, we analyze the signaling abilities and higher order organization of complexes containing native receptors, as well as receptors belonging to different classes or engineered receptors with an altered cytoplasmic domain. The ability of heterologous receptors to form active signaling complexes with *E.coli* proteins highlights the striking conservation of the pathway and opens a way towards the elucidation of the specificity of hundreds of uncharacterized receptors present in nature.

MI-S04.
BLUE LIGHT ACTIVATED HISTIDINE KINASE: A NOVEL TWO-COMPONENT SENSORIN *Brucella* spp.
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Flavin containing LOV (light, oxygen, or voltage) domains are distributed in the three kingdoms of life (Eukarya, Archaea, and Bacteria). LOV domains function as light-sensory modules in plant and algal phototropins and in fungal blue-light receptors. In most cases, the LOV domain is the primary sensory module that conveys a signal to protein domains with known or putative functions as diverse as regulation of gene expression, regulation of protein catabolism, and activation of serine/threonine kinases in eukaryotes and histidine kinases in prokaryotes. Histidine kinases, used for environmental sensing by bacterial two-component system, are involved in regulation of bacterial gene expression, chemotaxis, phototaxis, and virulence. We have discovered that the prokaryotes *Brucella melitensis, Brucella abortus, Erythrobacter litoralis* and *Pseudomonas syringae* contain light-activated histidine kinases (LOV-HK) that bind a flavin chromophore and undergo photochemistry indicative of cysteinyl-flavin adduct formation. Infection of macrophages by *B. abortus* was stimulated by light in the wild type but was limited in photochemically inactive and null mutants of LOV-HK, indicating that the flavin-containing histidine kinase functions as a photoreceptor regulating *B. abortus* LOV-HK appears to function as a photoreceptor that is directly related to *Brucella* survival and replication within macrophages. We are studying the downstream signaling partners of the LOV-HK photosensor. Recently, we identified a responseregulator that is specifically phosphorylated by light-activated LOV-HK. Although high-resolution structures are available for a number of histidine kinase domains, it is not known how signal information is communicated from the sensor domain to the kinase domain, and how autophosphorylation is initiated. We are also conducting structural studies of the *Brucella* LOV-HK in order to answer this question.

LI-S01.
FUNCTIONS OF PLASMA MEMBRANE PHOSPHATIDYLINOSITOL 4-PHOSPHATE
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Phosphatidylinositol 4-phosphate (PI4P) was first discovered in the 1960s, and its principal function was (and still is) believed to be to act as the essential synthetic precursor to the multi-functional lipid PI(4,5)P2. Since then a range of functions for, and effectors of, PI4P have been described in intracellular compartments, particularly the Golgi apparatus, and PI4P has been quantified there, and the regulation of its synthesis studied. However, PI4P in the plasma membrane has eluded unambiguous identification, although it has always been assumed that it must be there (not least, to make PI4,5)P2).
We recently described protocols that enable us to quantify PI4P in the plasma membrane (Hammond *et al.,* Biochem J. 422, 23-35; 2009), and in this talk we will discuss advances in our understanding of plama membrane PI4P that have ensued from this quantification. In brief, we show that the detectable PI4P is not acting as a precursor to PI(4,5)P2: the PI4P that is 5-phosphorylated appears to be synthesised when required and then immediately phosphorylated. Rather, the majority of PI4P acts together with PI(4,5)P2 as an essential but independent lipid determinant of plasma membrane identity. We suggest that it may act as a stable negative charge to keep a wide range of signalling proteins attached to the plasma membrane while PI(4,5)P2 levels vary during its many signalling functions.

LI-S02.
c-Fos PHYSICALLY ASSOCIATES AND ACTIVATES SPECIFIC ENZYMES OF THE PATHWAY OF PHOSPHOLIPID SYNTHESIS
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We previously showed that c-Fos has, in addition to its well known AP-1 transcription factor activity, the capacity to associating to the endoplasmic reticulum (ER) and activating key enzymes of phospholipid and glycolipid synthesis in events related to normal growth or the exacerbated growth characteristic of tumor cells. c-Fos/ER-association and consequently its lipid activation capacity is regulated by the phosphorylation state of c-Fos tyrosine residues. Herein we show that c-Fos physically associates to the enzymes that it activates; no interaction is observed between c-Fos and enzymes it does not activate.
c-Fos activates and immunoprecipitates CDP-DAG synthase (CDS) and PtdIn 4K (PI4K) but not PtdIn Synthase (PIS). Direct physical association was confirmed by FRET (Fluorescence Resonance Energy Transfer) assays.Positive FRET was observed between c-Fos and CDS and c-Fos with PI4K but not with PIS. The N-terminal of c-Fos is required for its association to these enzymes whereas its basic domain (amino acids 139-159) is responsible for enzyme activation.
c-Fos expression is tightly regulated by specific environmental cues, assuring that lipid metabolism activation will occur as a response to cell requirements. Thus, c-Fos emerges as an important regulator of key membrane metabolisms in membrane-biogenesis demanding processes such as normal and exacerbated growth.

LI-S03.
CHOLINE KINASE , A NOVEL MOLECULAR TARGET IN CANCER THERAPY
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Human choline kinase (ChoK) and (ChoK) are members of the choline kinase family. In humans, this family is encoded by two genes, CHKA and CHKB, resulting in three different proteins with a choline kinase domain: ChoK 1, ChoK 2 and ChoK 1. The presence of the ChoK domain confers the capacity to phosphorylate choline (Cho) to phosphocholine (PCho), the first step in the biosynthesis of phosphatidylcholine (PC), the major phospholipid in eukaryotic membranes. The implication of ChoK in cell proliferation, initiation and progression of cancer is well documented. Furthermore, it is overexpressed in breast, lung, colon, prostate, bladder and ovary cancers.
Pharmacological inhibition of ChoK has been proposed as a novel antitumoral strategy. ChoK inhibitors have potent antiproliferative activity in tumor cells, and induce a strong reduction of tumor growth in mice xenografts. The first ChoK inhibitor has recently entered Phase I clinical trials. Therefore ChoK has become an important subject in cancer research, both as a tumor marker and as a new molecular target for the design of novel promising anticancer strategies. Determination of ChoK levels or activity may become an important novel strategy for the diagnosis and prognosis of a large variety of human tumors. The development of effective and specific approaches to inhibit ChoK may become an important anticancer treatment.

LI-S04.
STAYING ALIVE IN THE RETINA: CRUCIAL ROLES FOR SIMPLE SPHINGOLIPIDS
Rotstein NP, Abrahan CE, Agnolazza D, Navallas S, German OL, Politi LE, Miranda GE.
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The apoptotic death of photoreceptors (PHRs) is a hallmark of retina neurodegenerative diseases that lead to irreversible visual loss. Uncovering the pathways and mediators that induce or prevent this death is essential for finding new treatments for these diseases. In the last two decades several simple sphingolipids were shown to act as bioactive lipids, regulating vital cellular processes. We have uncovered crucial roles for these sphingolipids in the regulation of PHR survival and development in the retina. We showed that both ceramide (Cer) and sphingosine (Sph) are key mediators of PHR apoptosis upon oxidative stress; blocking their synthesis or decreasing their levels effectively prevents PHRs death. On the contrary, their phosphorylated derivatives, sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) promote PHR survival, stimulate the proliferation of PHR progenitors and enhance their differentiation. Moreover, PHR trophic factors, such as docosahexaenoic acid, modulate the levels or activity of several enzymes in the complex sphingolipid metabolism to lower Cer and Sph content and increase S1P levels in PHRs, thus promoting their survival and differentiation. Hence, we propose that regulation of the metabolism of bioactive sphingolipids to tilt the balance towards the synthesis of S1P and C1P might provide a critical tool to rescue PHRs in retina degenerations

CB-S01.
ErbB RECEPTOR SIGNALING IN CANCER: NOVEL ROLES FOR PKC AND THE Rac-GEF P-Rex1
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A main goal in our laboratory is the identification of signaling molecules implicated in oncogenic transformation. In recent studies we established key roles for the diacylglycerol-regulated kinase PKC in cancer cell proliferation driven by ErbB receptors. PKC is up-regulated in epithelial cancers and controls the expression of genes involved in cell cycle progression and survival. Prostate-specific PKC transgenic mice develop proneoplastic lesions, and invasive adenocarcinomas when expressed in a Pten-null background. These lesions display hyperactivation of the Akt, NF- B, and Stat3 pathways. PKC is also required for Rac activation and motility in response to ErbB ligands. In a search for novel ErbB receptor effectors we identified the Rac exchange factor (Rac-GEF) P-Rex1 as a key player of mammary cell transformation and motility. P-Rex1 is highly expressed in human breast tumors, particularly those with high ErbB2 and ER levels, and in lymph node metastases. Depletion of P-Rex1 from breast cancer cells affects actin cytoskeleton reorganization, growth in soft agar, and tumorigenesis. Remarkably, activation of P-Rex1 in breast cancer cells requires the convergence of inputs from ErbB receptors and a CXCR4/G /PI3K -dependent pathway. In summary, our studies identified novel ErbB receptor signaling effectors that play key roles in cell cycle progression, survival and motility.

CB-S02.
FBH1 IS REQUIRED TO INACTIVATE STALLED DNA REPLICATION FORKS UPON REPLICATION STRESS
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Proper resolution of stalled replication forks is essential for genome stability. Purifications of the FBH1 helicase identified the RPA complex as novel interacting proteins. Following replication stress, such as hydroxyurea (HU) treatment, the RPA complex binds single stranded DNA (ssDNA). While both control and FBH1-depleted cells exhibited equal ATR-mediated signaling following extended HU treatment, when compared to control cells, FBH1-depleted cells showed significantly fewer double strand breaks, no activation of ATM or DNA-PK, and no phopshorylation of RPA2 on Ser4/8. Proper phopshorylation of RPA2 on Ser4 and Ser8 required FBH1 helicase activity. During recovery from prolonged HU treatment, FBH1-depleted cells displayed rapid RPA unloading and decreased levels of fork inactivation, ssDNA, g-H2AX foci, and cell death. While less sensitive to chronic DNA replication stress, FBH1-depleted cells exhibited a striking increase in mutation rate. Finally, deletions of FBH1 itself, including focal and a helicase domain point mutation, were detected in 47% of the melanoma cell lines. Significantly, melanoma cell lines in which FBH1 was hemizygously deleted or mutated displayed the same HU-resistant phenotype as FBH1-depleted cells. We propose that FBH1 is required for the inactivation of stalled replication forks and maintenance of genome stability.

CB-S03.
TELOMERE MAINTENANCE DURING THE CELL CYCLE
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During the last few years we have learned that normal long telomeres require an interaction with DNA damage and repair proteins for their replication and maintenance. It has been shown that DNA-PK and Ku70/Ku80, essential components of the Non-Homologous End Joining (NHEJ) pathway, not only interact with human telomeres but also are essential in keeping their stability. Other PI3-like kinases such as ATM and ATR have been linked to specific steps of the replication or processing of normal long telomeres, however there is no molecular model that explains the role of the DNA-PK complex in the maintenance of chromosome ends. Here we show that DNA-PKcs recruitment to the telomeric chromatin occurs in a biphasic manner during the cell cycle in primary human cells. Knockdown of Ku70 confirmed that DNA-PK could be recruited to the telomeres without an interaction with the Ku70/Ku80 heterodimer. Also an increase in DNA repair proteins on the telomeres during their replication was observed when DNA-PKcs levels were lowered via shRNA. Finally we found that knockdown of DNA-PKcs and Ku70/86 proteins but not DNA LigaseIV affects the TERRA (telomeric-repeat containing RNA) steady-state in human cells. Thus, these findings suggest that DNA-PKcs plays a role in telomere replication and processing and that some of its functions at the chromosome ends are independent of the KU70/86 proteins.

CB-S04.
NOVEL REGULATORS OF THE REPLICATION OF DAMAGED DNA
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While it is clear that DNA lesions trigger the activation of many signalling pathways that aid DNA replication, the levels of coordination between such pathways are unknown. On one hand, stalled forks trigger the activation of checkpoint signals to globally reduce DNA synthesis. On the other hand, translesion DNA synthesis (TLS) avoids fork stalling using DNA lesions as templates. While TLS promotes the maintenance of DNA replication on individual replicating forks, one single stalling event spreads a checkpoint-induced warning signal to other replicating forks. Both pathways are activated in the same cells but it is unknown if they can coordinate their action at single replicating forks.
We have recently found that two molecules associated to checkpoint activation can influence TLS activation. We have accumulated evidence indicating that a checkpoint target, the cyclin kinases inhibitor p21, is a negative regulator of TLS associated events. Also, we have found that a central checkpoint effector kinase, Chk1, positively modulates the same events. Intriguingly the PCNA domain of both proteins is required for their action on TLS. Thus, we propose that the crosstalk between molecules in both pathways might determine the timing of their activation at forks that encounter DNA lesions.

PL-S01.
HOW PLANTS SURVIVE THE NIGHT
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Although plants make sugars from atmospheric carbon dioxide in the process of photosynthesis, they face problems of carbohydrate supply on a daily basis. Plants can photosynthesise only during the day - every night all of the cells of the plant become dependent upon the mobilisation of carbohydrate (in the form of starch) synthesised and stored during the day. Mutant plants that cannot synthesise starch during the day or cannot degrade it at night usually have reduced growth rates. My lab is trying to understand the diurnal control of starch storage and mobilisation in leaves of the model plant Arabidopsis, using forward and reverse genetic approaches. I will present our progress in defining the surprisingly complex pathway of starch degradation at night, and discuss how flux through this pathway is controlled to ensure that supplies of carbohydrate last until dawn. Our recent work shows that the circadian clock plays a central role in controlling carbohydrate availability at night, and this in turn determines the overall productivity of the plant.

PL-S02.
STARCH DEGRADING ENZYMES, INHIBITORS AND THE THIOREDOXIN SYSTEM IN GERMINATING BARLEY SEEDS
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The germinating barley seed represents a highly dynamic biological system where dedicated enzymes and regulatory proteins participate in the mobilisation of storage compounds and growth of the emerging plantlet. Insight at the molecular level in structure, function and protein-protein interactions for the starch degrading enzymes ?-amylase and limit dextrinase and their endogenous specific proteinaceous inhibitors will be presented and the connection to the thioredoxin/NADPH-dependent thioredoxin reductase system will be discussed. This involves protein crystallography, mutational analysis of structure/function relationships, binding analysis by aid of surface plasmon resonance and proteomics-based identification of thioredoxin target disulfide bonds of different barley seed proteins.
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Maeda, K. *et al*. Structure 14, 1701-1710 (2006).
Shahpiri A. *et al*. Plant Physiol. 146, 789-799 (2008).
Vester-Christensen, M.B. *et al*. J. Mol. Biol. 403, 739-750 (2010).

PL-S03.
HOMOLOGUES OF COX ASSEMBLY FACTORS MODULATE COPPER HOMEOSTASIS AND STRESS RESPONSES IN PLANTS
Gonzalez DH, Garcia L, Welchen E, Attallah CV.
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Plant genomes encode putative homologues of mitochondrial proteins involved in copper delivery for cytochrome c oxidase (COX) biogenesis. Two Arabidopsis HCC genes, originated by an ancient duplication in plants, encode proteins with homology to SCO proteins, involved in copper insertion into COX2. Mutation of HCC1 is embryo lethal and produces a loss of COX activity. In turn, HCC2 mutants develop normally and do not show changes in COX2 levels. These plants display increased sensitivity to copper and higher expression of MIR398 and other genes induced by copper limitation, even if they have a higher copper content than wild-type. They also show increased expression of stress-responsive genes. The results suggest that HCC1 is the protein involved in COX biogenesis while HCC2, that lacks a conserved copper binding motif, functions in copper sensing and redox homeostasis. We also used artificial miRNAs to obtain plants with reduced expression of COX17 and COX19, that encode copper chaperones from the intermembrane space. These plants show normal COX2 levels, but altered expression of copper- and stress-responsive genes, increased lipid peroxidation and increased sensitivity to salt and high-light stress. We postulate that plant mitochondrial copper chaperones have roles beyond COX biogenesis and participate in the regulation of copper homeostasis and stress responses within plant cells

PL-S04.
LIGHT SIGNALLING NETWORKS IN PLANTS
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Light and temperature are arguably the most important variables of the environment providing cues for the adjustment of plant body form and function to the prevailing conditions. Light and temperature changes represent both a challenge and an opportunity for plants; a challenge, because they can generate physiological stress; an opportunity, because the anticipated perception of changes in the environment provides information that helps plants to adjust to the incoming stressful conditions. Phytochromes and cryptochromes are the main plant photoreceptors. Downstream signalling involves a complex network where the photoreceptors repress negative regulators of photomorphogenesis. These pathways are connected to the hormone signalling pathways, creating complex networks that link light signals to physiological responses. Our current interest is to understand the molecular mechanisms by which plants can integrate different environmental signals controlling the shape and function of their body

ST-S01.
SPATIOTEMPORAL REGULATION OF PROTEIN KINASES IN LIVING CELLS
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The complexity and specificity of many forms of signal transduction are widely suspected to require spatial microcompartmentation and dynamic modulation of the activities of protein kinases. To achieve dynamic tracking of signaling activities in living cells, genetically encoded fluorescent reporters for protein kinase activities have been engineered. These powerful tools revealed oscillatory activity of Protein Kinase A in living cells, and differential regulation of Protein Kinase B in plasma membrane microdomains. These studies provided new insights into the spatiotemporal regulation of protein kinases in their native biological contexts.

ST-S02.
INFORMATION TRANSMISSION AND GRADIENT SENSING IN THE *Saccharomyces cerevisiae* PHEROMONE RESPONSE PATHWAY
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We studied the quantitative behavior of a prototypical information sensing and decision-making system, the mating pheromone response pathway in yeast. To locate a partner, yeast measure external mean concentration and direction of the pheromone gradient. To study concentration determination, we used fluorescent protein fusions, and monitored signal propagation in single cells with seconds to minutes time resolution. We quantified by microscope-based cytometry membrane recruitment of the MAP kinase cascade scaffold Ste5 and activation of the transcription factor Ste12. We found that this system relays information precisely and that this behavior relies on negative feedback originating on the MAP kinases. For gradient determination, yeast measures on which side there are more bound receptors. This sensing modality only works in non-saturating concentrations. However, yeast orient in the direction of the gradient even in very high external average concentrations. Surprisingly, our numerical simulations show that the slow binding dynamics of pheromone to its receptor enhances gradient determination in saturating conditions, resulting in more precise polarizations than comparatively faster binding rates. Our results show that eukaryotic systems employ system level regulatory mechanisms to ensure faithful information transmission even in saturating conditions.

ST-S03.
SPERM EXOCYTOSIS REQUIRES cAMP, Epac, INTRACELLULAR CALCIUM AND MULTIPLE PROTEIN-PROTEIN INTERACTIONS
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Exocytosis consists of multiple stages that include tethering and docking of the vesicle to the plasma membrane, priming of the fusion machinery, and calcium-triggered opening of fusion pores. Our lab is interested in unveiling the molecular mechanisms that drive exocytosis in sperm, a model where signaling cascades are mostly unidirectional and irreversible. We have established a plasma membrane permeabilization protocol that grants access to intracellular compartments and permits monitoring of the secretory activity following sequential application of different molecular tools, a procedure that is not readily feasible in more complex exocytotic cells or in intact sperm. Thanks to this protocol, we have shown that human sperm exocytosis relies on the assembly of a proteinaceous fusion machinery that includes Rab3 and 27, -SNAP, NSF, SNAREs, complexin, Munc18 and synaptotagmin VI as well as kinases and phosphatases. This exocytosis also requires cAMP and calcium (from the extracellular medium and from IP3-sensitive intracellular stores). The relevant cAMP target is Epac, a GEF for Rap. We have identified a novel connection between Epac and another small GTPase, Rab3. Epac sits at a critical point during the exocytotic cascade after which the pathway splits into two limbs, one that assembles the fusion machinery into place and another that elicits intracellular calcium release

ST-S04.
PROTEINS INTERACTING WITH PROTEIN KINASE A FROM *Saccharomyces cerevisiae*
Rossi S, Galello F, Gonzalez Bardeci N, Moreno S.
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Subcellular targeting through the association with adaptor and scaffolding proteins has emerged as a key mechanism in signaling specificity. Compartmentalization of cAMP-PKA pathway is maintained by A kinase anchoring proteins (AKAPs) which interact with PKA regulatory subunit (R). PKA AKAPs in mammals are classified in RII, RI or dual specificity. In *S.cerevisiae* the anchoring of PKA is poorly understood. We have identified proteins that tether the R subunit (Bcy1) from yeast PKA, *in vitro* and *in vivo*. The characterization of the Bcy1-binding domains showed that although -helices were predicted, they do not show the characteristics of the amphipathic helix of PKA AKAPs. The key residues for the interaction are positively charged residues. These characteristics are shared with RISR (RI Specifier Region) present in dual specificity AKAPs. The amino termini of R subunits constitute the so called D/D domain, responsible for its dimerization and its interaction with anchoring proteins. Chemical crosslinking experiments confirm that Bcy1 exists as a dimer, via its N-terminus. The N-terminus of Bcy1 is necessary for the interaction with the tethering proteins of yeast PKA. The modelling by homology of the N-terminus of Bcy1 shows that it shares with its counterparts the residues important for dimerization but that the exposed surface has characteristics of its own.

CB-C01.

A NOVEL MURINE XENOGRAFT MODEL FOR THE STUDY OF HUMAN BREAST CANCER

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A number of studies investigating the role of COX-2 in breast cancer are based on models exhibiting endogenously high COX-2 levels. The benefit of COX-2 inhibitors is being seen as a great promise in cancer therapy however, there have been recent concerns about potential cardiotoxicity. The aim of our study was to develop a novel murine xenograft model of human breast cancer based on a cell line, MCF-7, that fails to form tumors when injected into female athymic mice due to its low aggressive phenotype and on the MDA-MB-231 cell line, known to form tumors upon injection. Stable transfection of MCF-7 cells with the acyl-CoA synthetase 4 (ACSL4) cDNA transformed cells from a non-aggressive into a highly aggressive phenotype providing first-time evidence on the key role of ACSL4 in the regulation of COX-2 expression and on the proliferation and metastatic potential of cancer cells. Injection of ACSL4-transformed MCF-7 cells to female athymic mice resulted in the development of mammary tumors. Treatment of MDA-MB-231 tumor-bearing mice with a combination of ACSL4, LOX and COX-2 inhibitors resulted in a significant reduction of tumor growth with a sinergystic effect of the inhibitors. We conclude that this murine xenograft model of breast cancer may be useful for the evaluation of preclinical safety and efficacy of novel adjuvant therapies for women with metastastic breast cancer.

CB-C02.

HORMONE-DEPENDENT MITOCHONDRIAL FUSION REGULATES THE TRANSLOCATION OF ERK TO THE MITOCHONDRIA

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Mitochondrial dynamics (fusion-fission) is important for maintaining the integrity of these organelles and many cellular processes. The activation of the cAMP signal leads to a translocation of PKA and ERK to the mitochondria to form a multiprotein complex necessary for cholesterol transport. However, neither PKA nor ERK present mitochondrial targeting signal. The aim of this study is to evaluate if hormonal stimulation could promote mitochondrial morphology changes that might regulate specialized cellular functions. We transfected MA-10 Leydig cells with a specific mitochondrial marker, mtYFP and visualized the samples by confocal microscopy. Hormonal stimulation, with hCG or cAMP (permeable analogue of cAMP) promotes a clear rearrangement of the mitochondrial network, from a cluster type in control cells to a fusion-like tubular shape. Treatment of the cells with CCCP, a proton ionophore, demonstrates that the mitochondrial membrane potential is required for this reorganization. Also, mitochondrial rearrangement produces the association of the acyl-CoA synthetase 4 with the organelle. The protein tyrosine phosphatase SHP2 knock-down abolishes the morphological changes and translocation of ERK. Thus, it seems that SHP2 may control mitochondrial dynamics allowing the recruitment of crucial mitochondrial proteins to generate a multiprotein complex that regulates cellular functions.

CB-C03.

REGULATION OF CHKA EXPRESSION DURING RA-INDUCED NEURONAL DIFFERENTIATION. A MAJOR ROLE FOR C/EBP

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Neuronal differentiation is accompanied by changes in gene expression that mediate the redirection leading to neurite formation and function. Acceleration of membrane phospholipid synthesis is associated with neurite elongation, and phosphatidylcholine (PtdCho) is the major membrane phospholipid in mammalian cells. The transcription of two genes encoding key enzymes in the CDP-choline pathway for PtdCho biosynthesis are stimulated; the Chka gene for choline kinase (CK) alpha isoform and the Pcyt1a gene for the CTP:phosphocholine cytidylyltransferase (CCT) alpha isoform. By analysis of reporter constructs we demonstrated that the increased binding of the transcription factor C/EBPb to the Chka-proximal promoter is in part responsible for the increased transcription during RA-induced differentiation of Neuro-2a cells. Besides, C/EBPb overexpression is able to induce CKa expression and promote neuronal differentiation.
This is the first elucidation of the mechanism by which the expression of Chka is regulated during neuronal differentiation.

CB-C04.

DECIPHERING THE ROLE OF CNBP DURING ZEBRAFISH CRANIOFACIAL DEVELOPMENT

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The zinc-finger cellular nucleic acid binding protein (CNBP) plays essential roles during rostral head development. Knock down of CNBP adversely affects craniofacial cartilage development. Craniofacial cartilages derive from the cranial neural crest (CNC). Our main goal was to elucidate the CNBP role in CNC development using zebrafish (*Danio rerio*) as animal model. Transiently constitutive CNBP gain-of-function (by over-expressing wild-type CNBP) and loss-of-function (by over-expressing a CNBP dominant negative isoform) adversely affected the zebrafish development. Mild affected larvae (over-expressing both CNBP variants) displayed most of the head cartilages shorter in length while those larvae with stronger phenotypes had their cartilaginous craniofacial skeleton highly malformed. Moreover, over-expression of both forms of CNBP specifically modified the expression patterns of typical CNC marker genes, as seen in whole-mount in situ hybridization. Zebrafish transgenic lines over-expressing both variants were generated by using the Tol2 transposon vector system. Heterozygous F1 siblings of up to three months showed no visible phenotypes. Further analysis of these transgenic lines, together with the generation of cold-shock inducible lines over-expressing the above-mentioned CNBP forms, will allow us to complete our study about the *in vivo* participation of CNBP in CNC development.

CB-C05.
ANALYSIS AND VALIDATION OF CNBP TARGET GENES IN *Danio rerio*
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CNBP is a nucleic acid binding protein capable of chaperoning the formation of single-stranded DNA and RNA G-enriched sequences into guanine quadruplex structures (G4). CNBP is a conserved protein required for craniofacial development in zebrafish, mouse and chicken; however, its molecular targets remain unknown. By yeast inverse one hybrid assays with genomic *Danio rerio* and *Mus musculus* libraries we found 96 sequences as putative CNBP targets. We searched for a consensus motif between these sequences using several algorithms with the TMOD software and found a 14-nucleotide consensus string (NGGGGG(A/T)GGGGGGN). This sequence may fold as G4, thus reinforcing the notion that CNBP controls gene expression through G4 binding and/or folding promotion. On the other hand, a list of putative CNBP targets was retrieved from data of genes co-expressed with CNBP obtained from zebrafish, mouse and chicken microarrays (http://coxpresdb.jp/) and whole-mount *in situ* hybridization spatiotemporal patterns (http://zfin.org for zebrafish, http://www.emouseatlas.org/emage/ for mouse, and http://geisha.arizona.edu/ for chicken). The overall analysis resulted in a reduced list of nine putative CNBP targets. We have initiated the validation of these genes *in vivo* by studying their mRNA level and pattern by real time RT-PCR and whole-mount *in situ* hybridization in CNBP-depleted zebrafish embryos.

CB-C06.
E2F1 AND E2F2 CONTRIBUTE TO THE DNA DAMAGE RESPONSE IN NEURONAL CELLS
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The E2F transciption factors are key regulators of multiple cellular processes including proliferation, development and apoptosis. In the last years, E2F1 has been described to participate in the DNA damage response, an event that requires its post-translational modification and subsequent protein stabilization. We have previously reported that the E2F1 and E2F2 genes are also regulated at the transcriptional level and become induced following different genotoxic stimuli (neocarzinostatin, H2O2 and UV) in human and murine neuronal cells. Consistently, here we show that DNA damage increased E2F transcriptional activity 4-fold, as assessed by reporter assays. Our goal was to characterize and evaluate the significance of E2F1 and E2F2 induction in the maintenance of genome integrity. Inhibition of the MAPK and ATM/ATR pathways prevented the upregulation of E2F1 and E2F2 mRNA levels in response to genotoxic stress. E2F1 and E2F2 ablation using antisense oligonucleotides resulted in increased levels of H2AX following DNA damage, as observed by immunofluorescence microscopy. Moreover, clonogenic assays showed that cells with reduced E2F1 and E2F2 mRNA levels have 33% and 59% diminished proliferative capacity after genotoxic stimuli, respectively.
Our results suggest that the enhanced transcription of E2F1 and E2F2 plays a relevant role during the DNA damage response.

CB-C07.
ENDOCYTIC RECYCLING OF LRP1 IN ALPHA 2-MACROGLOBULIN-STIMULATED CELLS
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The LDL receptor-related protein 1 (LRP1) is an endocytic and signaling receptor, which play an key role in the cellular migration and proliferation. Previously we demonstrated that 2-Macroglubulin (2M*) induced intracellular signaling activation via LRP1, which is characterized by PKC and MAPK activation. Our hypothesis is that the cellular function of LRP1 involves the endocytic recycling and cell surface sorting of this receptor in 2M*-stimulated cells. Hence, in this work we tried to characterize the endocytic recycling and cell membrane sorting of LRP1 in MIO-M1 cells stimulated with 2M*. Using confocal microscopy, flow cytometry and a recombinant mini-receptor version of LRP1 (mLRP4/GFP) we demonstrated that 2M* induced the LRP1 localization in Rab11-recycling compartments between 15-30 min after 2M* stimulation. Then, by TIRF microscopy and LRP1 immunoprecipitation techniques of biotin-labeled cell surface proteins we showed that 2M* promoted (after 15 min) the intracellular sorting of the constitutive LRP1 and mLRP4 to the cell membrane. This sorting was partially blocked by the negative dominant mutant form of Rab11. However, other Rab forms, probably Rab8 and Rab6, could be involved in this sorting process. Our data suggest that the LRP1 function in 2M*-stimulated cells is dependent on the endocytic recycling of this receptor

CB-C08.
VAMP7 IS INVOLVED IN HOMOTYPIC AND HETEROTYPIC FUSION OF *C. Burnetii* PHAGOSOMES
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Coxiella burnetii, is a Gram-negative obligate intracellular bacterium. It has been described that both the endocytic and the autophagic pathways contribute to Coxiella replicative vacuole (CRV) maturation. The large CRV shares characteristic of a phagolysosome-autophagolysosomal compartment. VAMP7, Vti1a and Vti1b are SNAREs involved in both the endocytic and secretory pathways, which particularly participates in the heterotypic fusion between late endosomes and lysosomes. We have previously observed that VAMP7 interacts with *C. burnetii* at different infection times (1h-72h p.i.). A truncated mutant of VAMP7 (VAMP7 NT) and siRNA against this SNARE protein affected the development of CRV, suggesting that VAMP7 mediates fusion events that are required for the biogenesis of the CRVs. In the present work, we observed that overexpression of VAMP7 NT affected the heterotypic fusion with late endosomes and also the homotypic fusion between Coxiella phagosomes and CRVs. On the other hand, we also detected the VAMP7 partners (Vti1a and Vti1b) in the vacuole membrane at different infection times. Moreover, treatment with chloramphenicol reduced the colocalization between *C. burnetii* and VAMP7, Vti1a or Vti1b, indicating that the recruitment of these SNAREs proteins is a bacteria driven process that favors the CRV biogenesis, likely by facilitating the interaction with the lysosomes.

CB-C09.

TRANS-ACTIVITY OF PLASMA MEMBRANE-ASSOCIATED GANGLIOSIDE SIALYLTRANSFERASE IN MAMMALIAN CELLS

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Gangliosides are acidic glycosphingolipids that are synthesized at the Golgi complex by a combination of glycosyltransferase activities followed by vesicular delivery to the plasma membrane, where they participate in a variety of physiological as well as pathological processes. Recently, a number of enzymes of ganglioside anabolism and catabolism have been shown to be associated with the plasma membrane. In particular, it was observed that CMP-NeuAc:GM3 sialyltransferase (Sial-T2) is able to sialylate GM3 at the plasma membrane (cis-catalytic activity). Here, we demonstrated that plasma membrane-integrated ecto-Sial-T2 also displays a trans-catalytic activity at the cell surface of epithelial and melanoma cells. By biochemical and cell biology techniques, we observed that ecto-Sial-T2 was able to sialylate hydrophobically or covalently immobilized GM3 onto a solid surface. More interestingly, we observed that ecto-Sial-T2 was able to sialylate GM3 exposed on the membrane of neighboring cells by using both the exogenous and endogenous donor substrate (CMP-NeuAc) available at the extracellular milieu. Our findings provide the first direct evidence that an ecto-sialyltransferase is able to trans-sialylate substrates exposed in the plasma membrane from mammalian cells, which represents a novel insight into the molecular events that regulate the local glycosphingolipid composition.

CB-C10.

STARVATION- INDUCED AUTOPHAGY CAUSES A RE-DISTRIBUTION OF VAMP7 VESICLES TO FOCAL ADHESIONS IN HELA

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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 which localizes to autophagosomes. SNAREs are key molecules of the vesicle fusion machinery. Our results indicate that a subset of Vamp7 (V-SNARE)-positive vacuoles colocalize with LC3 at the cell periphery upon starvation. Moreover, we have demonstrated that these Vamp7-positive structures are labeled by paxillin and vinculin (focal adhesion markers), indicating that the Vamp7-positive autophagosomes are present in these structures. We have also shown that the Vamp7 structures close to plasma membrane have late endosomal characteristics. Interestingly, the re-distribution of Vamp7 positive structures is a microtubule-dependent event, with the participation of the motor protein Kif5 and a Rab7 effector RILP. Furthermore, we have observed an increased number of ATP vesicles labeled with Vamp7 at the focal adhesions upon starvation. Taken together, our results suggest that VAMP7 is involved in the trafficking of amphisomes, which contain ATP, to the focal adhesions. It is likely that these structures may fuse with plasma membrane to release the nucleotide to the extracellular medium.

CB-C11.

RAB5 AND RAB11 ARE INVOLVED IN THE TRAFFICKING OF VINCULIN IN RENAL PAPILLARY COLLECTING DUCT CELLS

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Focal adhesions (FA) are structures of cell attachment to the extracellular matrix. We have showed that bradykinin (BK) induces a dissipation of vinculin-stained FA, with formation of vinculin and PIP2 containing vesicles. At least a fraction of vinculin was delivered to the recycling endosomal compartment. Now, we isolate the vinculin containing vesicles by immunomagnetic method from cultured papillary collecting duct (CD) cells treated or not with BK (1, 5 and 10 min). We analyzed by confocal microscopy, the colocalization of vinculin with Rab5 which is localized in early endosomes and with Rab11which mediates recycling of endocytic vesicles. Vinculin - Rab5 colocalization was found in control and in BK-treated cells in the marginal and perinuclear cell regions, showing stronger overlapping in control than in 1 min BK-treated-cells. No differences were observed at 5 and 10 min. Vinculin also colocalized with Rab11 in perinuclear region in both control and BK-treated cells showing stronger overlap after 5 and 10 min. In the marginal region, overlapping was observed only after 5 and 10 min of BK-treatment. The modulation of these vinculin containing vesicles could be a physiological mechanism to reuse the BK-induced internalized vinculin to be delivered for newly forming FA in CD cells

CB-C12.

ROLE OF 2-OXOGLUTARATE-DEPENDENT DI-OXIGENASES IN DROSOPHILA DEVELOPMENT

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The Hypoxia Inducible Factor (HIF), is a heterodimeric / transcription factor composed of two bHLH-PAS subunits that regulates the response to hypoxia. While HIF- is constitutively expressed, HIF- subunit is tightly regulated by oxygen. Oxygen regulation is mediated by specific Prolyl-4-hydroxilases (PHDs) that hydroxylate HIF- in two proline residues utilizing O_2 as a co substrate of the reaction. Hydroxylated HIF- is targeted for degradation at the 26S proteasome. In our lab we have identified Sima and Fatiga (Fga) as the HIF- and PHD fly homologues respectively. *sima* mutants are fully viable and fertile in normoxia and *fga* mutants are lethal at different developmental stages. *fga* lethality is due to Sima over accumulation as *fga-sima* duble mutants recover viability. Despite being fully viable, *fga-sima* double mutants are sterile indicating that an alternative Fga target, different from sima, is involved in the *Drosophila* ovary development. We found that Fga is required in the germline but not in follicle cells for oogenesis progression. Over-activation of the transcription factor FOXO in germ cells accounts for the ovary phenotype of the *fga sima* double mutants, since in *fga-sima-foxo* triple mutant ovaries were totally normal. Our results therefore suggest that FOXO is negatively regulated by Fga in the germline, thereby allowing oogenesis progression.

CB-C13.

AIR POLLUTION AND OBESITY ASSOCIATED METABOLIC COMPLICATIONS

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Air pollution might worsen obesity-associated metabolic complications. We found that the lung from obese mice has more neutrophils and myeloperoxidase (MPO) than lean mice. This led us to hypothesize that inhalation of air pollutants makes the obese lung an important source of systemic inflammation due to local MPO-triggered oxidative processes. To test this hypothesis we used male B6 mice acutely exposed to intratracheal instillation of either saline, LPS, DMPO or LPS/DMPO (a spin trap). These treatments did not cause death of animals. Interestingly, DMPO treatment greatly reduced LPS-induced cachexia and asthenia produced by LPS. LPS increased neutrophils and markers of oxidative stress (carbonyls, nitrotyrosine and chlorotyrosine), inflammation (TNF-a, IL-1b, nitrite/nitrate, and IL-6) and tissue damage (LDH) in the BALF supernatant, serum and lung tissue homogenates. These changes were prevented by DMPO with concurrent formation of protein-DMPO nitrone adducts, and reduced neutrophils and ICAM-1 in the lung parenchyma. These results suggest that DMPO blocks lung inflammation by reducing neutrophil's homing in the lung and thereby blocking further local oxidation and subsequent systemic inflammation. Prevention of neutrophil homing in the lung might be useful to reduce obesity-associated metabolic complications in obese patients exposed to air pollutants.

CB-C14.

INFLAMMATORY MACROPHAGES, MYELOPEROXIDASE AND ADIPOSE TISSUE DYSFUNCTION

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Macrophages-mediated fat tissue inflammation contributes to low-grade chronic systemic inflammation and insulin resistance (IR) in adipocytes during obesity. However, the agents, targets and mechanistic bases of the oxidation process that occurs in fat during obesity remain unclear. In this study we sought to determine whether myeloperoxidase (MPO) causes inflammation and IR in adipocytes. We used a mouse model of diet-induced obesity and an in vitro model of human adipocytes loaded with MPO and stressed with H2O2. The visceral fat of obese mice was infiltrated with macrophages that express MPO, located in crown-like structures in hypoxic areas. Immuno-staining and RT-PCR showed MPO also inside the adipocytes, especially around the lipid droplets, but not in other stromal vascular cells. Luminol showed that MPO produced HOCl inside adipocytes that was prevented by the MPO inhibitors or resveratrol—a cell permeable scavenger of HOCl. HOCl produced inside the adipocyte caused adipokine deregulation and IR. Immuno-spin trapping with anti-DMPO of adipocytes loaded with MPO and treated with H2O2 and DMPO showed a number of proteins targets of oxidation in the adipocyte and reduced adipokine deregulation and IR. Treatments aimed at preventing or stopping MPO-driven oxidation inside adipocytes might offer new therapeutic avenues for preventing obese patients from becoming diabetic.

CB-C15.

ROLE OF SPHINGOLIPID METABOLISM IN MDCK CELLS TRANSITION FROM POLARIZED TO DIFFERENTIATED PHENOTYPE

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Cell-Cell adhesion, by establishment of adherens junctions (AJ) is an early event for epithelial cell polarization/differentiation and maintenance of cell differentiation. We have previously demonstrated that AJ complexes are located in a sphingomyelin enriched lipid membrane domain. We aim to study the importance of SM synthesis in the acquisition of the polarized-differentiated MDCK cells phenotype. Confluent MDCK cells were submitted to hypertonicity and concomitantly treated or not (control) with different concentration of D609, an SM synthase inhibitor. After 48 h, cell phenotype was visualized by confocal microscopy of actin cytoskeleton and cell–cell adhesion structures. As inhibitor concentration rise, the cell–cell adhesions were impaired, and the characteristic polarized phenotype of the cells was lost. First cells appeared lengthened, and thereafter acquired a fibroblast like phenotype. E-cadherin signal decreased and appeared discontinued. Polymerized actin seemed not to be altered, cortically located and increased stress fibers were observed at the highest D609 concentration. Moreover, we determined that augment in the SMS1/SMS2 ratio was involved in the cell-cell adhesion instauration. From these results we suggest that, instead to differentiate, polarized MDCK cells undergo mesenchymal transition when induced to differentiate in a condition of inhibition of SM synthesis.

CB-C16.

IDENTIFICATION OF POTENTIAL TRANSCRIPTIONAL REGULATORY SEQUENCES IN INTRON5 OF THE RUNX1 GENE

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The RUNX1 gene codes for the RUNX1 transcription factor, a critical hematopoietic regulator. This gene is one of the most frequent target of leukemia causing translocations, with t(8;21) being one of the most commonly found in myeloid leukemia patients. Until now, all the breakpoints mapped in the RUNX1 gene for t(8;21) are grouped together in three regions of the intron5, denominated the breakpoint cluster regions (BCRs). Interestingly, several chromatin structural elements such as DNaseI hypersensitive sites and topoisomerase II cleavage sites co-localize with these BCRs. The DHS sites are commonly associated with regulatory elements such as promoters, enhancers and silencers, suggesting that some regulatory sequences may be harbored in intron5. In order to identify potential regulatory sequences in intron5 of the RUNX1 gene, we used a combination of comparative genomics and chromatin analysis. Our genomic analysis identified nine potential enhancer regions that are evolutionarily conserved. Using chromatin immunoprecipitation assays we evaluate the histone acetylation status of these regions in myeloid cells. Our results show a high association of H3K4me1, H3K27ac, and H3K9/K14ac, all marks of enhancer modules. Therefore our data suggested that intron5 contains several potential enhancers active in the myeloid lineage.

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CB-C17.

HISTONE ACETYLATION ANALYSIS OF ETO GENE BREAKPOINT REGIONS INVOLVED IN T(8;21) FORMATION

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One of the most frequent chromosomal translocation found in approximately 15% of acute myeloid leukemia (AML) patients is the t(8;21). This translocation involves the RUNX1 and ETO genes. The breakpoints regions (BCR) for t(8;21) are located at intron5 and intrón1 of the RUNX1 and ETO gene respectively. To date, no homologous sequences have been found in these regions to explain their recombination. However, the BCR involved in t(8;21) colocalize with DNasaI hypersensitive sites and *in vivo* topoisomerase II cleavage sites, suggesting that chromatin conformation maybe crucial for DNA breaks and translocation formation. In order to analyze the chromatin structure of the BCR at the ETO gene, we performed chromatin immunoprecipitation (ChIP) using antibodies against acetylated histone H3, acetylated histone H4 and histone H1. We also used an antibody against total histone H3 to evaluate the presence of nucleosomes. Our data show that in promyeloid cells, the BCR at the ETO gene are enriched in hyperacetylated histone H3 and H4 compared to a control region of similar size, but where no translocations have been described. However, we observed no H1 association at the BCR of the ETO gene or the control region. These results suggest that acetylation of H3 and H4 could facilitate DNA breaks and t(8;21) formation.

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CB-C18.

CROSSTALK BETWEEN AND PKC ISOFORMS AND RETINOIC ACID SYSTEM IN MALIGNANT PHENOTYPE REVERSION

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Retinoic acid (RA) may exert some of their effects on cell differentiation and malignant phenotype reversion through the interaction with some PKCs. We used mammary tumor cell linesLM3 and MDA-MB231 as models. Objetive: study crosstalk between retinoid system and PKC signaling pathway on mechanisms implicated in malignant phenotype reversion. In LM3 cell line, all trans RA induced growth inhibition associated with reduction of pERK and increase nuclear p27. No modulations were observed in MDA-MB231 cells. RA induced an increase in PKC and PKC together with nuclear translocation only of PKC isoform in the murine cell line. In MDA-MB231 cells, both isoforms of PKC were reduced after RA exposure. Pharmacological silencing of PKC Gö6976 (Gö) and PKC (Rottlerin) prevented retinoic receptors (RAR) activation by RA in LM3 cells (gene reporter assay). Only PKC inhibition impaired RA-induced RAR translocation to the nucleus and co-immunoprecipitated with RAR 1 after RA exposure. Gö-RA cotreatment decreased cell duplication rate in additive manner respect to each one separately. In *in vivo* assay the combination therapy reduces the number of lung metastasis. Conclusion: crosstalk PKC/retinoids is implicated in growth inhibition through RA transcripcion genes induction. Combined treatment with Retinoids and PKC modulators could be considered for PKC positive breast cancer patients

CB-C19.

UNDERSTANDING POSTTRANSLATIONAL MODIFICATIONS IN *G. Lamblia*, ONE OF EARLIEST DIVERGENT EUKARYOTES

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Posttranslational modifications are one of the most effective ways by which evolution has increased the versatility in protein function. In eukaryotic cells, SUMOylation of highly conserved proteins occurs. In the intestinal parasite *G. lamblia* we showed that gSentrin protein localize mainly in the cytoplasm of *Giardia* trophozoites, though also surrounding the nuclei, even inside it. By Western Blot we observed several sumoylated proteins but, at this time, only Arginine Deiminase (gADI) was confirmed as a sumoylated substrate. gADI is an important enzyme in the survival and differentiation of the parasite. During the differentiation process, gADI translocated to the nuclei and the production of cysts was reduced. Recently, we demonstrated that in the nuclei gADI citrullinates histones. Moreover, when the enzyme had mutations in the site of sumoylation, the translocation to the nuclei did not occur, nor the citrullination of histones and the number of cysts produced were similar to wild type trophozoites. The characterization of SUMOylation and citrulination in *Giardia* are crucial to understand the role of these posttranslational modifications in the evolution history.

CB-C20.

ALTERATION OF POLYAMINE METABOLISM IN THE EFFECT OF AZINPHOS-METHYL ON *Rhinella arenarum* DEVELOPMENT

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The objective of this study was to evaluate the impact of the pesticide azinphos-methyl (AM) on polyamine (PA) metabolism along embryonic development of the toad *Rhinella arenarum*. HPLC analysis of extracts from exposed embryos showed an increase in putrescine (Put) and a decrease in spermidine (Spd) and spermine (Spm) levels at late embryonic development. Ornithine decarboxylase (ODC) and diamine oxidase (DAO) activities measured by $^{14}CO_2$ release and spectrophotometry respectively were increased at intermediate and late embryonic stages by AM exposure. The transcription factor c-Fos, which participates in the transcriptional control of *Odc* gene, showed an increase in its translocation to the nucleus in response to AM exposure at intermediate stage (Western Blot analysis). At the end of embryonic development, a significant decrease in GSH levels and a significant increase in the percentage of malformations were also observed in AM-exposed embryos. We conclude that AM affects PA metabolism decreasing Spd and Spm levels, which alter embryo development and may also trigger ODC induction and Put synthesis. Both a decreased synthesis and an increased degradation of Spd/Spm may contribute to Put accumulation leading to an increased degradation through DAO. PA increased degradation generates oxyradicals which could contribute to oxidative stress and altered development.

CB-C21.

Chlamydia trachomatis USES HOST AKT/AS160 PATHWAY TO ENSURE ITS DEVELOPMENT

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Chlamydia trachomatis is a Gram negative obligate intracellular bacterium that causes genital and ocular infections in humans. This bacterium replicates in a vacuole named inclusion. We demonstrated 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 the Golgi to the inclusion. Furthermore, new results show that infection with *C. trachomatis* causes an increase in Rab14 expression and activates AKT. On the other hand, it has been described that active AKT causes the inhibition of AS160, a GAP (GTPase Activating Protein) for Rab14. We postulate that *C. trachomatis* manipulates PI3K/Akt/AS160 pathway to ensure the arrival of sphingolipids departed from the Golgi through Rab14-positive vesicles. We analyzed the effect of a specific AKT inhibitor (iAkt) on *Chlamydia*-infected cells. Treatment with iAkt decreases chlamydial inclusion size and reduces Rab14 recruitment to the inclusion in a doses-dependent manner. Moreover, iAKT treatment of infected cells generates abnormal bacterial forms assessed by electron microscopy. Likewise, inclusion forming unit analysis shows that iAKT treatment significantly decreases bacterial multiplication and infectivity. These data suggest that *Chlamydia trachomatis* usurps PI3K/Akt/AS160 pathway to ensure its survival.

CB-C22.

SEPARATION OF ACROSOME REACTED LIVING HUMAN SPERM BASED ON THE USE OF PSA-FITC AND FACS

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The acrosome reaction (AR) in mammalian sperm is a regulated secretion absolutely necessary for fertilization. In this study we evaluated the possibility of assessing AR in living human sperm by using *Pisum sativum* agglutinin (PSA) conjugated with fluorescein isothiocyanate (FITC). This lectin binds with high affinity glycoproteins present in the lumen of the acrosome. Capacitated sperms were stimulated with the calcium ionophore A23187 in the presence of PSA-FITC to detect the accumulation of the lectin in the interior of the granule as the fusion pores opened. Propidium iodide was included in the assay to label dead sperms. We observed by confocal microscopy that the acrosome of living sperm became suddenly fluorescent. Surprisingly, the label was not lost with time, as it would be expected because of the dispersion of the acrosome content after exocytosis. By electron microscopy we corroborated that in the presence of the lectin, the hybrid vesicles formed during exocytosis and the acrosomal content remain attached to the cells. With this procedure, we have generated a population of fluorescent live reacted sperm. Next, we succeeded in separating this population by fluorescence-activated cell sorting (FACS). Few techniques to assess AR in living cells have been developed, so we expect that these two new techniques will contribute to unveil new aspects of the AR.

SB-C01.

ANALYSIS OF PROTEIN-PROTEIN/PROTEIN-DNA INTERFACES OF *Pseudomonas aeruginosa* MUTL N-TERMINAL DOMAIN

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Mismatch Repair System (MRS) corrects mutations arising from DNA replication that escape from DNA polymerase proofreading activity. MutL coordinates the action of most of the proteins involved in repair. MutL is a dimeric protein that contains a C-terminal (CTD) dimerization domain connected by a linker to an N-terminal (NTD) ATPase domain. MutL is a member of GHL ATPase family and the ATPase cycle has been proposed to modulate MutL´s activity during the repair process.
We used an experimental and in-silico approach to determine interaction surfaces of *P. aeruginosa* NTD (paNTD). Unlike *E. coli* NTD (ecNTD), paNTD is dimeric in absence of nucleotide. Since the crystal structure of paNTD is not known, we constructed a 3D homology model. MD simulations of paNTD models and ecNTD crystal structures with or without ATP were carried out in order to analyze structural differences. Therefore, in silico analysis allowed us to identify and characterize the paNTD dimerization interface and compared it with that of ecNTD. Also, simulations in mixed solvent were performed to identify areas with a high tendency to desolvate, allowing us to identify other two putative interaction interfaces. One corresponds to the known homologue region of *E. coli* DNA binding patch, whereas the second interface corresponds to a potential paNTD protein-protein interaction site.

SB-C02.

SET-UP OF A REPRODUCIBLE PROCEDURE TO PURIFY CNBP SUITABLE FOR STRUCTURAL AND BIOCHEMICAL STUDIES

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Cellular nucleic acid binding protein (CNBP) is a conserved zinc knuckle-containing single-stranded nucleic acid binding protein involved in human muscular diseases and craniofacial development. Recombinant CNBPs fused to different tags were used for previous biochemical studies. However, tag-free CNBP is needed to perform spectroscopic approaches. Here we describe a purification procedure that yielded 5 mg of soluble tag-free recombinant CNBP per liter of LB. Electrophoretic mobility shift assays and intrinsic fluorescence quenching experiments showed that purified CNBP is biochemically active. Size exclusion chromatography revealed homodimeric and monomeric forms coexisting in solution independently of CNBP binding to its targets. CD spectra predicted that CNBP secondary structure is dominated by ß-sheet, probably comprising zinc knuckles, and has high content of random-coil. CNBP zinc knuckles are implicated in its structure and functionality since Zn^{2+}-depleted CNBP failed to bind single-stranded nucleic acids and was more sensitive to proteolysis. Moreover, CNBP was less sensitive to proteolysis in the presence of single-stranded DNA or RNA, suggesting that CNBP gains structure upon binding to its targets. The availability of the purification method presented here will be useful for further structural and biochemical characterization to shed light on CNBP biological function.

PL-C01.
EXPRESSION AND REGULATION OF FLAVONOL SYNTHASES FROM MAIZE

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Flavonoids are specialized compounds widely distributed throughout the plant kingdom with diverse functions in plants and benefits for human health. In particular, flavonols, synthesized by flavonol synthase (FLS), protect plants against UV-B radiation and are essential for male fertility in maize. We have recently characterized a UV-B inducible *ZmFLS1* which was the first enzyme to be described in monocot plants. Interestingly, the new assembly of the B73 genome revealed the presence of a second putative *FLS* gene (*ZmFLS2*) with extremely high similarity both at the nucleotide and amino acid level. Expression analysis showed that both genes are UV-B regulated in inbred lines and high altitude landraces. By combining EMSA assays and transient expression experiments, we were able to show that both genes are direct targets of the transcription factors P1, C1 and R involved in flavonoid biosynthesis regulation. Moreover, the high sequence conservation of the *ZmFLS* promoters between maize lines would indicate that the differences observed in *ZmFLS* expression could be explained by allelic variations in the transcription factor activities. Finally, the presence of two *FLS* genes in maize and sorghum, and one in rice and *Brachypodium*, in addition to the evolutionary distances across the grasses suggest that duplication of the *FLS* gene occurred early in evolution.

PL-C02.
CHARACTERIZATION AND cDNA SEQUENCING OF A NOVEL FUNGAL SUBTILISIN WITH DEFENSE-ELICITING ACTIVITY

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One of the possible disease biocontrol strategies in crops consists in activating the plant innate immunity by using defense elicitor molecules. In this work the purification and characterization of an extracellular elicitor protein produced by the pathogen *Acremonium strictum* is reported. Culture filtrate was fractioned by ultrafiltration (cut-off 30 kDa), followed by anionic exchange (Q sepharose, pH 7.5) and hydrophobic interaction (Phenyl Superose) chromatographies. 2D-SDS/PAGE of the active fraction revealed a single spot of 34 kDa and pI 8.8. Phytopathogenicity tests confirmed that the purified protein induced total protection against anthracnose in strawberry plants. Based on the use of degenerate primers designed from the partial aminoacid sequences and RACE technology, the complete nucleotide sequence of the elicitor-encoding cDNA of 1167 nucleotides was obtained. The deduced aminoacid sequence showed significant identity with fungal serine proteinases of the subtilisin family, suggesting that elicitor is synthesized as a larger precursor that contains in addition to the 283-residue of mature protein, a 15-residue secretory signal peptide and a 90-residue peptidase inhibitor domain I9. The elicitor exhibited proteolytic activity *in vitro* and was inhibited by PMSF but not by TPCK. This is the first report of a fungal subtilisin that shows defense-eliciting activity in plants.

PL-C03.
DC1 DOMAIN PROTEINS INVOLVED IN DEFENSE RESPONSE AND FEMALE GAMETOPHYTE DEVELOPMENT IN *Arabidopsis thaliana*

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Disease resistance and reproductive development are two major aspects of the plant life cycle. Although these processes have been traditionally considered as independent and regulated by different groups of genes, new findings have linked defense related proteins to different aspects of plant development. In this work, we identified two genes containing a DC1 domain, named *AtDC1* and *AtDC2*, whose expression showed to be highly upregulated upon microbial elicitors and biotic stress in *Arabidopsis thaliana* plants. Analysis of mutant plants carrying insertions in *AtDC1* and *AtDC2* genes revealed that the expression of these genes is required for normal female gametophyte development. In these gametophytic mutants, embryo sac development is arrested at FG1 stage, showing a putative functional megaspore that does not undergo mitosis. Altogether, our results suggest that AtDC1 and AtDC2 might have dual roles participating both in female gametophyte development and in defense response to biotic stresses in *A. Thaliana*.
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PL-C04.
A NEW RETROGRADE PATHWAY THAT FUNCTIONS IN DROUGHT AND HIGH LIGHT SIGNALLING IN ARABIDOPSIS

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Compartmentation of the cell requires a complex set of subcellular messages including multiple retrograde signals from the organelles to the nucleus to regulate gene expression. Although some proteins that participate in different signalling cascades in higher plants have been identified, the actual mobile signals and their mechanism of action are debated. Here we demonstrate that a phosphonucleotide, PAP, accumulates in *Arabidopsis thaliana* in response to drought and high light stress and its levels are regulated by the phosphatase SAL1. SAL1 accumulates in both the chloroplast and mitochondria; but not in the cytosol. Transgenic nuclear targeting of SAL1 lowers PAP levels and complementation of a SAL1 mutant with the yeast SAL1 homologue targeted just to the chloroplast complements the expression of the *APX2* high light inducible gene somewhat proportionally to the level of PAP. The data are consistent with PAP moving from chloroplasts to the cytosol and nucleus where it likely inhibits the activity of exoribonucleases (XRN), as global expression analyses showed that both SAL1 and the nuclear XRNs modulate the expression of a very similar subset of HL, ABA and drought-inducible genes, including the high light inducible gene expression of *APX2* and *ELIP2*. Apparently, PAP can function as a retrograde signal altering nuclear gene expression, during high light and drought stress

PL-C05.

NON RACE-SPECIFIC RESISTANCE OF POTATO TO *Phytophthora infestans* INVOLVES DEVDASE ACTIVITY

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During *S. tuberosum- P. infestans* (Pi) interaction, two forms of genetic resistance have been characterized: race- or non race-specific. The latter is due to the expression of quantitative loci and thus, its field durability is greater than in race specific resistance. In the last years, plant caspase- like activities have been described in *Solanaceae*: caspase-1 (after viral infection in tobacco leaves) and caspase-3 and 9 (after heat shock in tomato fruit). The aim of this work was to analyze caspase-3 like activity (DEVDase) in potato cultivars with non race specific resistance to Pi. Protein extracts from potato leaves from cv. Pampeana (moderately resistant) and cv. Bintje (susceptible) infected with Pi for 0, 1, 6, 12, 24 and 48hs were prepared. DEVDase activity was detected after 24 and 48h of infection in the resistant cv. but not in the susceptible one. Biochemical characterization of DEVDase activity exhibited inhibition by PMSF and caspase-3 inhibitor (DEVD-CHO) and sensitiveness to Ca^{+2} and Zn^{+2} ions. Pre-treatment of Pampeana leaves with DEVD-CHO or the general caspase inhibitor VAD-FMK showed a greater Pi disease development compared to control condition. However, plant cell death was decreased in caspase inhibitors treated leaves. Finally, we conclude that caspase- 3 like activity is involved in partial nonrace specific resistance of potato leaves to *P. Infestans*.

PL-C06.

EXPLORING THE LINK BETWEEN EPIGENETICS AND ADAPTIVE STRESS RESPONSES IN PLANTS

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Epigenetics refers to chromatin marks that do not involve sequence changes, often reversible, heritable and triggered by environmental factors. In plants, there are cytosine methylation contexts other than the known CG dinucleotide, like CNG and CNN (asymmetric), the latter typically found in transposons and non-coding repetitive elements.

Our goal was to test the hypothesis that stress-induced adaptive physiological responses in plants depend on epigenetic alterations. We thus explored leaves and roots of tomato (harbouring a genome 10 times larger than that of Arabidopsis) under basal and water-deficit conditions.

We surveyed the epigenetic status (in the three methylation contexts) of our challenging model gene, *Asr1*, non-transposon, protein-coding and a member of a stress-inducible family absent in Arabidopsis. We used the bisulphite procedure to detect DNA methylation and for histone modifications, we did ChIP using anti-H3K4me3 and H3K27me3 antibodies. We found high basal levels of atypical methylation at CNN sites in leaves, whereas stress caused their removal all over the gene, concomitantly with a moderate increase of CG methylation marks in exon 1, removal of the repressive mark H3K27me3 and a 36-fold increase in *Asr1* transcript.

These findings may represent a general mechanism for the acquisition of new epialleles, pivotal for regulating gene expression in plants.

PL-C07.

GENOMIC ANALYSIS OF THE STILBENE SYNTHASE GENE FAMILY THROUGH THE ELICITATION OF GRAPE CELL CULTURES

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Resveratrol is a phytoalexin produced naturally by grapevine and other few plants when under different biotic and abiotic stress. Stilbene synthase (STS), the key enzyme in resveratrol biosynthesis, is codified by a gene family with about 40 similar members distributed in just two clusters on grapevine chromosomes 10 and 16. Our main goal is to determine if the different biotic and abiotic factors inducing resveratrol production regulate the different STS genes in a redundant or specific way. To do so, we are characterizing the STS gene family in terms of structure and number of genes as well as their different expression patterns. Moreover, we have performed a microarray analysis using the GrapeGen Affymetrix GeneChip on grape cell suspensions that accumulate high levels of stilbenoids in response to the presence of cyclodextrin (CD), methyl-jasmonate (MeJa) or both elicitors. Principal Component Analysis (PCA) showed that more that 70% of the global variation is explained by a principal component (PC1, 51%) determined by the synergistic gene regulation under the combined treatment, while a second component (PC2, 20%) is determined by the individual effect of MeJa. Focusing on the STS genes, we observed that the 15 members represented in the chip showed a common induction in the combined treatment while significant differences were detected in the individual treatments.

PL-C08.

INCREASED TOLERANCE TO OXIDATIVE STRESS IN PLANTS EXPRESSING *IN VIVO* SUBSTRATE AMPLIFICATION SYSTEM

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Ferredoxin $NADP^+$-reductase (FNR) and ferredoxin constitute the reducing side of photosystem I, producing the necessary NADPH for productive chloroplasts routes. Oxidative stress causes ferredoxin down-regulation and $NADP^+$ shortage, leading to over-reduction of the photosynthetic electron transport chain and reactive oxygen species generation. Expression of cyanobacterial flavodoxin in tobacco chloroplasts compensates for ferredoxin decline and enhances tolerance to oxidative stress. We postulate that higher degrees of tolerance could be achieved by improving the capacity for flavodoxin reduction *in planta* under stress conditions. Soluble FNR catalyzes flavodoxin reduction by NADPH with high efficiency. Plants expressing a soluble cyanobacterial FNR, a modified version of the reductase in which the phycobilisome-binding domain has been removed, were generated and crossed with those expressing flavodoxin. Co-expression of the two flavoproteins resulted in lines displaying enhanced tolerance to redox-cycling oxidants, lower damage to pigments and membranes, and decreased reactive oxygen species accumulation, generating an *in vivo* amplification system to optimise flavodoxin reduction under stress conditions. The results provide a tool to improve crop tolerance toward environmental hardships.

PL-C09.

METAL ACCUMULATION IN SOYBEAN: METALLOTHIONEIN FAMILY CHARACTERIZATION

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Plants produce different types of peptidic defences against heavy metals: phytochelatins –enzymatically synthesized peptides-, metallothioneins (MTs) –proteins deeply studied in the animal kingdom- and metallohistins –a new kind of vegetal protein thought to be involved in metal metabolism. Nevertheless, data on the last two systems (polymorphism, regulation, structure and function of these genes and corresponding proteins) in plants are scarce, so the aim of this study is the characterization of the molecular mechanisms involved in metal accumulation in plants of interest in agriculture, such as soybean.
In silico analysis of soybean genome and ESTs banks with known MT sequences or motifs has rendered 10 sequences of probable MT genes. Four of these genes, whose sequences codify for the 4 plant MT types described, are currently under studies. All of them are expressed at high levels: *MT1*, *MT2* and *MT3* in root, shoot and seeds and *MT4* only in seeds. Heterologous expression in *S.cerevisiae* restores Cd and Cu tolerance, only marginally enhances Zn tolerance, and improves resistance to oxidative stress in MT-null yeast strains. Soybean MTs, purified from *E.coli* cells cultured in different metal rich media, render better folded polypeptides with Cd and Cu than with Zn. These results point towards a role in metal sequestration and/or oxidative stress defence for all soybean MTs studied.

PL-C10.

BASAL GENE TRANSCRIPTION CONTRIBUTES TO DISEASE TOLERANCE IN *Lotus japonicus* ECOTYPE GIFU

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We previously reported the analysis of the transcriptional reprogramming that occurs in *Lotus japonicus* during the pathogenic interaction with *Pseudomonas syringae*. That study included the ecotypes MG20 and Gifu showing susceptible and tolerant phenotypes, respectively, and allowed us to evaluate candidate genes with a probable role in the establishment of an effective defense response. In an attempt to identify new genes involved, in the present work we studied the differences in gene expression that exist in both ecotypes in non-treated (control) conditions. In this case, our analysis shows 2874 differentially expressed genes, 60% of them showing greater expression in the ecotype Gifu. At least 691 genes are predicted to have a role during biotic stress and might contribute to tolerance. Most of them participate in general protein metabolism as well as synthesis of flavonoids and jasmonic acid, two compounds with an important role in plant defense. Interestingly, many of the genes that show a higher expression in the ecotype Gifu are consequently induced in the susceptible MG20 under bacterial infection. These observations suggest that basal gene expression in the ecotype Gifu might explain, at least partially, the higher tolerance to pathogen attack. The importance of these genes during plant defense and the similarities with previously published reports are discussed.

PL-C11.

RECENT HORIZONTAL TRANSFERS OF THE *COX1* INTRON IN SOLANACEAE DUE TO A FUNCTIONAL HOMING ENDONUCLEASE

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Plant mitochondria are unique in their propensity to acquire genes by horizontal transfer. The most stunning case of horizontal gene transfer (HGT) involves a group I intron in the mitochondrial gene *cox1*. We have previously estimated 80 transfer events among >800 angiosperms. This intron encodes a putatively functional homing endonuclease that may promote the intron's frequent horizontal acquisitions. To study the endonuclease activity, we designed an experimental approach based on somatic cell hybridization. Given that the ideal plant group for somatic genetics is the family Solanaceae, we first undertook an extensive survey to find members with the *cox1* intron. Out of 426 solanaceous species examined by PCR and sequencing, only three clades contained the intron. Analyses of intron evolution suggested three independent and recent intron acquisitions in Solanaceae. Cybrids (Cytoplasmic Hybrids), obtained from protoplast fusions between intron-containing and intron-lacking solanaceous plants, were analyzed to test the functionality of the intron-encoded homing endonuclease. Examination of *cox1* genes from cybrid indicated that the intron colonized the *cox1* copy from the intronless plant. These results constitute strong evidence indicating that the *cox1* intron-encoded homing endonuclease is functional and active in plants.

PL-C12.

GREAT EXPANSION OF THE *MI-1* GENE CLUSTER IN *Solanum tuberosum*

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Root-knot nematodes are able to attack and seriously damage all the major crops. Resistance to the nematode *Meloidogyne incognita* has been introduced in tomato, *Solanum lycopersicum*, and consists in a multigene locus called *Mi-1*. Homologs to the gene *Mi-1* in the genus *Solanum* have been reported from three potato species, *S. bulbocastanum*, *S. phureja* and *S. Tuberosum*.
The aim of this work is to study the evolutionary relationships among *Mi-1* homologs in the genus *Solanum*. We amplified, cloned and sequenced 13 homologs from the wild potato *S. kurtzianum*, which showed tolerance to *M. incognita* infection. Also, we analyzed more than 120 *Mi-1* homologs from *S. tuberosum*, obtained from Genbank databases using BLAST. These homologs were aligned and edited manually using MacClade. Maximum likelihood analyses were performed with Garli.
The phylogenetic tree showed a great expansion of the homologous *Mi-1* locus in *S. tuberosum* with two main gene clusters located in chromosomes 5 and 6. Sequences from *S. kurtzianum* grouped with *S. tuberosum Mi-1* homologs from chromosome 6. We also observed at least seven translocations to other chromosomes (chr 1, 8, 9 and 11) in the genome of *S. tuberosum*. Putative repetitive hybridizations in the origin of the cultivated potato may be responsible for the multiple duplication events of *Mi-1* homologs.

BT-C01.

PLANT HSP90 PROTEINS INTERACT WITH B-CELLS AND STIMULATE THEIR PROLIFERATION

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The molecular chaperone Hsp90 plays an important role in folding stabilization and activation of client proteins, also Hsp90 of mammals and mammalian pathogens displays immunostimulatory properties. We investigated the role of plant-derived Hsp90s as B-cell mitogens by measuring their proliferative responses *in vitro*. Plant cytosolic Hsp90 isoforms from *Arabidopsis thaliana* (AtHsp81.2) and *Nicotiana benthamiana* (NbHsp90.3) were expressed in *E.coli*. rAtHsp81.2 and rNbHsp90.3 proteins induced prominent proliferative responses in spleen cells form BALB/c mice. In vitro incubation of spleen cells with rpHsp90 led to the expansion of B lymphocytes. This effect was confirmed by immunofluorescence analysis, where a direct binding of rpHsp90 to B- but not to T-cells was observed in cells from BALB/c and C3H/HeN mice. Finally, we examined the involvement of Toll Like Receptor 4 (TLR4) in the rpHsp90s induction of B-cell proliferation. Spleen cells from C3H/HeJ mice responded poorly to prAtHsp90. However, the interaction between rpHsp90 and B-cells from C3H/HeJ mice was not altered, suggesting that the mutation on TLR4 would be affecting the signal cascade but not the rpHsp90-TLR4 receptor interaction. Our results show that spleen cell proliferation can be stimulated by a non-pathogen-derived Hsp90. Furthermore, our data provide a new example of a non-pathogen-derived ligand for TLRs.

BT-C02.

SELF-ASSEMBLED GANGLIOSIDE MICELLES AS NANODELIVERY VEHICLES OF TAXANES

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Background: Taxanes are anticancer agents that, due to their poor aqueous solubility, are formulated in vehicles associated with severe side effects. On the other side, gangliosides are amphipathic molecules that spontaneously self-assemble in water as micelles.
Objective: Characterization of the interactions between taxanes and ganglioside micelles (water-solubility, structure, stability, cytotoxicity).
Methods: UV-Vis spectrometry, DLS, EM and chromatography were used to characterize water-solubility and structure. Human larynx hepithelioma cell cultures were used to assess cytotoxicity.
Results: Water solubility of paclitaxel (Ptx) is 1 µg/mL and increases up to 7 mg/mL upon its association with GM1 micelles. The incorporation of Ptx in GM1 reaches an optimum at Ptx:GM1 1:20 molar ratio. Loading of the Ptx into the micelle induces a structural reorganization that leads to a reduction in size and an important protection of Ptx reducing its hydrolysis at alkaline pH. Diffusion of either GM1 or Ptx is restricted upon mixed-micelle formation. In vitro assays show that Ptx is incorporated by the cells and has antimytotic activity equivalent to that of free Ptx.
Conclusions: Ptx is spontaneously incorporated in GM1 micelles, increasing its water solubility by at least 7,000 times. Complexes thus formed are more stable and do not affect the biological antimytotic activity of Ptx.

BT-C03.

SEQUENTIAL LOADING OF PACLITAXEL (PTX) AND DOXORUBICIN (D) INTO GANGLIOSIDE MICELLES

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Background: Doxorubicin (D) is an anticancer agent that has important toxic effects and low water solubility. Gangliosides spontaneously self-assemble in water as micelles and have been shown to increase water solubility of Paclitaxel (Ptx).
Objective: Characterization of the interactions between Ptx, D and ganglioside micelles (water-solubility, structure, stability, cytotoxicity).
Methods: UV-Vis spectrometry, DLS, EM and chromatography were used to characterize water-solubility and structure. Hep-2 and HeLa cell cultures were used to assess cytotoxicity.
Results: GM1 micelles increase water solubility of D up to 20 mg/mL leading to a micellar structure that protects D from alkaline hydrolysis. Similar results are obtained with Ptx:GM1 (1:20 molar ratio) micelles. However, incorporation of D into GM1 micelles impairs the incorporation of Ptx. Ternary micelles have greater stability than any of the components. Micelles are reorganized upon drug loading leading to smaller structures with a mean diameter of 10 nm. *In vitro* assays show that both drugs penetrate into HeLa and Hep-2 cells and are directed to microtubules (Ptx) and the cell nucleus (D).
Conclusions: GM1 spontaneously loads Ptx and D into different nano-domains of stable and water soluble 10 nm micelles that deliver the drugs to HeLa and Hep-2 cells similarly than the respective free drugs. Co-delivery of Ptx and D enhance chemotherapy *in vitro*.

BT-C04.

CRITICAL ASPECTS ON SCALING UP PHYTOREMEDIATION PROCESSES

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Despite the promising results obtained on herbicides phytoremediation at lab scale, they are not in correlation with the successful field scale projects. When lab data are scaled up, researchers face new paradigms to cope with. So, in the lab scale performance, it is necessary to hold a global vision about the complexity and dynamism of phytoremediation. In this context, the selection and design of the experimental system are critical. In a first stage, it is necessary to consider the soil properties and the characteristics of the process, in order to select the analytical method to assess the contamination level and monitor the process. The experimental systems can be: cell cultures, organ cultures, hydroponics and microcosms. In a second stage, the selection and management of the biological agents are the main aspects to be considered. The sole presence of plants is an advantage since they generate a microenvironment favorable for microbial activity and usually reduce the run-off and leaching of the contaminant. In a third stage, the scaling up requires engineering to deal with large amounts of soil. Although the low cost is the main advantages of phytoremediation, field scale projects involve large budgets. So, full scale phytoremediation projects imply an interdisciplinary approach, which include from biochemistry and agronomy to engineering, legislation and economy aspects.

BT-C05.

THERMOPHILES BIOCATALYSTS FOR ANTIVIRAL COMPOUNDS SYNTHESIS

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Bioprocesses have become widely used in several fields of biotechnological industry, offering advantages over conventional chemical methods. The use of whole cells in biocatalysis can be improved by immobilization techniques. Immobilization allows the reuse of biocatalysts and simple purification methodology.

Thermophiles bacteria are very useful in industrial processes owing to their activity under hard conditions. In addition, high temperatures increase substrates solubility, decrease microbial contamination and appearance of sub-products.

Screening was performed using different thermopiles bacterial genus. *Geobacillus* genus has shown high yields in 6-modified 2'-deoxyribosides production in short periods of reaction. These microorganisms were able to yield 90% and 80% in two hours of 6-bromopurine and 2,6-diaminopurine 2'-deoxyribosides, respectively. 6-chloropurine and 6-chloro-2-fluoropurine 2'-deoxyribosides yields were about 70%. Agarose was the best matrix for immobilization. Parameters as thermal stability, mechanic resistance, cell retention, storage and reusability were evaluated. These compounds and their derivatives can be used as prodrugs or antiviral agents for hepatitis C and HIV treatments.

BT-C06.

IMMOBILIZATION OF LACTIC ACID BACTERIA AND THEIR APPLICATION IN GREEN BIOCATALYSIS

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Green Biocatalysis is an interesting alternative to replace traditional multistep chemical methods because it can develop simple reactions without environmental impact and satisfactory stereo and regiospecificity.

Immobilized biocatalysts show high chemical and mechanical stability and the products can be separated from medium for their reuse in a subsequent reaction. Additionally, these biocatalysts allow reactors' design which are easily handled and controlled.

Enzymatic synthesis of nucleoside analogues is catalyzed by two nucleosides phosphorilases (NPs). In lactic acid bacteria (LABs), this reaction could be carried out for nucleoside 2'-deoxyribosyltransferases (NDTs). NDT (2.4.2.6) provides an alternative to NPs because they catalyze the same reaction with one enzyme.

In this work, we have done a biocatalytic screening with different strains of LAB. The most active LAB was immobilized over different support by adsorption and entrapment techniques. The best immobilized biocatalysts (alginate and DEAE) have shown high activity, nearly 100% in 3 hs; and stability, with negligible loss of microorganisms (only 10%) after 50 hs of use. We used these biocatalytic systems to obtain halogenated nucleosides. These compounds are frequently used as very powerful chemotherapeutic agents.

NS-C01.

THE ROLE OF ALPHA-2M/LRP1 SYSTEM IN THE REGULATION OF MATRIX METALLOPROTEINASES IN MÜLLER CELLS

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Müller cells (MC) undergoes changes during retinal proliferative disorders. We have previously demonstrated that MC express the 2-Macroglobulin (2M) receptor, LRP1, and that under 2M treatment, MC regulate the MMP-2 activity and MC migration on different matrix-protein-coated surfaces through a LRP1-dependent mechanism, which suggest the participation of MT1-MMP. To test this hypothesis we evaluated whether LRP1 regulates the MT1-MMP function in an spontaneously immortalized Müller cell line (MIO-M1) stimulated by 2M. The cellular distribution of MT1-MMP and LRP1 was examined by confocal microscopy using GFP-MT1-MMP and specific antibodies against LRP1 and intracellular compartments of endocytosis and endocytic recycling. The molecular association of MT1-MMP/LRP1 was analyzed by immunoprecipitation (IP). MIO-M1 cells under 2M treatment showed that LRP1 and MT1-MMP were mainly co-localized in endosomal compartments characterized as sorting endosomes. By IP assay we showed a molecular association between MT1-MMP and LRP1, which was increased by 2M stimulation. Results from our experiments provided new knowledge to understand how LRP1 regulates the intracellular traffic of MT1-MMP to the plasma membrane.

LI-C01.

THE TYROSINE PHOSPHATASE SHP2 REGULATES THE EXPRESSION OF THE ACYL-COA SYNTHETASE ACSL4

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme implicated in fatty acid metabolism which preferes arachidonic acid. cAMP-stimulation of steroidogenic cells leads to increased ACSL4 protein levels, through a pathway that requires protein tyrosine phosphatase (PTP) activity. Since NSC87877, potent inhibitor of the PTP SHP2, reduced cAMP-stimulated progesterone production (cAMP: 77±15; NSC87877+cAMP 38±9 ng/ml, $p<0.001$) in MA-10 Leydig cells, we tested whether SHP2 is involved in ACSL4 expression. For that purpose, we used a plasmid-mediated gene transfer and RNAi-mediated gene silencing approach to modify intracellular levels of SHP2 in MA-10 cells and determined ACSL4 mRNA (RT-PCR) and protein levels (Western blot) and steroid production (radioimmunoassay). Overexpression of SHP2 increased ACSL4 protein levels in cAMP-stimulated cells ($p<0.001$ vs mock). The effect could be specifically attributed to SHP2 since knock-down of this PTP by specific shRNA reduced ACSL4 mRNA and protein levels (in both cases, $p<0.01$ vs mock). Modifications in SHP2 protein levels also affected the steroidogenic capacity of MA-10 cells: overexpression or knock-down of SHP2 led to increased or decreased steroid production respectively ($p<0.01$ and $p<0.05$ vs mock respectively). In conclusion, SHP2 is at least one of the PTPs involved in the regulation of the expression of the fatty acid-metabolizing enzyme ACSL4.

LI-C02.

PHOSPHATIDYLCHOLINE: STRUCTURAL AND SIGNALING ROLE IN NEURONAL DIFFERENTIATION

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Neuritogenesis is a dynamic process, involving the extension of long protrusions called neurites, and is critically dependent on membrane biosynthesis. Phosphatidylcholine (PC) is the most abundant phospholipid in eukaryotic cells. During retinoic acid (RA) induced differentiation of neuroblastoma cells, the augmented PC synthesis is supported by the sequential activation of two enzymes of the Kennedy pathway: choline kinase (CK) and CTP:phosphocholine cytydilyltransferase alpha (CCTa). In addition, enforced CK or CCTa expression promoted neuronal differentiation even in the absence of RA.

Interestingly, we found that the addition of PC liposomes promotes neuronal differentiation by activating ERK signaling cascade, mimicking RA effects. In addition, PC-treated neurons express bIII-tubulin as a differentiation marker.

In light of these results, chemical inhibitors or siRNAs designed to specifically inhibit CK or CCTa activity, significantly abrogate the extension of neurites, attenuate ERK signaling cascade and decrease the expression of bIII tubulin.

These results allow us to propose that PC, or any of its derivative metabolites, are not only important as structural membrane components but it could also stimulate neuronal differentiation, which means a significant progress in the identification of specific signals regulating neuritogenesis.

LI-C03.

BIOSYNTHESIS OF VERY LONG CHAIN POLYENOIC FATTY ACID-CONTAINING SPHINGOLIPIDS IN GERMINAL CELLS

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In mammalian testis, sphingomyelin (SM) and ceramide (Cer) were previously shown to be rich in very long chain (VLC) polyunsaturated fatty acids (PUFA). Recent work in rat pachytene spermatocytes and round spermatids demonstrated that these sphingolipids exclusively belong to germ cells. We also recently showed that these two cell types, highly enriched in 22:5n-6-containing glycerophospholipids, express mRNA transcripts of ELOVL5 and ELOVL2, two elongases involved in the biosynthesis of C18-C22 PUFA. Because such type of PUFA need to be further elongated to produce the VLCPUFA present in rat testicular sphingolipids (C26-C32), in this work we studied the expression of ELOVL4, the enzyme expected to catalyze such elongation. During postnatal development, ELOVL4 protein expression was first detectable in the rat testis at P25, in concomitance with the timing of appearance of the first spermatocytes. In adult rat seminiferous tubules, whereas the expression of this elongase was found to be only marginal in Sertoli cells, it was significant in spermatocytes and spermatids. The use of radiolabeled precursors and the fluorescent marker NBD C6-Cer showed that isolated spermatogenic cells in culture do synthesize sphingolipids. Both results are consistent in demonstrating that spermatogenic cells are able to biosynthesize the molecular species of SM and Cer that contain VLCPUFA

LI-C04.

THE LIPID PROFILE IS A ROBUST INDICATOR OF FUNCTIONAL SENESCENCE IN THE MEDFLY *Ceratitis capitata*

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The Medfly *Ceratitis capitata* is a global orchard pest of great economic importance. Population studies in this species have been essential to answer fundamental demographic questions. We used *C. capitata* as a model to study functional senescence, combining demographic, behavioral and biochemical parameters. We previously analyzed survival curves of *C. capitata* under standard laboratory conditions (23°C, 60% room humidity), as well as changes in spontaneous locomotor activity, negative geotaxis performance and lipid profile with age. Multivariate statistical analyses showed age-dependent changes of the lipid pattern that were mostly influenced by, e.g., sterol esters, isoprenoids and fatty acid esters. To evaluate if the lipid profile could be used as a biomarker of functional state, we compared these results with those of populations maintained under a mild thermal stress of 28°C. Mean longevity of females kept at 28°C was lower than 23°C controls, while males did not present differences in their survival curves. The performance in different behavioral assays of individuals kept at 28 ° C was worse than that of individuals at 23°C. The lipid pattern of young populations at 28°C was similar to the pattern of old populations at 23°C. In conclusion, we believe that the lipid profile is a good indicator of the functional state of flies, which declines with age and under a stress condition.

LI-C05.
NUCLEAR LIPID DROPLETS HAVE AN ACTIVE LIPID METABOLISM

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Nuclear lipids have an active role in cell proliferation, differentiation and apoptosis. We have already determined that nuclear lipids would have two main locations, the double nuclear membrane (mainly composed of GPL, SPL and C) and the Nuclear Lipid Droplets (NLD: mainly composed of TAG, CE and C); both of them correspond to alternative lipid sources with different chemical composition, physical properties, regulation and functions. We have also demonstrated that nuclear matrices (where NLD are located) have a more active lipid metabolism than that of whole liver cell nuclei. Taking into account that nuclear oleic acid is mainly esterified to TAG and CE, the aim of this work was to determine if exogenous 18:1n-9 could be incorporated into NLD lipids. With this purpose, rat liver isolated nuclei (N) were labeled *in vitro* with [1-^{14}C]18:1n-9 and NLD were isolated after incubation. In NLD, [1-^{14}C]18:1n-9 was incorporated as FFA and esterified in TAG>GPL>CE. Neither 18:1n-9 nor 16:0, 16:1n-7, 18:0 and 18:2n-6 were esterified to exogenous [4-^{14}C]C, in spite of [4-^{14}C]C being incorporated into NLD. Exogenous C must have been incorporated in an inadequate pool where it cannot be esterified. In conclusion, NLD have an active lipid metabolism since endogenous lipids FA can be remodeled. NLD oleic acid (TAG and CE) is a small cellular pool with a strategic localization.

LI-C06.
A COMPREHENSIVE STUDY OF PHOSPHOLIPID METABOLISM IN BREAST CANCER

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De-regulation of the enzymes involved in phospholipid metabolism has been previously related individually with human cancer. Previous results from our group showed the relevance of the specific alteration of phospholipid metabolism enzymes such as choline kinase (ChoK) and phospholipase D (PLD). However, a comprehensive study with all the enzymes, regulatory proteins and metabolites involved, to better understand their mutual functional interdependency and the effects resulting from individual alterations has not been approached yet. The objective of this study is the investigation in a comprehensive manner of the specific levels of expression of these functionally related genes in patients with breast tumours. Thus, the approach followed in this study, will provide unique comprehensive information on those key steps that are linked to the alterations on these critical enzymes required for the generation and progression of cancer cells.
Furthermore, the effects of inhibitors and specific siRNA downregulation of some of the key enzymes (such as ChoK) will be essential to understand better their mechanism of action. Our previous results showed that blockage of *de novo* synthesis of phosphocholine by ChoK inhibitors as a potent antitumoral activity, producing a drastic wobble in the metabolism of sphingolipids. The new technologies, such as HPLC-MS, may lead to identify the species of sphingolipids, subspecies of ceramides and dihydroceramides that participate in the action of these inhibitors.

MI-C01.

TRANSCRIPTIONAL CONTROL OF THE COPPER-RESISTANCE REGULON IN *Salmonella enterica*

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Copper (Cu) is an essential metal required as a cofactor in numerous essential biochemical reactions. However, free Cu ions can be toxic. In Gram-negative bacteria, Cu toxicity is handled by the *cue* regulon. This includes the Cu-sensor/regulator CueR that induces the expression of the Cu-efflux pump *copA* and the multicopper oxidase *cueO* coding genes. Under anaerobic conditions most species also require the *cus* system to cope with Cu excess. This includes CusR/CusS, a two-component system that senses periplasmic Cu and induces the expression of CusCFBA, an RND-based Cu-efflux pump. In *Salmonella* as well as in other species that do not harbor the *cus* locus, Cu resistance in anaerobiosis relies on CueP, a periplasmic protein encoded by a gene that belongs to the *Salmonella cue* regulon. We examined the transcriptional regulation of the *Salmonella cue* regulon in different environments and found a differential expression within its constituents. Using bioinformatic tools we detected putative cis-acting sequences responsible for this differential expression. *in vivo* transcription as well as *in vitro* footprinting experiments allowed us to confirm the existence of these regulatory elements, which ensure the correct supply of the copper-resistance components when the environment becomes unfavorable for cell survival.

MI-C02.

ABAR-TYPE RESISTANCE ISLANDS IN MULTIDRUG RESISTANCE *Acinetobacter baumannii* (MDRAB) ISOLATES

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Over the past years, MDRAb isolates have been reported with increasing frequency. The acquisition of resistance has been correlated with the large number of resistant determinants in its genome. The aim of this study was to determine the occurrence of the AbaR-type genomic island and its association with class 1 integrons MDRAb isolates.52 MDRAb isolates recovered from several hospitals at different years and belonging to unrelated clones were included. The presence and structure of the island were determined by an AbaR1-based PCR mapping. Class 1 integrons location was investigated by PCR amplification. AbaR-type genomic resistance islands were present in 37/52 MDRAb isolates. PCR mapping showed different arrays to those previously described, evidencing the high variability of the AbaR-type islands. Class 1 integrons were found in 13 isolates. In 9 isolates (69%) class 1 integrons were located inside the island, and in 3 isolates we identified its plasmid location. Accordingly to MLST, isolates carrying AbaR-type islands were grouped at least in 3 clonal complexes and 5 new singletons. A widespread distribution of the AbaR-type genomic island among novel clones of Ab has been identified. The high frequency of class 1 integrons inserted in the AbaR-type genomic island in our Ab population since 1986 suggests its preferential location and persistence over time of this genetic platform

MI-C03.

***Helicobacter pylori* PLASTICITY ZONE: VARIABLE PUTATIVE PATHOLOGY-SPECIFIC-GENES CONTENT**

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We investigated: inter-niche variation in a single host of *jhp0940*, *jhp0945*, *jhp0947* and *jhp0949* genes in populations with peptic ulcer (PU) and chronic gastritis (GC); in addition to the TnPZ types. A total of 193 isolates from 34 patients (19 with CG and 15 with PU) were obtained from multiples biopsies in the same endoscopy process. Neutral markers were used to identify mixt infections. The pathology-specific-genes and the others from the three types of TnPZ were identified by PCR. For statistical analysis, SPSS program vs-16 was used. None of the pathology-specific-genes was significantly associated with PU or CG ($p>0,05$). Inter-niche variations were observed with the following frequency: 1/4 (*jhp0949* and *jhp0940*), 1/6 (*jhp0947*) and 1/3 (*jhp0945*) patients. Considering the 193 isolates, 38% showed the three TnPZ types or their remains, 32% two types, 30% one type. Inter-niche variations were observed in 19/34 patients. Our results support the hypotesis that Hp uses the constant acquisition and loss of genes during persistence jhp0940, jhp0945, jhp0947 and jhp0949 genes located in the *Helycobacter pylori* (Hp) plasticity zone (PZ) were assumed as pathology-specific-genes. Recently, the PZ was recognized as a genomic island structure including also different Type IV Secretion Systems. Three class of island (transposone-PZ), called TnPZ type-1, TnPZ-type-1b and TnPZ-type-2 were described.

MI-C04.

MULTILOCUS SEQUENCING TYPING (MLST) SCHEME OF *Acinetobacter baumannii* ISOLATED IN THE LAST 3 DECADES

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Acinetobacter baumannii has emerged as a significant global pathogen, with a rapid acquisition of antimicrobial pan-resistance and spread within hospitals. This study was aimed to investigate *A. baumannii* lineage related to nosocomial infection during 1983 to 2010 in Argentinean and in borderline countries. MLST and Sequence type (ST) assignation for the 95 isolates studied were performed according to *A. baumannii* MLST website (http://pubmlst.org/abaumannii/). STs clonal complexes (CCs) assignation was assessed by the eBURST algorithm (http://eburst.mlst.net/).All isolates were classified in 20 well defined and 14 news STs, grouped in 5 CC and 14 singletons. Sixty-four isolates were placed in 3 CC: CC131 (Argentina and Uruguay), CC119 (Argentina and Chile), CC109 (Argentina and Uruguay). In argentinean hospitals CC131 was widespread and it persists since 1983, conversely CC119 was recovered until 1997. Albeit, the macroestriction previously demonstrated monoclonal pattern of most outbreaks and the inter- and intra-hospital transmission of defined clones and their endemic persistence, MLST demonstrated the temporal evolution and spatial distribution of the lineage. In addition, international comparison delineated our persistent CC131 as a proper South-American lineage. In contrast with Europe, in Argentina isolates with epidemic behaviour showed a highest range of STs within a CC.

MI-C05.

INSIGHTS INTO THE CO-EVOLUTION OF REGULATOR/OPERATOR SELECTIVITY AMONG MerR TRANSCRIPTION FACTORS

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Metal ions are essential cellular constituents but they can be toxic at high concentration. In *Salmonella*, two paralog metal sensors of the MerR family, CueR and GolS, direct the response to Cu, Au and Ag ions by inducing the expression of different set of transporters. Previously, we demonstrated that cross-activation between CueR- and GolS-controlled promoters is prevented in two ways: the presence of distinctive bases at the centre of the operator sequences that confer selectivity towards their innate regulator, and a tight control of the cytoplasmic concentration of each transcription factor. Here, we evaluated the role of the domain and the amino acid residues that privilege binding to CueR and GolS target operators. The interaction of each mutant regulator with target promoters from both regulons was evaluated both *in vivo* and *in vitro*. Our studies indicate that the selective operator activation relies on the a2-helix, in particular the residue at position 16 within this helix. Furthermore, we show that all GolS xenologes harbour an invariant M residue at this position while T, A or S, but not M, are present in CueR-like proteins. The co-evolution of distinctive features in both the transcription factor and its target promoters guarantee selective recognition and a proper response to metals.

MI-C06.

PBP2B CONTROLS THE SHAPE DETERMINATION AND CELL DIVISION MECHANISMS OF *Streptococcus pneumoniae*

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Mutations in penicillin-binding proteins (PBPs) PBP1a, PBP2x, and PBP2b confer ß-Lactam resistance in *S. pneumoniae*. These enzymes are involved in cell wall synthesis and cell division. We described that laboratory strain harboring *pbp2b* mutations showed morphological abnormalities (rod-shaped cells) with an abnormal septum pattern, suggesting alterations in the cell division mechanism. These cell alterations were compensated by the acquisition of *pbp2x* and *pbp1a* resistance-conferring mutations obtained from clinical strains.

Analyzing PBP-tag protein fusions, we demonstrated that all these pbp mutations conferred an increased stability to their respective mutant proteins, suggesting that a higher half-life of PBP2x and PBP1a mutant proteins is necessary to compensate the increased half-life of the PBP2b mutant protein (PBP2b*). Probably this compensation is related to a coordinated enzymatic activity between PBPs.

In the *pbp2b* mutants, we demonstrated that the FtsZ and PBP2B* were delocalized, and that PBP2b* displayed a helix manner, similar to that showed by FtsZ in the same *pbp2b* mutants. By two-hybrid system assays, we detected a protein-protein interaction between FtsZ and PBP2b*, suggesting that the cell division alterations are caused by this interaction. We propose that PBP2b participates in the control of shape determination and cell division mechanisms in *S.pneumoniae*.

MI-C07.

ANALYSIS OF A *Bordetella bronchiseptica* CYCLIC-DI-GMP-BINDING PROTEIN REVEALS A ROLE IN MOTILITYAND

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Bis (3',5')-cyclic diguanylic acid (c-di-GMP) is a cyclic dinucleotide that has recently been recognized as an important intracellular signaling molecule in diverse bacteria. The intracellular concentration of c-di-GMP is regulated by the opposing activities of diguanylate cyclase (DGC) and phosphodiesterase (PDE) enzymes. PilZ domain-containing proteins bind c-di-GMP and serve as important downstream effector proteins. Conformational changes in Pilz proteins after c-di-GMP binding are implicated in bacterial motility and pathogenesis of several pathogens.

Bordetella bronchiseptica is a pathogenic bacterium that causes respiratory infections in a wide variety of host. We determined that the only PilZ containing domain protein in *B. bronchiseptica* genome (BB1561) is able to bind c-di GMP. In a previous work we determined that high c-di-GMP enhances biofilm formation and decreases motility in *B. bronchiseptica*. We constructed a deletion mutant in BB1961 and determined that biofilm formation was enhanced and motility was reduced compared to parental strain. Furthermore, experimental mice colonization was reduced in BB1961 mutant compared to parental strain.

MI-C08.

FASR, A NOVEL TRANSCRIPTIONAL ACTIVATOR OF THE *FAS* GENE OF *Mycobacterium tuberculosis*

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M. tuberculosis remains a major human public health threat. The success of this pathogen largely stems from its remarkable capacity to survive within the infected host, being its unusual cell wall a key factor in this survival. Mycobacteria cell wall biosynthesis involves two structural distinct fatty acid synthase systems, FAS-I and FAS-II, which should work in a finely coordinate manner to keep lipid homeostasis tightly regulated. Our studies on the regulation of the *fasII* operon provided strong evidences of the existence of a sophisticated regulatory signalling cascade involved in the coordinate regulation of the mycobacterial FAS systems at the transcriptional level. Recently, we were able to identify and purify from *M. smegmatis* crude extracts, a new transcriptional regulator of the *fas* gene, named FasR. Footprinting assays demonstrated that FasR specifically recognize two regions in the *fas* gene promoter (p*fas*). The interaction of FasR with p*fas* is inhibited in the presence of long chain acyl-CoAs. Beta-galactosidase assays indicated that FasR is a positive regulator of *fas* gene and confirmed the modulatory role of long chain acyl-CoAs in the transcription of the *fas* gene. Construction of the mutant strain will help us to elucidate the complex regulatory network of fatty acids biosynthetic pathway, an attractive target for the development of new antimycobacterial drugs.

MI-C09.

IDENTIFICATION OF A PLANT EXTRACT WITH INHIBITORY ACTIVITY AGAINST *Salmonella enterica* PhoP/PhoQ SYSTEM

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Salmonella is an enteropathogen that causes a wide range of diseases in humans and animals. During infection, bacteria continuously interact with the surrounding media, in order to coordinate the expression of genes required for invasion and colonization of the host. In *S. typhimurium*, the PhoP/PhoQ two-component system (TCS) controls the virulence, the adaptation to limited Mg^{2+} conditions and the resistance to antimicrobial peptides. Because mammals lack TCS, histidine kinases are strategic targets for new antimicrobial agents. By using a bioguided strategy, consisting on a TLC-overlay with a strain carrying a PhoP-controlled reporter gene, we have determined that a purified fraction from a *Lamium amplexicaule* methanol extract represses the PhoP/PhoQ system, but has no effect on genes regulated by other homologous and heterologous systems. This was confirmed by quantitative ß-galactosidase activity assays. Using membrane vesicles enriched in the sensor protein we have also established that the target of action is the autokinase activity of PhoQ, and that the response is dose-dependent. Further studies are being carried out in order to determine the specificity of the inhibition and the molecular basis for this action. This methodology stands for a new strategy for the search and identification of antimicrobial agents in plants.

MI-C10.

TRANSCRIPTIONAL REGULATION OF AGMATINE DEIMINASE PATHWAY IN *Enterococcus faecalis*

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Enterococcus faecalis are found as nonstarter lactic acid bacteria in a wide variety of cheeses mostly produced from raw milk. This microorganism can be potentially hazardous to human health due to their pathogenicity or by the production of toxic compounds like biogenic amines. *E. faecalis* is able to metabolize agmatine with putrescine, ammonia and ATP as final products by means of agmatine deiminase pathway.
In this work we demonstrate that the transcriptional regulator AguR is essential for the agmatine metabolism in *E. faecalis*, since an AguR defective strain is unable to grow in presence of agmatine. Moreover, as a result of ß-galactosidase and Northern blot analysis we could demonstrate that agmatine is inducing the *agu* gene cluster. There are some differences in the mechanisms underlying agmatine metabolizing gene expression in *E. faecalis* with the related bacterium *Streptococcus mutants*. In the later, the *agu* cluster is induced under acid or high temperature conditions, whereas in *E. faecalis* its expression showed to be independent of pH and temperature. Finally, we investigated the role of catabolite repression on the activity of the AguR controlled promoter, showing that this promoter is regulated by a CcpA independent mechanism.

MI-C11.

ENTEROBACTIN PROTECTS *Escherichia coli* FROM THE ROS-MEDIATED TOXIC EFFECTS OF PYOCHELIN

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Many bacteria produce the uptake systems for siderophores that they do not synthesize, allowing them to benefit from resources produced by other bacteria. Under iron-limited conditions, *Escherichia coli* K-12 produces the catecholate siderophore enterobactin in addition to the transport systems for a variety of other siderophores. However, it is not known whether there is an uptake system for pyochelin, a pseudomonad siderophore. In this report, we analyzed the moderate toxicity we observed for pyochelin on *E. coli*. An *E. coli* enterobactin mutant (*entE*) showed elevated sensitivity to pyochelin and this was reversed by addition of enterobactin. Surprisingly, iron supplementation did not have a similar protective effect, ruling out iron starvation as an inhibitory mechanism. Addition of pyochelin to *entE E. coli* increased levels of reactive oxygen species (ROS), which were lowered in the presence of exogenous enterobactin and ascorbic acid. In addition, we observed that copper, but not iron or zinc, also reversed pyochelin toxicity. This reversal was not observed in a *cueO* background, thus implicating the multicopper oxidase CueO in protection from pyochelin. Together, these results suggest that siderophores may be used by bacteria to exert physiological and environmental roles other than iron acquisition.

MI-C12.

ROLE OF THE REPLICATION PROCESSIVITY FACTOR ß CLAMP IN THE MISMATCH REPAIR OF *Pseudomonas aeruginosa*

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The DNA mismatch repair system (MRS) removes nucleotides mis-incorporated during DNA replication and regulates the recombination between divergent sequences. It has been proposed that the replication processivity factor ß clamp can play a role in the initiation of MRS, by targeting the repair protein MutS to areas of active DNA replication, and in the discrimination of the newly synthesized strand. We have analyzed the role of MutS-ß clamp interaction in *Pseudomonas aeruginosa* by characterizing two MutS mutant versions that do not interact with the replication factor: a C-terminal deletion mutant (MutS798) and a ß clamp binding motif mutant ($MutS^{ß}$). A detailed analysis of MutS798 and $MutS^{ß}$ function demonstrated that both mutants were proficient in mismatch repair even under mutagenic conditions, as we determined by measuring mutation rates to rifampicin and ciprofloxacin resistance and reversion of a +1G mutation. These results clearly indicate that MutS-ß clamp interaction is not required for a proper MRS activity in *P. aeruginosa*. However, determination of the molecular basis of resistance to rifampicin and ciprofloxacin revealed that $MutS^{ß}$ produced a mutation spectrum different from wild type MutS and unrelated to *mutS* deficiency. At present, we are analyzing the biochemical properties of $MutS^{ß}$, and if the affinity of MutS for different heteroduplexes is regulated by ß clamp.

MI-C13.

BIOFILM DEVELOPMENT IS INFLUENCED BY THE GLOBAL REGULATOR ANR IN *Pseudomonas extremaustralis*

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Bacterial biofilms, known as complex communities, represent a protective life style. Biofilms have heterogeneous structure giving rise to nutritional gradients where oxygen is one of the most important. In *Pseudomonas* species, genes involved in microaerobic metabolism are controlled by Anr, a global redox regulator. In this study we analyze the influence of Anr in biofilm formation in *P. extremaustralis*, a highly stress resistant Antarctic bacteria, using an *anr*- strain. Biofilms were developed in a "glass bottom Petri dish" for 24, 48, 72 or 96 h and analyzed by using epifluorescence microscopy and COMSTAT2 software. The growth of planktonic cells, measured as OD600nm, was similar in both strains, however, the *anr*- biofilms presented lower biomass and thickness than the wild type strain biofilms. In addition, impairment in aggregation was observed in the mutant strain. Electron microscopy showed that in the *anr*- cells, flagella were more numerous and longer compared with those of the wild type strain, in line with a higher motility in the mutant strain using solid media. *In silico* analysis of the upstream region of flagella associated genes such as *fliK* ,*fliM* and *flgE* allowed to predict conserved Anr-boxes suggesting that Anr is involved in flagella formation and biofilm development.

MI-C14.

POLYPHOSPHATE DEGRADATION IN STATIONARY PHASE TRIGGERS BIOFILM FORMATION VIA AI-2 IN *Escherichia coli*

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Escherichia coli maintained a high polyphosphate (polyP) level even in stationary phase when cells were grown in media containing phosphate concentration ≥37 mM. Here, the effect of phosphate and polyP levels on the ability to form biofilm was analyzed. PolyP levels were modulated by the phosphate concentration and/or by using deficient strains in polyphosphate kinase (*ppk*) and exopolyphosphatase (*ppx*). Biofilm formation was carried out in MT media supplemented with different phosphate concentrations in polystyrene plates at 30ºC and it was quantified by the crystal violet method. At 48 h, maximal biofilm formation was achieved between 2-10 mM phosphate, being almost nule at concentrations ≥35 mM. Biofilm formation was not observed when *ppk*$^-$*ppx*$^-$ and *ppk*$^-$*ppx*$^-$ /pBC29(*ppk*$^+$) strains were analyzed in the whole range of phosphate concentrations. To determine if *luxS*/AI-2 or indol signaling quorum sensing systems are involved in the phosphate effects, deficient strains in *luxS*, *tnaA* and *mtr* genes were used. The *luxS* mutant was unable to form biofilm in sufficient phosphate media (2 mM). However, biofilm formation was observed when *luxS*$^-$ cells were incubated with spent MT media of wild-type culture. According to our results, the polyP degradation in stationary phase is a key event that triggers AI-2 synthesis, a signal involved in biofilm formation.

MI-C15.

FIRST GENETIC EVIDENCE OF OXALOACETATE DECARBOXYLASE INVOLVEMENT IN CITRATE METABOLISM IN FIRMICUTES

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Citrate metabolism in *E. faecalis* involves the conversion of citrate to pyruvate by the sequential action of citrate lyase and oxaloacetate decarboxylase (OAD) enzymes. Our previous reports indicated that the gene cluster involved in citrate degradation in *E. faecalis* encodes an OAD membrane complex homologous to that reported for *Klebsiella pneumoniae*. In *K. pneumoniae* the complex is constituted by three subunits (Kpn- , and), whereas in *E. faecalis* a four subunit complex was found (Ef- , , and) in which the extra Ef- subunit may have functional properties analogous to the Kpn- subunit. Our analysis indicated that , and form a soluble complex that is capable of interacting with the membrane-bound ß subunit in a dynamic way. Moreover, construction of *E. faecalis* mutant strains for the different OAD subunits was performed. Diminished growth of the mutants in citrate supplemented media indicated that the activity of OAD is essential for citrate utilization. Also, the nature of the interaction between the subunits was analyzed. We observed that the complex was stable at acidic pH, but its integrity was compromised at incrementing basic pHs. Furthermore, analysis of single and double mutant strains allowed identifying interacting partners. In summary, we propose that the new subunit mediates the interaction between the remaining subunits of the complex.

MI-C16.

PURINE METABOLISM AND POLYKETIDE PRODUCTION IN *Streptomyces coelicolor*

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Bacteria from the genera *Streptomyces* produce a vast array of secondary metabolites of industrial and medical interest. Among these metabolites, polyketides compounds are especially important due to the diversity of biological activities they comprise (insecticides, inmunosupresors, antibiotics, anticancerous). These natural compounds are synthesized by a multienzymatic complex under certain conditions of growth and are regulated by specific transcriptional regulators. In turn, these transcriptional factors respond to intra and extracellular signals sensed by a set of global regulators. We have previously identified a gene in *S. coelicolor*, *allR*, encoding a transcriptional regulator that strongly impairs antibiotic production when inactivated. AllR negatively controls the expression of genes required for allantoin catabolism. This metabolic pathway is involved in the recycling of the carbon and nitrogen components of purines generated during nucleotide turnover. Proteome analysis of *allR* mutant strain has shown increase expression of proteins involved in the tricarboxylic acid cycle, biosynthesis of aminoacids and metabolism of purines among others. This result demonstrates that AllR is involved in the expression of other metabolic pathways and represents an important link between primary and secondary metabolism in *Streptomyces*.

MI-C17.

THE MCE1 PROTEINS OF *Mycobacterium tuberculosis* ARE INVOLVED IN THE UPTAKE AND METABOLISM OF LIPIDS

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The Mce proteins of *M. tuberculosis* have been implicated in the entry of the bacterium into the host cells. These proteins are codified in four locus in the *M. tuberculosis* genome: *mce1, -2, -3 and -4*, each one includes two *yrbE* and six *mce* genes. An *in silico* analysis have revealed them as transporter systems. Recently, it was demonstrated that Mce4 are involved in cholesterol uptake. Due to, one adaptation of *M. tuberculosis* to persist into the host cells is its changes to utilizes lipids as carbon source, we analyzed the role of Mce1 proteins into the uptake and metabolism of palmitic acid (pal). We have observed that *M. tuberculosis mce1* mutant incorpotes less [^{14}C]-pal compared to the wild type (WT) in the first 30min of an uptake experiment. However, after 30min [^{14C}]-pal accumulates in the mutant while the WT remains constant. In addition, by qRT-PCR experiment we observed a correlation between the uptake of pal and the *mce1* genes expression. Also, we identified two lipids accumulated in the mutant compared to the WT, when the bacteria were grown with pal as unique carbon source. In this study we also analysed the Mce1 proteins localization and the intracellular traffic of the *mce1* mutant. Taken together, the results presented here suggest that the Mce1 proteins are a palmitic acid transporter system and perhaps it would be related to the membrane lipids recycling.

MI-C18.

INHIBITION OF DES TRANSCRIPTION BY CERULENIN IS MEDIATED BY THE LENGTH OF PHOSPHOLIPID ACYL CHAINS

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The Des pathway of *B. subtilis* regulates the expression of the acyl-lipid desaturase, Des, thereby controlling the synthesis of unsaturated fatty acids from saturated phospholipid precursors. This pathway is regulated by a two-component system composed of a membrane-associated kinase, DesK, and a soluble transcriptional regulator, DesR, which stringently controls transcription of the *des* gene. This pathway is activated in response to a decrease in membrane fluidity provoked by a temperature downshift. However, very little is known about how DesK discriminates the surrounding lipid environment to promote membrane remodeling upon a drop in membrane fluidity. Here we report that inhibition of fatty acid (FA) biosynthesis by cerulenin represses the expression of *des* gene. The FA profiles strongly suggest that cerulenin affects the signaling state of DesK by increasing the incorporation of the lower-melting-point shorter-chain FA into membrane phospholipids. We confirmed this hypothesis analyzing *des* expression in a mutant that conditionally expresses *plsC* (which encodes an acyltransferase). We show that strains with *plsC* depletion possess membranes with very long chain FA which lead to *des* overexpression at higher temperatures. These results provide the first *in vivo* evidence that pinpointed the membrane thickness as a signal used by cold sensors to maintain thermal homeostasis.

ST-C01.

WHAT IS MORE IMPORTANT FOR DESK ACTIVITY REGULATION: HEADGROUPS OR ACYL CHAINS OF LIPIDS?

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DesK is the thermosensor of *B. subtilis* Des Pathway, the only example in nature where the mechanism of low temperature signals perception and transduction is well established and a functional connection between induced protein and cold adaptation demonstrated.
Since DesK is a membrane-integrated kinase with sensing mechanisms relying on its five transmembrane segments (TMS), it represents an ideal system for studying the molecular mechanism of thermodetection and signal transduction. Changes in environmental temperature may induce changes in lipid properties that in turn would affect the dynamics of DesK TMS, leading to changes in the extra-membraneous parts resulting in signaling.
To provide insight in this unique mechanism of signaling we studied the nature of the TMS-lipid interactions that are critical for sensing and signaling, analyzing the effect of bilayer thickness, lipid fluidity, lipid acyl chain packing as well as phospholipid headgroup on the autokinase activity of DesK. To this end, we biochemically characterized DesK proteoliposomes composed of different headgroup lipids containing variable fatty acyl chains. The results obtained *in vitro* complemented with *in vivo* experiments performed in *B. subtillis* phospholipids mutants, led us to conclude that the properties of the acyl chains of membrane phospholipids are the main clue that regulates DesK activity.

ST-C02.

HIV-1 TAT PROTEIN MAY DIRECTLY CONTRIBUTE TO NEUROAIDS : INHIBITORY EFFECT OF TAT ON NEUROSECRETION MECHANISM

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Tat is a small protein of HIV-1 involved in viral transcription. HIV-infected cells actively release Tat, and extracellular Tat can enter into uninfected cells potentially affecting various cellular functions. In the central nervous system (CNS), HIV-1 can infect astrocytes and microglia, but not neurons. However, HIV positive patients develop CNS dysfunctions (NeuroAIDS) like dementia that can also be correlated to the presence of Tat. When Tat is added to culture medium of neurons or neurosecretory cells, we found that it is actively internalized by endocytosis. Overexpression of Tat and Tat feeding strongly impairs exocytosis from chromaffin and PC12 cells, as revealed by carbon fiber amperometry and Growth Hormone (GH) release assay, respectively. Mutations that prevent binding of Tat to phosphatidylinositol 4,5 bisphophate ($PI(4,5)P_2$) does not affect secretion, whereas overexpression of phosphatidylinositol 4 phosphate 5-kinase (PI5K), the major $PI(4,5)P_2$ synthesizing enzyme, significantly rescued the inhibition of neurosecretion by Tat. Furthermore we found that the secretagogue-dependent recruitments of the $PI(4,5)P_2$-binding-protein Annexin2 (Anx2) at the plasma membrane is inhibited by Tat. Along the same line, Tat also impairs the Anx2-dependent plasma membrane recruitment of the major actin cytoskeleton regulator Cdc42. Hence, our results suggest that Tat blocks neurosecretion by preventing the reorganization of the actin cytoskeleton necessary for the movement of secretory vesicles towards their plasma membrane fusion sites. In agreement with this idea, we found a significant reduction of actin depolymerization in stimulated cells. Finally, we also found that the plasma membrane relocation of a DAG sensor in response to ATP stimulation is strongly delayed in the presence of Tat, suggesting a slower hydrolysis rate of $PI(4,5)P_2$ by phospholipase C. In conclusion we suggest that the inhibitory effect of Tat on neurosecretion relies on its tight binding to PI(4,5)P2, which is so strong that Tat competes with PI(4,5)P2-interacting proteins and thus blocks exocytosis. Hence impairment of neurosecretion would likely contribute to Neuro-AIDS. Preliminary data, using FM 1-43FX dye, indicate that HIV-1 Tat affects the turnover of presynaptic vesicles in primary mouse cortical neurons. Ongoing experiments concerning pre- and post-synaptic effects of the viral protein in neuron-pairs will also be illustrated.

ST-C03.

ANG II AT_2 RECEPTORS INDUCE APOPTOSIS IN HELA CELLS

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The signal transduction mechanism of Ang II AT_2 receptors in tissue remodeling and organogenesis remains unclear. Apoptosis is a mechanism involved in tissue differentiation. The aim of this project was to study apoptosis induction mediated by AT_2 in response to Ang II. HeLa cells over-expressing Ang II AT_2 receptors alone or together with RhoA WT or its mutants, were used as a model to study the signaling events triggering apoptosis. Apoptosis was evaluated by means of nuclear condensation, F-actin disassembly, caspases 3 and 8 cleavage and caspase 3/7 activation. In HeLa-AT_2 cells, cleavage of caspases 3 and 8 increased in a time dependent manner after 30 min stimulation. Caspase cleavage appeared earlier in cells co-expressing the constitutively active mutant of RhoA while the effect was absent in cells expressing the dominant negative RhoA N19. We also studied p38MAPK participation in the signaling pathway activated by AT_2 and found that inhibition with SB203580 increased apoptosis. To characterize the pathway, we determined JNK phosphorylation and FAK cleavage by western blot. Cleavage of FAK was time-dependent in Hela-AT_2 cells induced with Ang II. Co-expression of RhoA isoforms modulates FAK cleavage. The present results support a central role of RhoA in the signaling pathway of Ang II AT_2 receptors in apoptosis induction, with participation of JNK, p38MAPK and FAK.

ST-C04.

ENDODERM GENERATION FROM EMBRYONIC STEM CELLS IS REGULATED BY EXTRA-CELLULAR MATRIX REMODELING

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Adhesion of cells to the extra cellular matrix (ECM) has long been associated with homeostasis in adult tissues, enabling cell migration during development and tumour metastasis. As a result the role of ECM in cell fate specification during embryonic development has been largely seen as the indirect consequence of promoting the cellular association with particular environmental cues rather than directly inducing spatial and temporal gene expression programs. Here we use embryonic stem cells (ESC) differentiation to demonstrate a direct role for ECM in establishing the anterior-posterior (A-P) axis within the endoderm germ layer. ECM remodelling is driven by FGF signalling via phosphoinositide 3-kinase (PI3K)/Akt1 and is essential for anterior endoderm specification *in vitro* as well as *in vivo*. Isolated ECM modulates cell shape changes through a temporal restriction of Epithelial to Mesenchymal Transition (EMT) and simultaneously conveying positional identity. Based on Mass Spectrometry we identified Fibronectin (FN1) levels as a key determinant of ECM activity. Our data imply that ECM composition regulate both polarity and identity in the emerging endoderm, linking cellular morphogenesis directly to cell fate decisions.

ST-C05.

MARCKS REGULATES CALCIUM MOBILIZATION IN INTACT HUMAN SPERM

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Acrosomal exocytosis (AE) is a regulated Ca^{2+}-dependent secretion event required for sperm-egg interaction. To achieve this exocytosis, a Ca^{2+} efflux from the acrosome is required. This calcium mobilization is activated by IP_3-sensitive channels. PIP_2 is a precursor of IP_3. Our previous results, using the model of permeabilized human sperm, indicated that MARCKS plays a regulatory role on PIP_2 concentration and, in consequence, might also regulate Ca^{2+} mobilization from the acrosome. To test this hypothesis in a more physiological model, we used intact human sperm and AE was stimulated by calcium ionophore and progesterone. To analyze MARCKS function in intact sperm, we assayed the commercial MARCKS peptide corresponding to the effector domain conjugated with rhodamine (ED-TMR). The ED-TMR is a polybasic peptide and it has been described as a permeable peptide. Our results showed that ED-TMR peptide was able to translocate into intact sperm and inhibit the AE stimulated by A23187 and progesterone in a concentration-dependent manner. When calcium mobilization was assayed by spectrofluorometry, the preincubation of human sperm with ED-TMR abolished the increase in calcium levels caused by progesterone. Altogether, these results validate the hypothesis that MARCKS regulates Ca^{2+} mobilization through the regulation of PIP_2 concentration in intact human sperm.

ST-C06.

METABOLITE-IONIC FEEDBACK FOR SYNCHRONIZATION OF O_2 RELEASE AND SIGNAL TRANSDUCTION RESPONSIVENESS

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Objectives: To investigate the molecular level of the physiological relationships integrating fat cells and liver with a sensor response of red cells, to release O_2 to match glucose uptake. Methods: Characterization of metabolite-ionic hormonal responsiveness of adenylate cyclase (AC) and insulin receptor tyrosine-kinase (IRTK). Imaging of the dynamic structure of Hb. Results: the coordinative chemistry of Hb R-groups fits a unitary homo-hetero-tropic reactivity model. The pO2 saturation curve of Hb shows n=2.6 as a function of mutual inclusion of O_2 and/or Mg^{2+} plus mutual exclusion of 2,3-DPG. AC of brain, fat cells and liver IRTK show separable saturation curves for free Mg^{2+} dependence at 3 differentiable sites, with a cooperative value n=2.6: a) A site active only when free Mg^{2+} is in-excess of substrate MgATP. b) A free Mg^{2+} basal activatory site and c) As a requirement for hormonal responsiveness. Brain activity increases by 10 times glucose (G) consumption. In blood, G uptake by red cells, leads to sugar phosphate-dependent release of O_2 in cerebral spinal fluid. In nerve impulses, the Na^+-pump consumes ATP4-, releasing free Mg^{2+}. This, activate AC-responsiveness to norepinephrine and cAMP-dependent memory pathways. Conclusions: The molecular level shows free Mg^{2+} as integrating "in common" metabolic feedbacks, to prevent anaerobic glycolysis in brain tissue.

CB-P01.
NUCLEOCYTOPLASMIC TRANSPORT IN TRYPANOSOMATIDS
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The exchange of macromolecules between the cytoplasm and the cell nucleus is a vital process in eukaryotic cells. All nuclear proteins synthesized in the cytoplasm must be imported through the nuclear pore complexes generally with the aid of an active, carrier-mediated process. The import of proteins containing nuclear localization signals is facilitated by karyopherin proteins, also known as importins. Trypanosomatids are protozoan parasites with a complex life cycle that alternate between hosts and differentiation stages. They are causative agents of diseases such as Sleeping Sickness, Chagas Disease and leishmaniasis. We show here that nucleocytoplasmic transport seems to be conserved among trypanosomatids as many of the genes involved in it are found in their genomes. Although different importin variants have been discovered in higher eukaryotes, up to date only the canonical importin alpha and importin beta were found in these parasites. Homology modeling of these importins showed high conservation of three-dimensional structure albeit their sequence conservation is rather low. We found out using knock-down experiments that both importins are essential in procyclic forms of *Trypanosoma brucei*. In order to study their cellular localization, we tagged these proteins to YFP and we finally used yeast knock-out strains for complementation assays to confirm their foreseen function.

CB-P02.
OVEREXPRESSION OF TCAUK1 IN EPIMASTIGOTES OF *Trypanosoma cruzi* INDUCES CHROMATIN ALTERATIONS
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Aurora kinase family members coordinate a range of events associated with mitosis and cytokinesis. Yeast contain a single aurora kinase homologue (Ipl1), while mammals contain three (Aurora A, B and C). Aurora A is important for centrosome maturation, segregation and the assembly of the mitotic spindle. Aurora B and the yeast Ipl1 are each considered chromosomal passenger proteins. Early in mitosis Aurora B phosphorylates Ser-10 on histone H3 contributing in *Drosophila*, but not in humans, towards chromosome condensation. The function of Aurora C is less well understood. In *T. cruzi*, we have previously identified three aurora kinases paralogs (TcAUK1, 2 and 3) establishing TcAUK1 as a nuclear protein whose protein expression is regulated in a cell cycle-dependent way. In the present study we generate TcAUK1 overexpressing epimastigotes and begin to evaluate their phenotype. By Southern, northern and western blot was confirmed their overexpressing condition and by indirect immunofluorescence was determined the nuclear localization of TcAUK1 in transfected parasites. In addition, overexpressing-parasite extracts showed higher kinase activity levels than controls. Interestingly, DAPI DNA staining showed an altered chromatin distribution in the overexpressing parasites when compared with the empty vector control suggesting a possible role for this enzyme in cell cycle regulation.

CB-P03.
MOLECULAR AND MORPHOLOGICAL CHANGES IN TRANSPORT PLAYERS AFTER A SECRETORY STIMULATION
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The exocytic and endocytic pathways are ubiquitous and essential for the export and import of proteins. Secretory tissues required extensive membrane transport activity to accomplish their secretory function; however, how a secretory stimulus regulates molecular and morphological changes in transport players is unclear.
Here, a thyroid rat cell line (FRTL5) was used in order to study molecular and morphological changes of transport machinery after stimulation with thyroid stimulating hormone (TSH). TSH regulates thyroid growth and differentiation, and is required for the expression of thyroglobulin, thyroperoxidase and the Na/I symporter (NIS), necessary for the synthesis of thyroid hormones. Our results showed an increase of multiple transport proteins (Rab1b, GM130, Sec23a, Rab5a, Calreticulin and others) upon TSH addition. In agreement, an increase in their RNA levels was detected by Real Time PCR. Furthermore, morphological changes (analyzed by confocal microscopy) in the Golgi complex and COPII structures suggest that TSH induces their reorganization to deal with cargo increase.
FRTL5 cells provide a good model to study organelle biogenesis/reorganization in the secretory pathway and will allow us to define molecular mechanisms that regulate changes in transport machinery after a secretory stimulus

CB-P04.
EXPRESSION OF TUMOR NECROSIS FACTOR ALPHA RECEPTOR I MODULATES MELANOMA CELL MIGRATION
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Melanoma progression is suggested to depend on specific factors of the inflammatory microenvironment. When produced in this microenvironment, tumor necrosis factor alpha (TNF) promotes cancer. TNF signals through the p55 receptor (TNFRI) to activate nuclear factor-kappa B (NF- B). Precisely, NF- B has been pointed as a link between cancer and inflammation, which prompted us to investigate how the expression of TNFRI affects melanoma progression with special emphasis on NF- B induction. To this end, we isolated peritoneal macrophages from wild type and TNFRI-/- mice. Cells were stimulated with LPS and conditioned media (CM) was collected. By ELISA, we determined that interleukin (IL) 1, IL6, IL12, TNF and NO levels were higher in TNFRI-/- CM. The CM was used as a chemo attractant to evaluate migration of B16 melanoma cells in a modified Boyden Chamber. Interestingly, while CM obtained from wt macrophages inhibits migration, TNFRI-/- CM increases it. Thus, we suggest that an enhanced inflamed microenvironment in TNFRI-/- mice supports melanoma progression. Moreover, while we have previously shown that sphingosine-1-phosphate (S1P) is a bioactive lipid required for TNF-induced NF- B activation, our new preliminary data indicate that a differential expression of S1P receptors in melanoma cells may also regulate its invasive capability.
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CB-P05.

EFFECT OF TNFRp55 DEFICIENCY ON THE INFLAMMATORY PROCESS EXHIBITED AT THE END OF PREGNANCY

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Tumor necrosis factor is a pleiotropic pro-inflammatory cytokine and TNF receptor p55 (TNFRp55) mediates most of the TNF effects. Pregnancy, either with or without signs of infection, show elevated levels of pro-inflammatory cytokines in maternal serum and fetal membranes when parturition is imminent. How this inflammatory *milliu* could impact on the maternal synovial membrane has not been investigated. Our objective was to study the consequences of TNFRp55 deficiency on the interleukine(IL)-17 and progesterone (Pg) levels in the joint of C57BL/6 wild type (WT) and TNFRp55-/- (KO) mice. IL-17 and Pg were determined by ELISA and RIA, respectively. We found elevated levels of IL-17 at the end of pregnancy in WT mice compared to diestrous stage. Moreover, IL-17 levels were lower in KO than WT mice at the end of pregnancy ($p<0.01$). Interestingly, this cytokine exhibited a circadian profile in the joint of WT which was abolished in the deficient mice. On the other hand, Pg also showed a circadian rhythm in both strains. The rhythm acrophase was advanced in the KO mice. Although, there was no difference in the levels of this hormone at the end of pregnancy, we found lower levels of Pg in KO mice at the diestrous ($p<0.01$). These results suggest that TNFRp55 signaling plays a key role in the inflammatory process that constitutes normal-term labour as well as in its circadian modulation.

CB-P06.

TNFRp55 DEFICIENCY MODIFIES THE TEMPORAL EXPRESSION OF BMAL1 AND PG METABOLIZING ENZYMES IN OVARY

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We previously showed that Progesterone (Pg) levels exhibit a circadian rhythm in the corpus luteum. TNF exerts pleiotropic effects through its p55 and p75 receptors. Bmal1 is a key component of the cellular clock machinery. Our objective was to investigate the putative role of TNF in the modulation of the circadian expression of Bmal1 and two key Pg metabolizing enzymes (3ß-HSD and 20 -HSD) in C57BL/6, wild type and TNFRp55-/- mice, in the diestrus stage. Animals were maintained on a 12-h light: 12-h dark cycle, at 24±2°C, with irradiated food and water available ad libitum. Five days before the experiment mice were kept under constant darkness conditions. Ovaries were isolated every 6 h during a 24h period. Bmal1, 3ß-HSD and 20 -HSD transcripts levels were determined by RT-PCR. The expression of Bmal1, 3ß-HSD and 20 -HSD genes is circadian in the corpus luteum of wild type animals with maximal expression peaks occurring at CT 05:04±00:41, 18:27±00:04 and 19:15±02:18, respectively. TNFRp55 deficiency significantly increased the amplitude and modified the phase of the circadian rhythms. Circadian oscillation of Bmal1 in the corpus luteum would indicate a potential role of the endogenous clock in the regulation of Pg metabolism. In turn, clock-mediated regulation might be modulated by TNF through its p55 receptor pathway.

CB-P07.

ROLE OF REGULATORY T CELLS IN *Yersinia enterocolitica*-INDUCED REACTIVE ARTHRITIS IN TNFRP55-/- MICE

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Regulatory T (Treg) cells suppress physiological and pathological immune responses. We have demonstrated that TNFRp55-/-(KO) mice develop a severe and chronic reactive arthritis (ReA) after *Yersinia enterocolitica* (Ye) infection. Also, KO mice show significant differences of IL-10 levels at regional lymph nodes (RLN) to the joint. The aim of this work was to investigate whether Treg cells play a role in our ReA model. We determined the frequency of Treg cells and the Foxp3 mRNA expression. C57BL/6 wild-type (WT) and KO mice were infected with Ye O:3. Seven, 14 and 21 days after infection, CD4+CD25+Foxp3+ cells from RLN were analyzed by flow cytometry; Foxp3 mRNA expression in RLN CD4+T cells was determined by RT-qPCR. We found that on day 14 (arthritis onset), KO mice showed significantly lower frequency of CD4+CD25+Foxp3+ cells, compared to WT mice (3.7%±0.13; 5.1% ±0.46, respectively; $p<0.05$), this result was in line with relative expression of Foxp3 mRNA (0.78±0.03; 1.2±0.03, respectively; $p<0.005$). In contrast, despite KO mice showed arthritis on day 21 (chronic phase), a significantly higher frequency of Treg cells was found in these mice compared to WT (4.4%±0.27; 3.4%±0.29, respectively; $p<0.05$). We concluded that at local site, TNFRp55 signaling influences the Foxp3 expression and Treg frequency. Moreover, Treg decrease is involved in ReA onset in TNFRp55-/- mice.

CB-P08.

REGULATORY ROLE OF P120 CATENIN IN N-CADHERIN PRECURSOR TRAFFICKING

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N-cadherin, a cell-cell adhesion molecule, is synthesized in endoplasmic reticulum (ER)-bound ribosomes as a precursor protein, with a prodomain that is removed post-Golgi to generate an adhesive competent protein. P120 catenin binds to the cadherin precursor in the ER, and these proteins travel as a complex to the cell surface. Our previous data show that a mutation at the p120 binding site impairs N-cadherin precursor trafficking to the cell surface. We hypothesize that this effect reveals a requirement of p120 expression and binding to the precursor. To test this, we performed p120 RNAi knockdown (KD) in HeLa cells transfected with N-cadherin carrying an HA-tag inserted in the prodomain sequence. Western blot analysis of p120KD-HeLa cells reveals an accumulation of the N-cadherin precursor (HA detection) compared to control cells. In p120KD cells the N-cadherin precursor distributes in puncta scattered throughout the cell, while in control cells it accumulates at the perinuclear region. To determine more precisely the cytoplasmic localization site of the precursor in p120 KD cells we are carrying out colocalization assays with markers of different compartments of the trafficking pathway, such as Rab1, ergic-53, GalNacT, and Rab11. So far our results suggest a positive role of p120 in N-cadherin precursor trafficking, likely at the ER-Golgi step.

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CB-P09.
PTP1B REGULATES THE TRANSIENT ACTIVATION OF SRC IN NASCENT ADHESIONS
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PTP1B is an ER-bound tyrosine phosphatase implicated in the modulation of cell-matrix adhesion. Here we examined the mechanisms in which PTP1B is involved in this process. Immunofluorescence analysis of cells expressing PTP1B (WT) and plated on fibronectin revealed a transient accumulation of ß3 integrin, talin, phosphoactive FAK, phosphoactive Src, and paxillin in nascent adhesions at the cell periphery, which peaks at 5-10 min after plating. In contrast, PTP1B knockout (KO) cells exhibited uniform punctate distribution of these proteins at all times. Maximal Src activation in WT cells at 10 min after plating correlates with strong phosphorylation of paxillin at tyrosine 118. In contrast, Src activation and paxillin phosphorylation are poorly induced in KO cells. Phosphoactive Src accumulation at adhesions requires paxillin, and expression of non phosphorylatable Y118F paxillin in both paxillin- and PTP1B- KO cells rescued active Src at nascent adhesions. Our results are compatible with a model where PTP1B activates Src at adhesions, and subsequently Src phosphorylates paxillin. The transient nature of these events and the requirement of non phosphorylated paxillin for Src activation suggest a negative feedback loop which inactivates Src after paxillin phosphorylation.
Supported by CONICET and ANPCyT.

CB-P10.
PTP 1B REGULATES CELL MOTILITY THROUGH ITS INTERACTION WITH SRC AT FOCAL ADHESIONS
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Here we show, by time-lapse analysis, that Protein Tyrosine Phosphatase 1B (PTP1B) targets cell-matrix adhesions and modulate their behavior. Consistent with this, PTP1B deficiency decreased adhesion lifetimes and increased paxillin disassembly rates. Kymograph analysis of lamellar extensions in PTP1B null (KO) cells reveals higher frequency of switching between protrusion and retraction phases and threefold reduction in protrusion persistence time compared to WT cells. Migration of KO cells exhibited frequent changes of direction and pauses compared to WT cells. At molecular level, substrate trapping of PTP1BD181A at adhesions requires Src kinases, as revealed by removal and by reconstitution experiments using cells with triple knockout for the kinases Src, Fyn and Yes. Reconstitution of these cells with different wild type or Src mutants further revealed that PTP1B targets Src-pY527 in addition to phosphorylated Src substrates. This agrees with positive Bimolecular Fluorescence Complementation between Src and PTP1B in adhesions. Current pull down and FRET assays implicate PTP1B in the modulation of downstream rac and rho GTPases. Our results suggest that PTP1B activates a Src signaling pathway in cell-matrix adhesion sites, delaying the turnover of adhesions, promoting lamellar stability, and directional cell migration.
Supported by CONICET and ANPCyT.

CB-P11.
PERFRINGOLYSIN O: A USEFUL TOOL TO STUDY EXOCYTOSIS
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The acrosome overlies the nucleus of the spermatozoon. It undergoes calcium-dependent exocytosis termed acrosomal exocytosis (AE) required for fertilization. Because spermatozoa are transcriptional and translationally inactive overexpresion or interference RNA cannot be used. To study the role of different proteins in AE we took advantage of perfringolysin O (PFO) properties. This cytolysin assembles to membranes creating 25 nm aqueous pores. We measured pore forming activity of native PFO using eosin or propidium iodide. The latter fluorescent dye was used to analyze the dynamics of this process in real time. Dose-response curves using different incubation times demonstrated that 10 min incubation at 37ºC were enough for the cytolysin activity. Western blot experiments showed that PFO binds to sperm membranes in a cholesterol-dependent manner. We chose a 25 nM concentration to validate the use of the toxin for exocytosis studies. Calcium or Rab3A added to permeabilized sperm succeeded in inducing exocytosis while calcium-triggered exocytosis were abrogated by loading the permeabilized cells with synaptotagmin VI C2B domain, RIM-Rab3-GTP binding domain or antibodies anti Rab3A, complexin or Epac before adding calcium. Furthermore, we tested PFOC459A where the Cys459 was mutated to Ala and does not require thiol activation. Both toxins were valuable tools for exocytosis research

CB-P12.
POLYARGININE PEPTIDES, A POWERFUL TOOL FOR DELIVERING PROTEINS TO LIVING CELLS
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Sperm must be capacitated to undergo acrosomal reaction (AR). In capacitated sperm permeabilized with SLO, we have demonstrated that SNAREs are required for AR and that these proteins are assembled in neurotoxin resistant complexes. What is the condition of SNAREs before capacitation is unknown. For this, the effect of a neurotoxin needed to be assessed during capacitation in living cells. As previously shown, proteins containing a polyarginine peptide can cross membranes. To generate a permeant neurotoxin, the light chain of tetanus toxin was crosslinked with a polyarginine peptide. The activity of this toxin was evaluated by a functional assay and immunofluorescence techniques. We corroborated that, in resting non-permeabilized capacitated sperm, SNAREs were locked in cis complexes insensitive to the neurotoxin. Upon stimulation, SNAREs became sensible to the toxin. However, when we added the permeant neurotoxin to non-capacitated sperm, SNAREs were digested, indicating that they were not protected in stable cis complexes. This observation suggested that capacitation was important for stabilizing the complexes. When sperm were incubated in the absence of albumin, a key element for capacitation in vitro, SNARE remained toxin sensitive. Our results indicate that capacitation is important for the assembly of stable SNARE complexes required for acrosomal exocytosis.

CB-P13.

mDia1, AN ACTIN NUCLEATING FACTOR, IS INVOLVED IN *Coxiella burnetii* INTERNALIZATION

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Phagocytosis is an important host defence mechanism against pathogens. In this process the actin cytoskeleton and their main regulators, the GTPases of Rho family, play an important role. *Coxiella burnetii (Cb)* is an intracellular pathogen that enters into host cells by a mechanism partially characterized. We have evidences that RhoA is involved in the interaction *Cb*-host cells. In this work we investigate if Src, ROCK and mDia1, molecules belonging to the RhoA signalling pathway, participate in the *Cb* internalization. HeLa cells were treated with PP2 or Y27632, inhibitors of Src and ROCK kinases, respectively, before infection. Both inhibitors diminished *Cb* uptake. These results suggest that both kinases are involved in *Cb* entry. To study the role of mDia1, HeLa cells were transfected with pEGFPmDia1-full length (FL), - N3 or - N1 prior infection. mDia1- N3 contains the actin nucleation domain FH1-FH2, while mDia1- N1 contains the RhoA binding domain GBD. *Cb* internalization was significantly inhibited in cells overexpressing mDia1- N1. When cells overexpressing the different mDia1 constructs were incubated with the kinase inhibitors prior and during infection, *Cb* internalization was not affected in cell overexpressing mDia1-FL or - n3. These results suggest that mDia1 plays an important role in *Cb* entry through the Src-RhoA-mDia1/ROCK signalling route.

CB-P14.

CORTACTIN AND ERK1/2 KINASE ARE INVOLVED IN *Coxiella burnetii* ENTRY INTO HOST CELLS

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Cortactin (cttn) is involved in actin cytoskeleton organization regulating cell migration and also host cell-pathogen interaction. Cttn has domains to interact with actin, Arp2/3 and N-WASP. Its activity is regulated by Tyr and Ser phosphorylations catalyzed by Src and Erk kinases, respectively. *Coxiella burnetii* (Cb) is a pathogen that enters into host cell by a poorly characterized mechanism. We have evidences that cttn is involved in Cb entry. To determine the cttn domains that participate in this process, HeLa cells were transfected with plasmids encoding W22A (NTA domain) and W525K (SH3 domain) cttn mutants that do not interact with Arp2/3 and N-WASP, respectively. Internalization was inhibited in cell overexpressing W525K. We have previously observed that Src activation and cttn Tyr phosphorylation status are important for Cb internalization. To study if Ser-phosphorylation of cttn regulates Cb uptake, HeLa cells overexpressing the cttn mutants S405/418A (non-phosphorylatable) or S405/418D (phospho-mimetic) were infected with Cb. Overexpression of S405/418A caused a reduction in the internalization. We analyzed Erk activation in lysates of HeLa cells infected for different times. The Erk phosphorylation peak was observed at 15 min infection. These results suggest that Cb uptake is regulated by the N-WASP interacting SH3 domain and by Erk-dependent Ser phosphorylation of cttn.

CB-P15.

ANALYSIS OF HISTONE ACETYLATION LEVELS IN EXPERIMENTAL MODELS OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disorder caused by an aberrant expansion of polyQ stretch in the huntingtin (Htt) protein. An important feature of HD pathogenesis is transcriptional dysregulation. The sequestration of the CREB-binding protein (CBP), a transcriptional co-activator and acetyltransferase of histones, in Htt aggregates can contribute to these alterations. Also supporting a role for histone acetylation in HD pathogenesis, the inhibitors of histone deacethylases (HDACi) seem to ameliorate the progresión of neurodegeneration in HD mice.

We used three different models of HD: N171-82Q mice, HEK293 cells transfected with mutant htt (mHtt) and mice electroporated in utero with mHtt constructs.

In N171-82Q mice there is a dramatic increase in the number of Htt positive cells and intracellular inclusions in the cerebellum that correlates with the progression of the symptoms. We did not observed changes in bulk histone acetylation levels, but we detected important transcriptional alterations. We also obtained similar results in the other HD models.

Our results indicate that transcriptional dysregulation may be independent of the levels of bulk histone acetylation and open the possibility that the beneficial effects of HDACi are more related to non-histone substrates of HDAC enzymes.

CB-P16.

GEMININ AND CCAR1 ARE CRUCIAL FOR p19INK4D FUNCTION IN THE DNA DAMAGE RESPONSE

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p19INK4d (p19) is a member of the INK4 family of cell cycle inhibitors which also participates in the DNA damage response (DDR). We have previously reported that p19 is phosphorylated on Ser76 and Thr141 following treatment with different genotoxics. The phosphorylation is critical for p19 function in DDR. Here, we aimed to study p19 partners involved in DDR activity. We searched for proteins interacting with human p19 by a yeast two-hybrid screening of a HeLa cDNA library using Cytotrap system. 69 positives clones were obtained. The cDNA sequence of p19 interactors were PCR-amplified, sequenced and identified by comparison with GenBank database. Among them, BRD7, GMNN, MSL-1, CAND1, IRF2BP2, CCAR1 are particularly interesting since they participate in pathways related to p19 functions. *In vivo* phosphorylation assays showed that downregulation of GMNN and CCAR1 by specific antisense oligonucleotides impaired UV-induced-p19 phosphorylation. In addition, decreased levels of GMNN and CCAR1 negatively modified the protection from apoptosis conferred by the overexpression of wild type p19. In contrast, antisense treatments did not affect the resistance to apoptosis observed by the overexpression of a mutant p19 that mimicked the phosphorylation at Ser76 and Thr141 (p19S76ET141E). We conclude GMNN and CCAR1 are involved in the phosphorylation process necessary for p19 function in DDR.

CB-P17.

C-FOS DEPENDENT PI4P AND PI-4,5-P2 NUCLEAR SYNTHESIS INDUCE WIDE GLOBAL TRANSCRIPTIONAL CHANGES

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The immediate early protein c-Fos has been extensively described as a member of the AP-1 family of transcription factors. As such, it has been implicated in cellular events such as differentiation and proliferation. All data available failed to completely explain each particular process. In our lab an aditional activity for c-Fos was described that could eventually complete the story. c-Fos has the ability to activate the phospholipid synthesis machinery within the cytoplasm in an AP-1 independent manner. In the present report we show that PI4P and PI-4,5-P2 nuclear synthesis is also affected by the addition of recombinant c-Fos to the nuclear matrix model results in wide transcriptional changes (as determinated by RNA array). These changes were confirmed by qPCR in nuclear matrix and whole cell. A two fold increase in nuclear matrix size was observed upon treatment with c-Fos along with the appearance of PIP2 patches in specific locations, indicating that major structural changes are taking place in response to c-Fos addition. Nevertheless, if wortmannin is present in the incubation system, all mentioned changes are revoked. This report establishes that c-Fos transcriptional regulatory activities may not always be linked to AP-1 dimers; rather c-Fos can induce AP-1 independent changes, resulting in wide global transcriptional changes mediated by phosphoinositides.

CB-P18.

ROLE OF C-FOS DURING NEUROGENESIS

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Neurons of the mammalian CNS originate from progenitors dividing at the apical surface of the neuroepithelium. These cells show a high proliferation capacity and an adequate control of their growth is of key importance. The protein c-Fos is known as an AP-1 transcription factor and also as a protein capable activating phospholipid synthesis. *c-fos -/-* mice, although viable, die at approximately 7 months of age, are infertile and growth-retarded with respect to their WT littermates. We determined if the absence of c-Fos have an effect during neurogenesis. NCFC assays showed fewer neural stem cells in the telencephalon of *c-fos -/-* embryos compared to *c-fos+/+*, and neurospheres cultures show differences in their proliferation kinetics. Assays using dual injections of EdU and BrdU in *c-fos-/-* and *c-fos+/+* E14,5 showed an increased number of cells at the S-phase in the embryonic cortical telencephalon of *c-fos-/-* mice, while immunofluorescence for ßIII tubulin indicated a smaller number of differentiated cells. Determination of the mitosis angle in apical progenitors reflected a predominant symmetric division in the knock out condition. These results indicate that the absence of c-Fos: increases the average cell-cycle length of progenitor cells and/or decreases their differentiation capacity (avoiding their exit from the undifferentiated state).

CB-P19.

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

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In addition to its AP-1 activity, c-Fos activates phospholipid synthesis supporting membrane biogenesis for cell growth. c-Fos basic domain (BD) is essential for this activity. Fos-related antigen 1 (Fra-1), another Fos family member, shares an almost identical BD and also exerts lipid synthesis activating capacity. We studied if Fra-1 participates in breast cancer cell growth by activating phospholipid synthesis, a situation in which its overexpression has been recently reported.
Growing MDA-MB 231 and MCF7 cells overexpress Fra-1 and c-Fos which co-localize with calnexin, an endoplasmic reticulum marker. Stripping membranes of associated proteins (1 M KCl treatment), results in quiescent cell phospholipid synthesis rates which are restored to initial activated values upon addition of recombinant Fra-1 or c-Fos to the assays. Similar results were verified in human breast tumor samples: phospholipid synthesis was significantly higher in tumors as compared to normal tissue but it was significantly reduced when subjecting tumor samples to 1M KCl treatment, whereas the addition of recombinant Fra-1 or c-Fos restored phospholipid synthesis to initial rates. Moreover, both phospholipid synthesis and cell proliferation were abrogated by neutralizing Fra-1 and/or c-Fos activity.
Our results indicate that both Fra-1 and c-Fos support breast tumor growth by activating phospholipid synthesis.

CB-P20.

THE *Caenoharbditis elegans* UDP-GLUCOSE: GLYCOPROTEIN GLUCOSYLTRANSFERASE HOMOLOGUES HAVE NOT IDENTICAL FUNCTIONS

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The UDP-Glc:glycoprotein glucosyltransferase (UGGT) is the key component of the glycoprotein folding quality control mechanism in the endoplasmic reticulum (ER). It behaves as a sensor of glycoprotein conformation as it exclusively glucosylates glycoproteins not displaying their native conformations. Most species have only one gene coding for UGGT-like proteins while Euteleostomi, *Caenorhabditis* and humans have two homologues. In humans HUGT1 but not HUGT2 displayed UGGT activity and was upregulated under ER stress conditions. Bioinformatics analysis showed that in *C.elegans* there are two open reading frames F48E3.3 (*uggt-1*) and F26H9.8 (*uggt-2*) coding for UGGT homologues. Here we report that *C.elegans* expresses an active UGGT protein localized in the ER encoded by the *uggt-1* gene. We constructed transgenic worms carrying the *Puggt-1::gfp* construct and found that UGGT-1 is expressed in cells of the nervous system and is upregulated under ER stress. Real time PCR analysis showed that both *uggt-1* and *uggt-2* are expressed during the entire *C. elegans* life cycle but at very different levels, being *uggt-2* expression at most 3% of *uggt-1*. Depletion of UGGT-1 by RNAi resulted in a reduced lifespan and that of UGGT-1 and UGGT-2 in a delay in development. With an ER stressor drug the delay was increased. We conclude that UGGT-1 and UGGT-2 play a protective role under ER stress.

CB-P21.

ALPHA-2-M/LRP1 INDUCES DIFFERENTIAL MACROPHAGE MIGRATION IN NORMOXIC AND HYPOXIC CONDITIONS

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Alpha2-Macroglobulin (2M) is a proteinase inhibitor, which is recognized by LDL receptor-related protein 1 (LRP1). Previously, we demonstrated that 2M/LRP1 system induces intracellular signaling activation, MMP-9 expression and cellular migration in Raw264.7 macrophage-derived cell lines. It is known that tissue macrophages play several inflammatory functions under hypoxic conditions. Hypoxia is an inductor of LRP1 gene expression in tumoral cells. However, the hypoxic effect on the LRP1 expression and cell migration in macrophages has not been established. Herein we investigated the 2M effect on the Raw264.7 cell migration under cell cultured conditions of normoxia (O_2: 21%) and hypoxia (O_2: 1.5%) using wound-healing assays. In addition, we have examined the LRP1 gene and protein expression in Raw264.7 cells using RT-PCR and Western blot. We observed that 2M induced an increased cellular motility of RAW 264.7 cells in hypoxia compared to normoxia. Under hypoxia, LRP1 presented an increased mRNA and protein level compared to these cells cultured under normoxia. In conclusion, our data demonstrate that 2M promotes an increased cell migration of macrophages under hypoxia, which together with the hypoxia-induced LRP1 expression may be significant in the macrophage activity during inflammatory processes.

CB-P22.

cAMP AND EPAC PARTICIPATES IN THE REGULATION OF THE AUTOPHAGIC RESPONSE INDUCED BY ALPHA-HEMOLYSIN

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Staphylococcus aureus induces a caspase-independent cell death in which participates the autophagic pathway. Autophagy consists in the sequestration of cytosolic components, like organelles or microorganisms, in a vacuole called autophagosome. This vacuole finally fuses with the lysosome to degrade the trapped material. We have recently shown that the pore forming toxin alpha-Hemolysin (Hla), which is the main virulence factor secreted by *S. aureus*, is able to induce an autophagic response in CHO cells. We have shown that the LC3-positive vesicles generated in response to the toxin are not acidic and non-degradative compartments. In addition we have found that Hla-induced autophagy is independent of the PI3K/Beclin 1 complex, indicating that the autophagy activation occurs through a "no canonical" pathway. We have recently identified some of the essential molecules of the signaling pathway involved in this autophagic response induced by the toxin.Our results have reveal that cAMP is able to inhibit the autophagy induced by Hla. Moreover EPAC and Rap2b, through calpain activation, are key players in the regulation of this autophagic pathway. Interestingly, EPAC and Rap2b, are recruited to the membrane of the *S. aureus*-containing phagosome. Thus, our data provide the bases to unveil a new signaling pathway regulating pathogen-induced autophagic responses.

CB-P23.

GLYCOSPHINGOLIPID BIOSINTHETIC PATHWAY AS TARGET FOR NEW ANTIPARASITIC DRUGS

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Sphingolipids are important components of eukaryotic cells. Besides serving as structural components, they regulate cell proliferation, differentiation, cell migration, signaling, trafficking, cell-cell interactions, etc. Tamoxifen ((Z)-1-(4-(2-(dimethylamino)ethoxyl-phenyl)-1,2-diphenyl-1-butane, TAM), a nonsteroidal antiestrogen widely used for the treatment of breast cancer, has been involved with lipid metabolism in intact cells, however little experimental evidence exist up to date, related to the real effect of this drug in sphingolipid biosynthesis. Continuing with our work on the structure of *Trypanosoma cruzi* glycoconjugates we have shown that TAM inhibits epimastigote and trypomastigote development. We have performed a metabolic incorporation of NBD-ceramide into parasites treated or not with TAM. Sphingolipids were extracted and purified. TLC analysis showed that neutral sphingolipid biosynthesis was enhanced in treated parasites. When an HPLC analysis was performed, four labelled components were observed and interestingly three of them of the treated parasites presented a double area when compared with the untreated ones. Each component was isolated and further analyzed by UV-MALDI-TOF mass spectrometry. In addition, as it is well known that ceramide induces cell apoptosis, we further analyzed by flow cytometry if this event is taking place in *T. cruzi*.

CB-P24.

AMPHIBIAN OOCYTES RELEASE HSP70 DURING SPAWNING: POSSIBLE ROLE IN FERTILIZATION

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We recently found evidence suggesting a role for HSP70 proteins (HSP70s) in sperm-oocyte interaction in the amphibian *Bufo arenarum*. In this work our aim was to study their expression and behavior during fertilization. We detected HSP70s present in *B. arenarum* oocyte in the absence of any stress by Western-blot using two different antibodies. 2D electrophoresis experiments detected two isoforms with pIs of 5.25 and 5.45. We studied their subcellular distribution isolating total membranes, cytosol and plasma membranes. HSP70s were present in all these fractions. We confirmed these results by immunofluorescence microscopy and also found that HSP70 signal was present in the vitelline envelope (extracellular matrix). To further test this, we performed Western blot analysis in isolated vitelline envelopes (crosslinked and not) and in egg water (diffusible material from deposited oocytes). HSP70s were present in these two fractions, while actin or tubulin could not be detected. *In vitro* fertilization assays in the presence of anti-HSP70 polyclonal antibodies showed diminished fertilization scores at low sperm concentrations (10^5 cells/ml). Human recombinant His-tagged HSP70 was able to specifically bind to sperm *in vitro*. Our results suggest for the first time i) the presence of HSP70s in the extracellular structures of the oocyte and ii) a role for oocyte HSP70s in fertilization.

CB-P25.

HOMOPHILIC INTERACTIONS OF ALPHA PROTOCADHERINS

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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 Ca^{2+} dependent cell adhesion, but their function remains unknown. They consist of over 50 genes arranged in three clusters (alpha, beta and gamma). Combinatorial expression of these variants could help to establish neuronal identity. 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 and whether these trigger cell adhesion and/or signaling processes. Our model consists of a mouse neuronal cell line expressing one Pcdh isoform at a time, fused to a fluorescent protein or tag. We have described that they engage in homophilic interactions at the cell surface, and that these are calcium independent. Now we report that isoforms of the alpha cluster differ in the dynamics of formation of contacts and that the proteins undergo processing along their membrane transit. We have noted variations between isoforms in the amount of unprocessed protein while the contacts mature. We want to relate these data to the involvement of Pcdhs in cell signaling cascades.

CB-P26.

FIP2, NEW LINKER BETWEEN RAB11 AND RAB14 AT THE CHLAMYDIAL INCLUSION MEMBRANE

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Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium that modulates relevant eukaryotic trafficking pathways as an adaptive strategy. Chlamydial proteins might be involved in the recruitment of host Rab GTPases to the inclusion. Rabs are the molecules in charge of intracellular traffic control. We showed that Rab11 and Rab14 are required for bacterial nutrition and replication. A recently described eukaryotic protein, FIP2 possesses a *R*ab *B*inding *D*omain (RBD) that interacts with both Rabs. We examined by confocal microscopy the intracellular localization and role of these proteins in *Chlamydia*-infected cells. FIP2 was recruited to chlamydial inclusions, and colocalized with Rab11 and Rab14 at the inclusion membrane differentially depending on chlamydial developmental cycle stage. FIP2 association to the inclusions was through its RBD domain. Silencing of FIP2 by siRNA reduced *Chlamydia trachomatis* multiplication, assessed by the counting of inclusion forming units (UFI). Our results demonstrated the requirement of FIP2 for the development and replication of these bacteria. FIP2 might be involved in the formation of macromolecular platforms at the chlamydial inclusion. These data could contribute to the understanding of the complex molecular machinery used by the pathogen to manipulate host trafficking pathways for their own benefit.

CB-P27.

ENDOCYTOSIS OF ANTIBODY TO GANGLIOSIDE GD3: A POTENTIAL DRUG DELIVERY ROUTE FOR TUMOR CELLS?

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The expression of ganglioside GD3, which plays essential roles in normal brain development, decreases in adults but is up regulated in neuroectoderm-derived cancers, where it enhances malignant properties. Antibodies to tumor-associated gangliosides are being used as therapeutic agents. Here, we demonstrated in human (SK-Mel-28) and mouse (B16) melanoma cells and hamster ovary cells (CHO-K1) that the anti GD3 antibody R24 is endocytosed and accumulated in recycling endosomes, precluding its use as a "naked" therapeutic because when internalized it cannot activate pathways of immune-mediated anticancer activity. However, it would be possible to exploit the internalization feature for the selective delivery of cytotoxic agents to $GD3^+$ cancer cells. We have used antibody R24 to deliver saporin toxin-conjugated secondary antibody. We incubated cells in a 96-well plate with primary (3-60 nM) and secondary (1-8 nM) antibodies for 72 h, and cell viability determined. Wells with both antibody showed a decreasing of 70, 40 and 30% of cell viability in CHO-K1, SK-Mel-28 and B16 cells, respectively. The effect of the R24-saporin antibody conjugate was further evident by the lack of any effect on GD3-negative cells. Similar results were obtained using biotin-R24 coupled to streptavidin-saporin in SK-Mel-28 cells. The data suggest that GD3 may be a viable target for drug delivery for tumor cells.

CB-P28.

THE PARTICIPATION OF THE FANCONI ANEMIA PATHWAY IN THE REPLICATION OF UV-DAMAGED DNA

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When cells are challenged with genotoxic agents, replicating cells must use damaged DNA as templates. In this way, active replication forks do not collapse and cell viability is protected. After UV irradiation a specialized DNA polymerase pol eta uses UV-damaged DNA as template. Intriguingly, Pol eta lost in human cells does not steeply increase UV sensitivity. This suggests that compensatory mechanisms promote cell survival when pol eta is absent.
We have found an increase and sustained FANCD2 ubiquitination and focal formation after UV irradiation when pol eta is lost. FANCD2 is a key marker of the activation of the FANCONI ANEMIA (FA) pathway. While there is limited information regarding a role of the FA pathway after UV irradiation, it is well established that FANCD2 ubiquitination is linked to the recruitment of homologous recombination (HR) specific markers to other lesions. We therefore thought that cell viability in the absence of pol eta might result from the activation of FANDC2-dependent HR at collapsed replication forks. We are currently analyzing markers of damage such as H2AX phosphorylation, markers of HR such as Rad51, markers of double strand breaks accumulation such as 53BP1 and setting up viability assays. This information might allow us to predict if FANCD2 can trigger HR after UV and if this contributes to cell viability when pol eta is absent.

CB-P29.

THE ROLE OF GLUCOSIDASE II (G2Bß) AND MRH DOMAINS ON N-GLYCAN PROCESSING IN THE ENDOPLASMIC RETICULUM

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Glucosidase II (GII) removes the two innermost glucose (glc) residues from the glycan ($Glc_3Man_9GlcNAc_2$) transferred to proteins. GII also participates in cycles involving the lectin/chaperones calnexin and calreticulin as it removes the single glc unit added to folding intermediates and misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase (UGGT). GII is a heterodimer whose alpha subunit (GII) bears the active site whereas its beta subunit (GIIß) modulates GII activity through its C-terminus Mannose 6-Phosphate Receptor Homologous (MRH) domain. Here we report that as already described in cell-free assays, also in live *S.pombe* cells a decrease in the number of mannoses in the glycan results in a decreased GII activity. However, lack of mannose residues in the oligosaccharide did not affect *in vivo* UGGT activity. Thus, *N*-glycan demannosylation of misfolded/slow folding species favors their interaction with the lectin/chaperones by prolonging the existence of monoglucosylated glycans. Moreover, we show that even *N*-glycans bearing five mannoses may interact *in vivo* with the GIIß MRH domain and that the N-terminus GIIß G2B domain is involved in GII -GIIß interaction. Finally we confirm that the abscence of the canonical endoplasmic reticulum (ER) retention signal (VDEL) in GIIß did not prevent GII ER localization or activity, suggesting that VDEL is not the only ER localization signal present in GIIß.

CB-P30.

HEMIN INDUCES AUTOPHAGY IN LEUKEMIC ERYTHROBLASTS

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In eukaryotic cells, autophagy is a conserved intracellular pathway in which cytoplasmic components and some organelles are sequestered in a double-membrane vacuole (autophagosome) and degraded in the lysosomal compartment. Autophagy has been associated with several physiologic processes as erythroid maturation. LC3 is a protein present in autophagosomal membrane, therefore is considerate as a bonafide marker of this structure. Our results have shown that hemin (an erythroblast maturation inductor) produced an increased number and enlargement of GFP-LC3 positive vesicles labeled with lysotracker or DQ-BSA compared with others inductors as phorbol ester, sodium butyrate and dihydroxyurea. Moreover, we have demonstrated, in K562 cells incubated with hemin, an enlargement GFP-Lamp1 positive vesicles labeled with mitotracker. We have also shown in erythroblastic leukemic cells co-expressing RFP-LC3 and GFP-Rab11 (a multivesicular bodies marker) incubated in the presence of hemin an enlarged vesicles labeled with both markers. Likewise, we have performed a western blot to detect the processing of LC3 protein upon hemin incubation. We have observed in this assay that GFP-LC3 protein was cleaved, generating low molecular weight products. Taken together, our results suggest that hemin could induce an autophagic response in K562 cells, probably allowing an efficient and faster maturation.

CB-P31.

CONSERVATION OF POLY(ADP-RIBOSE) GLYCOHYDROLASE ACTIVITY IN TRYPANOSOME PARASITES

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Poly(ADP-ribose) polymers (PAR) are attached to specific target proteins in an early cellular response to DNA damage. Poly(ADP-ribose)glycohydrolase (PARG) represents the main PAR hydrolyzing activity in the cell via endo- and exo-glycosidic cleavage. The biological functions of PARG involve the PAR cycling required for structural chromatin remodeling during DNA repair, transcription, DNA replication and cell death pathways. *Trypanosoma cruzi* (Tc) and *T. brucei parg* (Tb) single-copy genes were identified in our laboratory. *In silico* analysis showed a high conservation of PARG catalytic domain and the presence of the three essential acidic residues (D-E-E). TcPARG mRNA was down regulated in mammalian host-stages amastigotes and trypomastigotes, while in the insect stage (epimastigote) it was up-regulated, in accordance with the protein expression determined by western blot. TbPARG was also expressed in bloodstream and procyclic forms of *T. brucei*. In both parasites, these enzymes showed nuclear localization despite the absence of DNA damage. *T. cruzi* overexpressing PARG increased the growth ratio about 40% when compared to wild type, suggesting a role even under physiological conditions. Native PARG activity was evaluated by measuring PAR turnover by dot blot and using specific inhibitors, which are currently being investigated on their effect on *T. cruzi* and *T. brucei* growth.

CB-P32.

A SURROGATE P53 REPORTER IN *Drosophila* REVEALS THE INTERACTION OF EIF4E AND P53

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eIF4E promotes translation upon binding the mRNA 5'cap and it is required for cell proliferation. p53 is a proapoptotic protein which is activated in response to DNA damage. There is evidence that suggests that eIF4E and p53 are connected in a mechanism that regulates their function. We propose a model for that such a mechanism to explain the equilibrium between apoptosis and cell proliferation. Our data shows a correlation between the overexpresion of eIF4E and the supression of apoptosis triggered by the overexpresion of p53 in *Drosophila* imaginal discs. We also studied a reporter transgene which expresses GFP in response to p53 activation by gamma radiation. We could confirm that this p53 surrogate works in imaginal discs as well as in embryos. This provided us a tool to quantify the effect on the GFP signal by overexpresion of eIF4E to confirm how these two proteins could interact *in vivo*. Our results suggest that p53 and eIF4E are indeed in an equilibrium that decides if a cell shall proliferate or die.

CB-P33.
CHARACTERIZATION OF LIPID METABOLISM DURING *Salmonella* INFECTION
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Salmonella is a facultative intracellular pathogen capable of infecting a wide range of animals, causing diseases ranging from self-limiting enteritis to typhoid fever. The virulence of this pathogen relies on its ability to establish a replicative niche, named *Salmonella*-containing vacuole (SCV), inside host cells. However, the microenvironment of the SCV and the bacteria and the metabolic pathways required during infection are largely undefined. The objective of this work is to characterize the bacterial and eukaryotic lipid metabolism during the infectious process. We use different reporter systems, fluorescence probes, TLC and confocal microscopy to study the environment and the physiological state of the bacterium in various cell lines. From the eukaryotic perspective, no changes were found in the phosphatidylcholine and phosphatidylethanolamine profiles when HeLa cells were infected. However, the expression level of enzymes involve in phosphatidylcholine biosynthesis was slightly induced after infection. Also, an increase in oleic acid incorporation was observed in infected HeLa cells. *Salmonella* strains presenting mutation in ß-oxidation pathway have shown a defect in replication on RAW-macrophages. A better understanding of the vacuolar environment and adaptive response use by *Salmonella* to survives and proliferates, will be the major outcome of this study.

CB-P34.
CIRCADIAN REGULATION OF CYTOPLASMIC RNA-GRANULES
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About 5-20% of the mRNAs expressed in a given tissue show daily oscillations in their levels. Presumably, this is largely generated by changes in the transcription of those genes; however, the contribution of mRNA posttranscriptional processing is unknown. In the cytoplasm, messenger ribonucleoprotein (mRNP) complexes can assemble into cytoplasmic mRNP granules as P-bodies and stress granules (SG). These foci are involved in the regulation of mRNA decay and storage, as well as translation. They are formed by RNA and a number of factors involved in mRNA processing. We have analyzed whether these foci show rhythmic changes. NIH3T3 cells were synchronized by serum shock. SG and P-bodies were detected at different times by immunocytochemistry (ICC) with antibodies against eIF3 and GE-1 respectively. P-body per cell (number) showed fluctuations peaking 28 h after synchronization and reaching highest areas 14 h after the shock. The number of arsenite-induced SG/cell did not change through time; however the area and perimeter of them showed daily variations with peak 14 h after serum shock. These rhythms are independent of the cell cycle since were observed in quiescent cells. We have also analyzed the temporal expression of several factors that could be responsible of the generation of these rhythms. Our results suggest that P-bodies and SGs, or a subpopulation of them, are regulated by a circadian clock.

CB-P35.
THE INVOLVMENT OF *Giardia lamblia* SF2 OF RNA HELICASES DURING ENCYSTATION AND ANTIGENIC VARIATION
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Helicases are encoded by a large fraction of eukaryotic and prokaryotic genomes and they play important roles in almost every cellular mechanism that involves nucleic acids. On the other hand, RNA is involved in virtually all aspects of gene expression, playing important regulatory roles in biological reactions. *Giardia* shows two important mechanisms of adaptation to survive both outside and inside the host´s intestine, which are “encystation” and “antigenic variation”, respectively. We analyzed the expression of some RNA helicases by qPCR, looking for the involvement of these genes on both process. The relative expressions of these genes were determined in trophozoites after incubation for 16 hours in encystation medium. For the antigenic variation, trophozoites were incubated at short times with a specific antibody recognizing the unique variable small protein (VSP) expressed in their surface. We found 4 genes down-regulated and 14 genes up-regulated during encystation. And during antigenic variation at short times, we found 3 genes up-regulated and 5 down-regulated within 1 hour, and 8 genes that were up-regulated and 4 down-regulated at 4 hours after the incubation with the specific antibody. The large DExD/H-box helicase family in *Giardia* could present this differential expression and regulation, being the cornerstone for the RNA metabolism and signaling in this parasite.

CB-P36.
TUMOR MICROENVIRONMENT INDUCES c-FOS OVEREXPRESSION AND BREAST CANCER STEM CELLS ENRICHMENT
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The Epithelial Mesenchimal Transition (EMT) program depends on a series of intracellular signaling networks involving, among others signal-transduction proteins, ERK, MAPK, PI3K, and c-Fos. Induction of c-Fos expression in normal mouse mammary epithelial cells induces EMT and is associated with a decrease in E-cadherin expression. Previous to EMT, CD44-/CD24+ cells correspond to the phenotype of the majority of cells found in breast carcinomas whereas a shift to CD44+/CD24- cells, promotes EMT and a profile associated with human breast cancer stem cells leading to the acquisition of mesenchymal qualities and an increased ability to form mammospheres and increased metastatic potential.
We found, that the microenvironment formed by macrophages induces both EMT in breast cancer cells with a stem cell–like phenotype and overexpression of c-Fos. Furthermore, in cultures, this microenvironment induces proliferation, invasiveness; and migration of these breast cancer cells. *In vivo*, this microenvironment increases breast tumor growth when macrophages are co-injected with MCF-7 cells into immunocompromised mice.

CB-P37.
HYPOTHYROIDISM, NUCLEAR RECEPTORS AND ATP-SENSITIVE POTASSIUM CHANNELS

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Nuclear receptors include many transcription factors that regulate the expression of specific genes that control heart metabolism. We have shown that hypothyroidism modifies heart lipid metabolism and redox balance. It is know that heart physiology depends of normal function of potassium channels. Now, we study if hypothyroidism affects the expressions both nuclear receptors and subunits of potassium channels in heart ventricle. Hypothyroid state was induced by 6-n-propyl-2-thiouracil (100mg/g body wt) in drinking water given to female Wistar rats (160 g body wt) starting 8 days before mating until day 21 of pregnancy or for 30 days in virgin rats. In heart ventricle, the protein levels of retinoic acid receptor (RAR and RARß) were determined by Western blot, and mRNA levels of TR , TRß, RXR , RXRß and subunits of ATP-sensitive potassium channel (Kir 6.1, Kir6.2 and SUR2) by RT-PCR. Pregnancy increased the expression of RAR , RARß, RXRß, and Kir 6.1 and Kir 6.2 subunits, and it decreased the expression of TR and TRß. Hypothyroidism in pregnancy decreased RAR , RXR , TR and Kir 6.2 expressions, without change in RXRß and Kir 6.1 expressions, compared with euthyroid pregnant rats. Hypothyroid virgin rats showed a decrease in RXR , TR , TRß, and Kir 6.2 and SUR2 expressions. These alterations could contribute to the myocardial dysfunction that occurs during hypothyroidism.

CB-P38.
F-ACTIN DEPOLYMERIZATION PRECEDES VACUOLAR BREAKDOWN DURING POLLEN REJECTION IN *Nicotiana alata*

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We studied the *in vivo* changes of F-actin cytoskeleton in pollen tubes after compatible and incompatible pollinations in *Nicotiana alata*. The integrity of actin filaments is essential for polar growth in pollen tubes. F-actin provides the track along which organelle and vesicle move, carrying the necessary material to sustain the pollen tip growth. In a previous work we have shown that incompatible pollen tubes underwent gradual depolymerization which continued for several days after complete cessation of pollen growth. To assess whether F-actin fragmentation was an early event in pollen rejection, double labeling of F-actin and the vacuolar compartments was performed in incompatible pollination. Vacuolar breakdown was reported as the crucial step in pollen rejection in *Nicotiana* because it releases cytotoxic S-RNase to the cytoplasm. At 1 and 3 days after pollination, pollen tubes with depolymerized F-actin were 45% and 70%, respectively. However, less than 10% showed disorganized both F-actin and vacuolar compartments. At 8 days after pollination 90% of pollen tubes had disorganized the F-actin but only 33% displayed vacuolar rupture. No pollen tubes with intact F-actin and disorganized vacuolar compartments were seen at any time. These results suggest that F-actin depolymerization occurs upstream in pollen rejection and precedes S-RNase releasing from vacuole to cytoplasm.

CB-P39.
A RELATION BETWEEN ALTERNATIVE SPLICING CHOICES AND CHROMATIN STRUCTURE IN NEURONAL DIFFERENTIATION

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RNA polymerase II elongation rates modulate alternative splicing choices and affect the timing at which nascent pre-mRNA splice sites are presented to the spliceosome. This is relevant for regulation of endogenous alternative splicing events by dynamic changes in intragenic chromatin structure that could affect RNA processivity or elongation rate. Using the alternative exon 18 of the NCAM gene as a model, we have shown that neuronal differentiation triggers transcription-repressive intragenic histone modifications in the NCAM gene, correlating with an increase of its alternative exon 18 inclusion. This alternative exon inclusion determines the expression of the NCAM 180 isoform, typical of mature and stable synapses. We study this regulation using DMSO-induced differentiation of N2a cells, a murine neuronal cell line. Treatment of differentiated N2a cells with an inhibitor of DNA methylation or with a histone hyper-acetylating drug revert the effect of differentiation on the exon 18 inclusion. To extend the analysis to other alternative splicing events, we used available ChIP-seq data to search genes with correlated modulation of both histone marks and AS patterns during neuronal differentiation, and selected the Tcf7l2 and the fibronectin genes. This regulation represents an example of chromatin-dependent alternative splicing modulation associated with development.

CB-P40.
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, 320-400nm) and high (UVB, 290-320nm) energy light. UVB light causes two types of mutagenic DNA lesions: thymine dimers and (6-4)photo-products. UVB mutagenesis is critical in the generation of skin cancer. 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 favouring the synthesis of pro-apoptotic isoforms of key proteins like Bcl-x or Caspase 9 (C9). As 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 (302nm) light. We observed an increase in pol II hyperphosphorylation, being this modification necessary for the change in AS of a model cassette exon. Moreover, UVB irradiation induces the pro-apoptotic mRNA isoforms of Bcl-x and C9 being these consistent with a key role of AS in response to DNA damage. Our results suggest that UVC and UVB light affect AS decisions through a similar mechanism. This indicates that lower energy irradiation, causing more limited DNA damage than UVC light, is sufficient to alter qualitatively patterns of gene expression in skin cells.

CB-P41.

ORDER OF INTRON REMOVAL AROUND CONSTITUTIVE AND ALTERNATIVE EXONS

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A better understanding of the mechanisms involved in alternative splicing regulation and of the role of transcription on alternative splicing decisions can be achieved by analyzing the order of removal of the introns surrounding particular exons. We studied the order of removal of the introns flanking constitutive and alternative exons in conditions that affect exon inclusion rate. We transfected Hep3B cells with plasmids with a constitutive or alternative exon minigene and we analyzed the relative order of removal of the flanking introns 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. Both for fibronectin (FN) EDI, a model alternative cassette exon, and for FN exon 28 (E28), a constitutive exon similar to EDI, intron 2-first pathway prevails. Cis mutations that enhance EDI inclusion change the pattern of intron removal in ways that are consistent with their mechanism of action. 5,6-dichloro-1- -d-ribofuranosylbenzi midasole (DRB) or camptothecin (CPT) augment EDI inclusion without changing the order of intron removal in either minigene, while flavopiridol, which also promotes EDI inclusion by slowing down Pol II, promotes the intron 1-first pathway in EDI and the intron 2-first pathway in E28. Thus, order of intron removal can point at differences in the action of drugs thought to affect alternative splicing by the same pathway.

CB-P42.

DEHYDROLEUCODINE INDUCES A TRANSIENT ARREST OF HeLa CELLS IN MITOSIS

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Cancer is a set of diseases in which the body´s cells become abnormal and divide without control. There are countless efforts to find agents to block the uncontrolled cell proliferation. Many sesquiterpene lactones (SL) have been isolated from medicinal plants and present a wide spectrum of biological activity. Dehydroleucodine (DhL) is a SL isolated from *Artemisia douglasiana Besser*, a plant that grows up in the west of Argentina. Previous results from our laboratory showed that DhL delays the proliferation of HeLa cells, arresting them in G2/M phase of the cell cycle. In this work we analyzed whether DhL affects M phase of HeLa cells. Cells synchronized in G1/S by double thymidine treatment, were stimulated to proliferate with 10% fetal bovine serum in the presence of 0-20 µM DhL. Images of the cells were taken from time 0h to 32h of treatment every 15 min using a Nikon Eclipse TE 2000-U microscope. The treatment with 10 and 20 µM DhL delayed the entry of cells to mitosis (4.33 ± 1.85 and 4.52 ± 1.06 h respectively) and increased the time that the cells spend in division (7.01 ± 1.07 and 6.43 ± 0.58 h respectively). DhL extended 0.17 ± 0.04, 5.07 ± 0.58 and 1.78 ± 0.24 h the time of Prophase, Metaphase to Telophase, and Cytokinesis respectively. These results show that DhL generated a delay of HeLa cells to enter to mitosis and an increase of the time that spend in division.

CB-P43.

DEHYDROLEUCODINE AFFECTS THE ORGANIZATION OF THE CYTOSKELETON AND THE FOCAL ADHESIONS OF HeLa CELLS

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Metastasis is the main cause of death in patients with cancer, therefore, it is relevant to find drugs to attack the cell invasion. Previously, we have demonstrated that Dehydroleucodine (DhL) has an inhibitory effect in the proliferation of HeLa cells. In this work we studied the effect of DhL on the migration of HeLa cells. Wound healing assay was performed in HeLa cells treated with 0 and 20µM DhL. We measured the speed and direction of migration and analyzed the cytoskeleton by immunofluorescence. DhL decreased the migration velocity (40%) and changed the dynamics of the advancing front of the cells. While control cells generated large and wide lamellipodia and migrated perpendicularly to the wound, DhL treated cells generated small lamellipodia and migrated erratically. Also, DhL treatment changed the organization of the cytoskeleton. In control cells the microtubules were concentrated in the central region and actin filaments formed an extensive network in the lamellipodia. In DhL treated cells, the microtubules were uniformly distributed in the cytoplasm and the actin filaments were packed in a thin line in the cell periphery. These results indicate that DhL affects the migration and the cytoskeleton of HeLa cells. DhL would be a potential agent to stop the invasion of tumor cells in the body.

CB-P44.

THE CONTRACTIBILITY OF PERITUBULAR MYOID CELL CHANGES ALONG THE SEMINIFEROUS TUBULE

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The myoid cells (MC) have contractile activity and express typical proteins of smooth muscle cells such as -actin and miosin II. Along the seminiferous tubules (ST), the germinal epithelium presents specific cell asotiations called stages (numbered from I to XIV in rat). In a previous work, we observed that MC contain an external layer actin filaments bundles (AF) distributed homogeneously in the cytoplasm in cells of the ST segments with VII-VIII stages, but only along of nuclear zone in cells of the ST segments with II-III and IX-X stages. We asked wether the MC cells have specific contraction features in each ST segment. We analyzed the morphology of the MC in each ST segment and the changes produced by the contraction. ST from adult rats were incubated with 0 and 50 nM endothelin-1 (ET-1) and fixed. The segments corresponding to II-III, VII-VIII and IX-X stages (segments A, B and C respectively) were isolated by transillumination, incubated with -actin-FITC and observed by confocal microscopy. The area of MC was of 2068.58±63.24, 1892.52±64.40, 1770.95±62.39 µm2 (x̄±sem).in the segments A, B and C respectively. By the contraction with ET-1 the MC area was reduced 36.5±2.3, 20.4±1.6 and 37.9±5.1% respectively. These results indicate that the contractibility of MC changes along the ST and probably is related to the distribution in the cytoplasm of the external layer of AF.

CB-P45.

CHARACTERIZATION OF ADP RIBOSYLATION FACTORS EVIDENCE AN ATYPICAL GOLGI APPARATUS IN *Giardia lamblia*

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Giardia lamblia is an intestinal parasite that belongs to the earliest diverging branch of the eukaryotic lineage of descent. *Giardia* appears as a "living fossil" since it has neither typical mitochondria, peroxisomes, nor a classical Golgi apparatus (GA). Although both constitutive and regulated pathways for protein secretion exist in *Giardia*, little is known about the molecular mechanisms involved in vesicular traffic. An exhaustive search of the complete *Giardia* genome identified genes for seven giardial ARF proteins (ADP Ribosylation Factors), one RER1 (Retention in Endoplasmic Reticulum 1) and one p24 (cargo receptor). The expression and localization of these proteins evidenced colocalization with specific markers for distinct subcellular organelles. Interestingly, ARF4 (ORF:13930) and RER1 (ORF:15413), in addition to SNAREs Qb1 and Qb2, displayed a vesicular pattern different from typical Giardia subcompartments, and colocalized with heterologous markers for the GA. Additionally, treatment with fungal toxin Brefeldin-A evidenced partial dissociation from these vesicular structures and redistribution of ARF4 and RER1 into the ER. Our results not only contribute to understanding the complex system of vesicular trafficking but also suggest the existence of a structurally atypical Golgi apparatus, capable of carrying out the functions of packaging and sorting of proteins in *Giardia*

CB-P46.

PLASMID DNA CLEAVAGE AND CYTOTOXICITY OF VO(oda)phen COMPLEX ON NORMAL AND TUMOR LINES IN CULTURE

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Vanadium is a transition metal with chemical, biological and pharmacological interest due to insulin mimetics, antitumoral and osteogenic actions. Nevertheless, vanadium compounds may also show cytotoxic effects mainly due to the generation of oxidative stress and DNA damage. In this work we report the biological effects of a complex of vanadyl (IV) cation with two ligands (oxodiacetate and phenanthroline) of biological interest, on two osteoblastic cell lines, one normal (MC3T3-E1) and the other tumoral (UMR 106). The effects on cell proliferation (crystal violet bioassay), metabolic activity (MTT bioassay), lysosomal activity (neutral red) and morphology were investigated. Besides, we studied the mechanisms involved in the cyto- and genotoxicity (oxidative stress and DNA cleavage). The complex caused an inhibitory effect on both line proliferation at low doses with a stronger effect on MC3T3-E1 ($p<0.001$). Moreover it altered the lysosomal and mitochondria metabolisms ($p< 0.001$). Morphological studies showed important transformations and a decrease in the number of cells in a dose response manner. Besides, VO(oda)phen interacted with plasmidic DNA (pA1) causing single and double strand cleavage. Altogether, these results suggest that the complex is a candidate to be further evaluated for alternative therapeutics in cancer treatment

CB-P47.

EXPRESSION AND LOCALIZATION OF COMPLEXIN IN MOUSE EGGS

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Cortical granule exocytosis is one of the most important steps, which occurs after sperm-egg fusion, at fertilization. Cortical granule exocytosis is induced by the spermatozoon and blocks polyspermy to enable a successful embryonic development. Induction of cortical granule exocytosis depends on intracellular calcium mobilization in the egg; however little is known about the signal transduction pathway downstream of calcium mobilization. Our general aim is to identify the proteins involved in cortical granule exocytosis, analyze their functions, and arrange them in a working model. We hypothesized that cortical granule exocytosis is controlled by a conserved protein machinery that involves SNAREs, Rabs, and additional proteins that are key for calcium-regulated exocytosis, such as complexin. The aim of this work was to investigate the expression and localization of complexin in mouse eggs. Using an antibody that recognizes two isoforms of complexin, Western blots results demonstrated that complexin is expressed in mouse eggs. Immunofluorescence studies showed that complexin localized specifically at the cortical region of mouse eggs, which is enriched in cortical granules. Our results suggest that complexin might be involved in cortical granule exocytosis. However, further studies are necessary to identify the specific isoform/s of complexin expressed in mouse eggs.

CB-P48.

KRÜPPEL LIKE FACTOR 6 IS AN IMPORTANT TRANSCRIPTIONAL REGULATOR OF TROPHOBLAST DIFFERENTIATION

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KLF6 is a ubiquitous zinc finger transcription factor involved in differentiation, cell cycle control and proliferation in several cell systems. Even though its highest expression has been detected in human and mice placenta, its function in trophoblast physiology is still unknown. During pregnancy, placental villous cytotrophoblast cells (CTB) proliferate and differentiate by fusion to form the syncytiotrophoblasts layer characterized by a high metabolic and biosynthetic activity. In this work, we postulate KLF6 as an important transcription factor that regulates the expression of genes implicated in trophoblast differentiation. KLF6 expression raised within the first hours of *in-vitro* syncytialization preceding cell fusion and expression of ßhCG and PSG genes, early markers of trophoblast differentiation. Over expression of KLF6 in trophoblast-like JEG-3 cells increased the mRNA and protein levels of ßhCG and PSG, as revealed by qRT-PCR and western blot assays. Most importantly, down-regulation of KLF6 expression by siRNA experiments before the beginning of *in-vitro* syncytialization resulted in lower mRNA expression of trophoblast differentiation marker genes and inhibition of the CTB fusion process. In sum, these results represent the first direct evidence to propose KLF6 as a regulator of trophoblast differentiation.

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CB-P49.

DIPHENYL DISELENIDE MODULATES MACROPHAGE ACTIVATION BY DECREASING PRO- AND ANTI-INFLAMMATORY MARKERS

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The biological importance of selenium led to the development of pharmacologically active organoselenium compounds. Diphenyl diselenide (PhSe)2 is an organoselenium compound whose biological activities have been poorly described. Previously, we evaluated its antioxidant and anti-inflammatory properties in an *in vitro* model of inflammation. We found that (PhSe)2 was able to prevent the production of reactive oxygen species, nitric oxide, the expression of iNOS, the peroxynitrite modification of proteins (nitrotyrosine immunostaining) and the antigen presentation capacity of LPS stimulated macrophages (Mph). Next, we focused on studying the ability of (PhSe)2 to modulate the alternative activation of Mph, which encompasses an upregulation of anti-inflammatory mediators. We isolated peritoneal Mph and stimulated them with dexamethasone to induce the alternative activation phenotype. We observed that activated Mph presented higher expression of IL-10, which was downregulated by (PhSe)2. Then, we examined the surface expression of mannose receptor (CD206), and found that dexamethasone treatment augmented the expression of CD206, while (PhSe)2 was able to downregulate this molecule. Finally, preliminary results indicate that (PhSe)2 decreased the ratio of IL-10/IL-12 mRNA in dexamethasone treated Mph. These findings suggest that (PhSe)2 could be used to modulate the activation of Mph.

CB-P50.

NICOTINIC ACETYLCHOLINE RECEPTOR CLUSTERS LOCALIZATION ON LIPID DOMAINS OF THE PLASMALEMMA

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In the present work we have attempted to establish whether there is a correlation between antibody-induced nicotinic acetylcholine receptor (AChR)-rich clusters and the physical state of the underlying cell membrane in living cells. For this purpose di-4-ANEPPDHQ, a fluorescent probe that differentiates liquid-ordered from liquid-disordered phases in model membranes was used in combination with labeling of the AChR in CHO-K1/A5, a clonal cell line expressing adult muscle-type AChR. The so-called generalized polarization ("GP") of di-4-ANEPPDHQ was measured in regions of the cell-surface membrane associated with the AChR platforms clearly identified using conventional wide-field fluorescence microscopy. Under control conditions AChR clusters are roughly equally distributed among liquid-ordered and liquid-disordered domains. This distribution changes upon cyclodextrin-mediated cholesterol depletion or by Latrunculin disruption of the actin cytoskeleton. Association of AChR clusters with lipid domains with different biophysical properties may have consequences on AChR trafficking processes.

CB-P51.

YACON LEAVES AMELLIORATE TGF-ß1/SMAD SIGNALING IN DIABETIC KIDNEY

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Nephropathy is a common cause of morbidity and mortality in diabetic patients. Prevention of this complication is clearly required. *Smallanthus sonchifolius* (yacon) leaves containing mainly polyphenolic acids and the lactone enhydrin have been shown to ameliorate hyperglycemia in streptozotocin-induced diabetic rats. In the present study, we examined the beneficial effects of yacon leaves decoction in diabetic kidney and explored the possible underlying mechanism.
Diabetic rats were orally administered with 10% yacon leaves water decoction (140mg dry extract/kg b.w.) for 30 days. Biochemical parameters in blood and urine, immunohistochemistry, western immunoblotting and qRT-PCR analysis were developed.
Yacon decoction significantly decreased high blood glucose level in diabetic rats and improved insulin production. Diabetic-dependent alterations in urinary albumin excretion, creatinine clearance, kidney hypertrophy and basement membrane thickening were attenuated by yacon decoction. These findings were associated with a marked decrease in TGFß1, TGFRII and p-Smad2/3. The expression of extracelullar matrix proteins as collagen IV, laminin-1, fibronectin and collagen III were also diminished in the yacon-treated group. These results suggest that yacon leaves is a protective agent against renal damage in diabetic nephropathy, whose action can be mediated by TGFß/Smads signals.

CB-P52.

IMPLICATION OF PKC ISOFORMS IN REVERSION OF MAMMARY TUMORS MALIGNANCY IN RESPONSE TO RETINOIC ACID

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In this work we have overeexpressed in LM3 murine mammary cells the and isoforms of PKC in order to study whether these genetically modified sublines are more sensitive to retinoid treatment (ATRA).
Through a reporter gene assay, using the retinoic acid responsive elements upstream luciferase gene (RARE-Luciferase), we could determine that only PKC overexpression induced an increase in the activity of these sites. This result correlates with previous assays showing that PKC traslocates to the nucleus coupled to retinoid receptors. ATRA effect was also studied *in vivo* and *in vitro*, evaluating parameters related to tumor growth and dissemination. While PKC overexpression induced an important increase in the *in vitro* proliferative capacity, only these overexpressors become sensitive to ATRA treatment showing a proliferative delay. Moreover, LM3-PKC cells also showed a higher migratory capacity, also reversed by retinoid treatment. *In vivo* assays showed that only PKC overexpression induced an increase in tumor growth and metastatic potential, and ATRA treatment was able to limit the malignant progression of these tumors. Our results suggest that, PKC overexpression confers a more aggressive phenotype but make the cells sensitive to ATRA effects, while PKC is necessary for retinoid receptors traslocation but is insufficient to alter cellular response to retinoid treatment.

CB-P53.

THE MODULAR ORGANIZATION OF PROTEIN-PROTEIN INTERACTION NETWORKS OF UNICELLULAR EUKARYOTES

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Network theory provides a framework to analyze, from a topological point of view, large amounts of data regarding molecular interactions and provide an integrated view of cellular function and evolution. In the past decades, complex network approaches to cell and molecular biology have been crucial to reveal the underlying architecture of organismal complexity. Among all of the topological properties that can be extracted, modularity (the tendency of elements or links to form more connected subsets) is considered to be one of the main organizing principles in biological networks. It has been suggested, for example, that the modularity of bacterial metabolic networks had experimented a reduction during evolution. It has been also pointed out that modularity is reduced in simbiont bacteria, but the opposite is true for obligate mammalian pathogens. In addition, the diameter of metabolic networks of parasitic eukaryotes tends to be similar to those of free-living eukaryotes, suggesting that network integrity had been a structural constraint during evolution. In this work we compare protein-protein interaction networks of unicellular eukaryotes extracted from STRING database, by calculating two different modularities. At first glance, parasitic/early divergent unicellular organisms tend to have high modularity values than non-parasitic/more derived ones.

CB-P54.

THE TRAFFIC OF SOLUBLE HYDROLASES IS MEDIATED BY VPS10P-LIKE RECEPTOR IN *Giardia lamblia*

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The targeting of lysosomal enzymes to their final destination is directed by a series of protein and recognition signals. Although, *Giardia lamblia* lacks a recognized Golgi apparatus or a typical endosome/lysosome system, it might have a specific and conserved mechanism by means the soluble hydrolases are sorted. In this work, we describe the presence of a Vps10p -like receptor and its function in sorting of lysosomal enzymes. Bioinformatics and proteinase K assays allowed us to determine that the gVps10p receptor is a type I transmembrana protein with a WD40 motif in the luminal portion and a cytoplasmic tyrosine motif (YQII). Immunoblot assay showed a ˜60 kDa but also a ˜120 kDa band, possibly related to dimerization. IFA and confocal microscopy of trophozoites expressing gVps10p-HA fusion protein, showed that it localize around the nuclei colocalizing with the endoplasmic reticulum marker BIP (Immunoglobulin Binding Protein). Co-expression of gVPS10p-HA and gAcPh-V5 fusion proteins showed colocalization around nuclei and in the lysosomal peripheral vacuoles. On other hand, gVps10p-YQII-HA fusion protein, lacking the cytoplasmic tyrosine motif, showed a different subcelular distribution possibly related with changes in protein trafficking. This work will contribute to understand how the endosomal/lysosomal pathway works in the early divergent parasite *G. lamblia*.

CB-P55.

CLATHRIN-ASSOCIATED PROTEIN IN *Giardia lamblia*

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The presence of clathrin-associated proteins (CLASP) has not yet been reported in the protozoan *G. lamblia*. However, by searching in the GDB, we found a protein that contains an ENTH domain characteristic of the CLASP epsin. The analysis of the participation of the ENTH protein (ENTHp) in the trafficking towards lysosomal–like peripheral vacuoles (PVs) was initiated by cloning and expression of the HA-fusion protein and its mutants in *Giardia* trophozoites. Using IFA and confocal microscopy we showed that ENTHp localize mainly in the cytosol and around the nuclei, partially colocalizing with BIP and AP-2. Additionally, we developed monoclonal antibodies directed against clathrin heavy chain (CHC) to disclose the interaction and function of these particular proteins. Clathrin positive polyclonal Ab (pAb) production was tested in wild-type trophozoites observing that the pAb recognized a protein localized in the PVs and a band of 203 kDa corresponding to the CHC. Using this pAb we observed the colocalization of ENTHp and CHC in the PVs and also validate the interaction between both proteins. Antibody-secreting hybridomas for CLH will allowed a better characterization of clathrin and also corroborate whether the ENTHp is a CLASP acting as a dual epsin-EpsinR protein. These results will greatly increase our understanding of unique aspects of lysosomal trafficking in *Giardia*.

CB-P56.

ROLE OF PALMITOYLATION DURING *Giardia lamblia* ENCYSTATION

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A key step in the life cycle of *G. lamblia* is the differentiation of the trophozoites into cysts. This process is induced in response to host-specific factors such as high levels of bile, low levels of cholesterol and a basic pH. One of the early events that occur during encystation is the synthesis of cyst wall proteins (CWP). Post-translational modifications (PTM) such as palmitoylation, are important to regulate molecular mechanisms involved in cell homeostasis and intracellular signaling. It has been shown that Variant-specific surface proteins of *G. lamblia* are palmitoylated, and the enzyme responsible for this activity has been characterized. However, the role of palmitoylation in encystation has not been reported yet. So, the aim of this work is to elucidate the role of this PTM in encystation in *G. lamblia*. We observed that when palmitoylation is inhibited by adding 2-Fluoro palmitic acid to the encystation media the percentage of cells expressing CWP1+ decreased. Moreover, we observed an increase of CWP1+ parasites in encysting *G. lamblia* trophozoites that overexpressed a palmitoyl transferase, compared to wild type *G. lamblia*. Due to the many intracellular signals involved in the encystation process is possible that PTM such as palmitoylation have an important role. Further studies are needed to find out the molecular mechanisms in which palmitoylation may be involved.

CB-P57.
TL(I) AND TL(III) DIFFERENTIALLY AFFECT THE ANTIOXIDANT DEFENSE SYSTEM IN PC12 CELLS
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Previously, we demonstrated that the exposure of rat pheochromocytoma (PC12) cells to a single dose of Tl(I) or Tl(III) (10-100 µM) resulted in an early oxidative stress status due to higher mitochondrial H_2O_2 production. In the current work we evaluated the effects of a short-term exposition (6 h) to Tl(I) or Tl(III) (10-100 µM) affected PC12 cells antioxidant defense system, measuring the expression and activity of the enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). While CAT expression was not affected by Tl, a significant and concentration-dependent decrease in its activity was observed for both cations. Mn-SOD expression was increased in Tl(I)-treated cells, and decreased in Tl(III)-treated cells. Total SOD activity was increased by Tl(I) (10-25 µM), while higher Tl(I) concentrations or Tl(III) decreased its activity. Accordingly to its function as H_2O_2-metabolizing enzyme, GPx activity was increased regardless the cation assessed. However, GR activity was slightly increased only in cells exposed to Tl(III). These results suggest an early misbalance between H_2O_2 production and degradation in Tl-exposed cells, effect that could be partially responsible for the toxic effects of these cations.
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CB-P58.
MARCKS PARTICIPATES IN CORTICAL GRANULE EXOCYTOSIS IN MOUSE EGGS
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Cortical granule (CG) exocytosis is a secretory process that blocks polyspermy and enables successful embryonic development. Nevertheless, the molecular mechanism of this particular exocytosis remains unknown. We have reported that MARCKS, a prominent substrate of PKC involved in exocytosis in different cell types, is expressed in mouse eggs. We hypothesized that MARCKS is involved in cortical granule exocytosis. To test this hypothesis, we first, analyzed the colocalization of MARCKS and CG by confocal microscopy during mouse oocyte maturation and egg activation, and, second, microinjected GST-MARCKS effector domain to analize MARCKS function in a functional assay of CG exocytosis. During oocyte maturation, confocal microscopy results showed that MARCKS localized uniformly in the cytoplasm of germinal vesicle oocytes; however, in metaphase II eggs, its localization was mainly associated to the cortical region, which is enriched in CG. During egg activation, CG exocytosis was associated with a dramatic decrease in MARCKS staining. Microinjection experiments showed that the microinjection of GST-MARCKS effector domain was able to inhibit CG exocytosis stimulated by $SrCl_2$ when compared to control, and this inhibition was concentration-dependent. Our data suggest that MARCKS is involved in the signal transduction pathways that lead to cortical granule exocytosis in mouse eggs.

CB-P59.
***Chlamydia trachomatis* RECRUITS Rab39, A NOVEL GOLGI-ASSOCIATED GTPase**
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Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium, which multiplies in a single vacuole called inclusion. This bacterium early dissociates from the endocytic pathway but continues the interaction with the biosynthetic route. This bacterium intercepts vesicles full of nutrients departed from the Golgi apparatus essential for its survival and development. We have described that *Chlamydia* re-directs Rab11- and Rab14-mediated vesicular transport to capture endogenous lipids synthesized in the Golgi. Rab39 is a recently described host GTPase, localized at the Golgi apparatus, which precise function is still poorly understood. The aim of this study was to investigate if *Chlamydia* manipulates Rab39-mediated vesicular trafficking for its own nutrition and benefit. Cells overexpressing Rab39a wt and its negative mutant: Rab39a S25N (a GDP-bound inactive form) and its positive mutant: Rab39a Q70L (a GTP-anchored active form) were analyzed by confocal and electron microscopy after bacterial infection. Our results showed that Rab39a wt is recruited to the chlamydial membrane inclusion in a time-dependent fashion. Furthermore, our results demonstrated that Rab39b wt, an isoform of Rab39a wt, did not display the same pattern of recruitment. These results suggest that host Rab39a-mediated transport is subverted by *Chlamydia trachomatis* to generate a safe intracellular niche where survive and replicate.

PL-P01.

ANTIOXIDANT – POLYPHENOLS IN ANDEAN POTATO VARIETIES OF THE ARGENTINEAN NORTHWEST

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Potato, and particularly wild varieties and native landraces, produces a variety of secondary metabolites, such as the antioxidant polyphenolic flavonoids, beneficial to human health. QTL – assisted breeding allows for the introduction of selected genotypic features from native to processing potatoes, directed at the increase of levels of flavonoids in processing varieties. However, before starting labor– intensive plant breeding programs, a profound knowledge of the regulation of polyphenol production is required. In order study the flavonoid metabolism, we started a comparative analysis between processing and Andean potatoes. We previously studied the levels of total phenolics, anthocyanins and the antioxidant capacity in pulp and peel of tubers of 12 processing potato varieties. Here we determine their levels in nine Andean potato varieties. All levels are higher as compared to the levels we reported for processing varieties. The phenolics acid content as well as the antioxidant capacity are higher in peel than in pulp. As expected anthocyanins were only detected in the pigmented varieties. Currently we are determining transcript levels of the key enzymes in polyphenol metabolism. Future analyses will also be directed at enzyme characteristics. Taken together these results will be integrated to generate a kinetic model of metabolic network of antioxidant-polyphenols.

PL-P02.

ANALYSIS OF SRNAS PROFILES AT EARLY STAGE OF TWO CONTRASTING *Tobamovirus* INFECTIONS

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Plant viral infections induce changes in gene expression and metabolic components. Our previous work demonstrated that tobacco infection by different *Tobamovirus* altered accumulation of miRNA, correlating with symptom severity. We also demonstrated alterations of miRNA levels at early stage of infection, suggesting that a systemic signal induces miRNA alteration changes since no virus were detected systemically. The aim of this work is to characterize sRNAs profiles at early stage (no virus present in sampled leaf) of two contrasting *Tobamovirus* infections (ORMV (severe) and TMV-Cg (mild)) in Arabidopsis using sRNA deep-sequencing, in order to establish correlations between miRNAs alteration and symptoms. Comparison between Arabidopsis-encoded sRNA obtained for mock and virus-treated samples reveals a significant impact of virus infection. The lenght distribution of sRNA-mappable reads shows a significant increase in the proportion of 24mer sRNAs for TMV-Cg infection, whereas the proportion of 21 mer is increased in ORMV treatment. Global miRNA differential expression patterns between virus-treated and mock exhibited alteration in several of the 52 miRNA families detected. Interestingly, certain miRNAs had a contrasting level of accumulation between ORMV and TMV-Cg treatment, pointing them as possible keys to explain the symptoms severity differences observed on the strains tested.

PL-P03.

SNAKIN-1 PEPTIDE IS INVOLVED IN DEFENSE AND DEVELOPMENT

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Snakin-1 (StSN1) is an antimicrobial peptide isolated from *Solanum tuberosum* that was shown to be active against fungal and bacterial pathogens *in vitro* and *in vivo*. Others members of its family (snakin/GASA) have already been implied in diverse processes such as cell division/elongation, transition to flowering and signaling pathways and it has been demonstrated that some of them are transcriptionally regulated by gibberellic acid (GA) or paclobutrazol (PAC; an inhibitor of GA biosynthesis). We have previously obtained potato transgenic lines overexpressing or silencing StSN1 and even though overexpressing lines did not show remarkable phenotypic differences from wild type, silenced plants exhibited altered leaf morphology, internode elongation, flowering timing and tuberization. In this work we demonstrated that internode elongation of plants grown either *in vitro* or pot-cultured was rescued by exogenous GA3. We also suggested that in potato StSN1 promoter was induced after GA treatments by analyzing transgenic lines harboring StSN1 promoter region fused to GUS reporter gene. Finally, we presented qRT-PCR analyses of StSN1 and genes involved in GA metabolism expression levels in treated or transgenic plants. Altogether, our results suggest that StSN1 is involved in GA metabolism and consequently it has a dual function in defense and development.

PL-P04.

TUMV STRAINS PRODUCING DIFFERENT SYMPTOMS CHANGE DIFFERENTIALLY miRNA AND miRNA-TARGET EXPRESSION

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Plant viral infections induce broad changes in plants like gene expression and metabolic profiles. These changes have a profound impact in plant physiology and morphology. Our previous work suggested that at an early stage of virus infection changes in the accumulation of microRNAs (miRNA) are observed in systemic virus-free tissue. Additionally, some hormone precursors are altered too, suggesting a possible signaling moving in advance to the virus itself. Occasionally, closely related viruses induce different symptoms, whose molecular mechanisms are largely unknown. In this work, we aimed to relate the different symptoms produced by two TuMV strains (JPN and UK1) on *A. thaliana* plants with gene expression changes in systemic leaves at two stages post-inoculation. We focused our study on some miRNAs which are either known to target transcription factors that are key regulators of leaf development and/or to be part of hormone regulatory networks. We studied also the accumulation of some precursors of miRNAs in order to analyze the possible impact of systemic signals on their transcription in developing leaves prior to virus invasion. Our results indicate the existence of differential changes in miRNAs precursors and target genes levels correlating with the differential symptoms produced by the two TuMV strains tested at both the early and late time points of the infection analyzed.

PL-P05.

EXPRESSION OF ANTIMICROBIAL SNAKIN-1 PEPTIDE IN INSECT CELLS

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Snakin-1 (StSN1) is a broad-spectrum antimicrobial cysteine-rich peptide isolated from *Solanum tuberosum*. Although it is active against fungal and bacterial pathogens, no information is available concerning the mechanism of action. Only some members of its family were expressed probably because its activity affects prokaryotic system viability. Even recombinant rSN1 could be expressed in *E.coli*, it was localized in inclusion bodies and displayed distinct activities than plant StSN1. In order to obtain purified protein for biochemical studies and subsequent research, we expressed SN1 in *Spodoptera frugiperda* (Sf9) insect cells using the Bac-to-Bac baculovirus expression system. This system allows expression of toxic proteins while the correct folding is more likely maintained because of their extensive and more accurate post-translational modifications. Here, the gene sequence codifying for the mature StSN1 peptide was subcloned into the pFastBacTMHT B, transposed into the bacmid and virus were amplified. Tagged-SN1 expression was detected in the nuclei fraction of infected Sf9 cells, recombinant protein was purified and mice were immunized to obtain polyclonal antibodies. More purifications are being carried out for biochemical experiments and for testing the protein on a wide variety of phytopathogens to assess the antimicrobial potential of rSN1 from baculovirus expression system.

PL-P06.

EXOSOME COMPONENTS ARE ALTERED IN TRANSGENIC TOBACCO PLANTS THAT CO-EXPRESS CP AND MP FROM TMV

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Viral diseases induce metabolic and physiological alterations probably associated to symptoms. Here we used transgenic plants that express TMV proteins to study the effect of each one on host gene expression. We studied the effects of capsid (CP) and movement protein (MP) and both CP and MP co-expression in tobacco. Neither CP nor MP exhibit silencing suppression effects, although plants that co-express them show altered levels of miRNAs and exhibit developmental changes that resemble symptoms. Gene expression patterns were analysed with a tomato 10K Affymetrix chip between CPxMP (altered phenotype) and cpxmp (silenced line with normal phenotype). We found many differentially expressed genes related to RNA turnover. In plants, this process is important for development and is rendered among others, by the exosome complex. We determined Rrp41, Rrp42 and Rrp43 expression levels (core subunits) in transgenic and TMV infected plants. We showed that GFP transient expression in CPxMP is enhanced, suggesting a modulation of transgene silencing in these plants. We silenced the expression of Rrp42 by means of VIGS in *N. benthamiana* and observed growth arrest, morphological defects and changes in miR156, miR164 and miR165 accumulation. Altogether, these data suggest that exosome components could be implicated in miRNAs alteration and symptom generation during plant-virus interaction.

PL-P07.

HEME OXYGENASE/CARBON MONOXIDE SYSTEM REGULATES GLUTATHIONE LEVELS IN PLANTS

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This objective of this work was to investigate whether heme oxygenase (HO) is involved in the regulation of GSH. Experiments were performed in plants treated with Zn-protoporphyrin (ZnPPIX) (specific inhibitor of HO) and then subjected to sodium nitroprusiate (SNP) concentrations (NO donor, HO inductor) or CO (HO reaction product) for 48 h. In soybean plants pretreated with ZnPPIX for 72 h before Hoagland (H) treatment (ZnPPIX/H), GSH level diminished 20% respect to controls. NO (ZnPPIX/SNP) as well as CO (ZnPPIX/H) enhanced GSH levels (40% and 15%, respectively). HO-1 expression decreased and oxidative stress parameters were increased. When the inhibitor was added before NO or CO treatment, HO-1 expression as well as GSH content and GR activity were increased. This increase is positively correlated with GSH content and GR activity. The enhancement of GSH is not related to oxidative stress. In contrast, HO inhibition brought about an enhancement (28%) in TBARS levels. In plants treated with ZnPPIX, CO does not induce HO-1, but an augmentation of GSH levels and GR activity was observed. CO released enhanced GR activity and in this way, GSH content was regulated. These data provide evidence of other possible roles that NO, as well as CO could play against the oxidative insult.

PL-P08.

EFFECT OF PHYTOHORMONES ON OXIDATIVE STRESS IN SOYBEAN ROOTS SUBJECTED TO DROUGHT

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The behaviour of catalase (CAT) and guayacol peroxidase (Gpox) was investigated in soybean plants pretreated with different phytohormones such as indol acetic (AIA), jasmonic (JA) and salicylic acids (SA) in soybean plants subjected to drought stress. Seeds were pretreated with 20 µM AIA, 20µM JA or 125 mM SA for 48 h before 4 days germination in vermiculite. Plants were then subjected to different polyethylene glycol (PEG) concentrations ranging from 0 to 8% (W/V). Our data showed that CAT as well as Gpox activity were enhanced (up to 57% and 69%, respectively) in plants treated with different PEG concentrations. Surprisingly, plants subjected to phytohormones revealed a biphasic behavior. On one hand, under a mild drought stress (2% PEG) an enhancement in CAT activity was observed in the presence of the three phytohormones (up to 100% in JA treated plants), but Gpox showed a different response. Activity diminution was assessed in the presence of AIA, JA or SA (40%, 30% and 40%, respectively). On the other hand, under PEG concentrations (4% or 8%) a significant diminution in both activities was detected. In order to get hints on these results, oxidative damage was detected in situ. Plants with diminished CAT and Gpox activities revealed a less oxidative damage respect to those with elevated peroxidases activities, indicating the possible protective role of phytohormones against drought

PL-P09.
CGMP AND ABSCISIC ACID ARE REQUIRED FOR SEED GERMINATION IN SOYBEAN PLANTS
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Cyclic guanosine 3,5 monophosphate (cGMP) is an important second messenger in animals, and is emerging as a player in regulatory functions in plants. In this study, we investigated the role of cGMP in seed germination in soybean. The seeds were pretreated with different concentrations of a membrane-permeant analogue of cGMP (100µM) (8-Br-cGMP), hemin (20µM) (an inducer of heme oxygenase), Zn-protoporphyrin (20µM) (ZnPPIX) (specific inhibitor of heme oxygenase) and abscisic acid (ABA) for 48 h. We demonstrated that both 8-Br-cGMP and hemine promoted seed germination. Experiments performed in seeds treated with ZnPPIX demonstrated that heme oxygenase (HO) activity is required for seed germination. When different concentrations of ABA (0.5, 1 and 2µM) were assessed, the highest concentration induced plant growth. These findings highlight that cGMP is a positive regulator and plays a crucial role in soybean seed germination. Furthermore, both ABA and cGMP are required for seed germination. These results infer that cGMP is involved in the HO signaling pathway.

PL-P10.
CELL WALL DISINTEGRATION ALTERS STARCH AND SUCROSE METABOLISM
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CBM (carbohydrate binding modules) are structures associated to catalytic domains which have the ability to bind to different polysaccharides. Among these, those with a strong affinity for starch (SBD) have an evolutionary advantage due to the presence of two substrate binding sites. We have fused the SSIII (AT1G11720) SBD to the signal peptide of EXPA8 (AT2G40610), and targeted to the cell wall of Arabidopsis plants. Transgenic plants showed an altered cell wall structure and composition. Interestingly, we observed an increase of transient starch levels from leaves collected at the end of the day. In addition, we found a modified amylose:amylopectin ratio. We also performed transcriptomic studies in order to analyze the expression of several genes that encode enzymes involved in starch and sucrose metabolism. We found evidence suggesting that the increase in starch content in the transgenic plants could be explained by a decrease in its degradation; while the alteration in the composition of this polysaccharide could be due to changes in the catalytic activity of other enzymes involved in the biosynthetic pathway. Finally, we have been detected a decrease in the levels of transcripts coding for key enzymes involved in the degradation of sucrose and therefore associated with decreased production of UDPGlc and fructose, two precursors of cell wall polysaccharides

PL-P11.
MITOCHONDRIAL DYSFUNCTION AFFECTS CHLOROPLAST FUNCTIONS
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There are several reports that address the relationship between mitochondrial respiration, photosynthesis and chloroplast functions. That process provides energy for biosynthesis, and its balance with photosynthesis determines the rate of plant biomass accumulation. These interactions involve transcriptional control, protein co-localization, distribution of biochemical pathways between organelles, and the impact of substrate and product concentrations (metabolic shuttles). Profound changes in gene expression of nuclear-encoded genes were observed in Arabidopsis plants exhibiting impaired mitochondrial function. This model, where the mitochondrial flaw was induced by the expression of the unedited form of the ATP synthase subunit 9 (*u-ATP9*), is useful to uncover the interactions between organelles in plant cells. An interesting point of our work is the fact that mitochondrial dysfunction in transgenic plants can affect photosynthesis by reducing the chlorophyll levels. In fact, degradation of these pigments may be due to the increase of Mg2+ dechelatase activity and ROS levels, inducing a dysfunction of the light-harvesting (antenna) complex. qRT-PCR analysis of *LCHI* (At1g19150) and *PSBQ2* (AT4g05180) mRNA levels shows an increase of about 5-fold in transgenic lines compared to wild type plants, confirming the induction observed in microarray experiments.

PL-P12.
ACTIVATION OF THE PRO/P5C CYCLE IN ARABIDOPSIS TISSUES DEVELOPING THE HYPERSENSITIVE RESPONSE
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L-proline (Pro) is converted into 1-pyrroline-5-carboxylate (P5C) by the mitochondrial enzyme Pro dehydrogenase (ProDH). In the same compartment P5C is transformed into glutamate (Glu) by P5C dehydrogenase (P5CDH). However, under particular conditions, P5C is reduced back to Pro by P5C reductase (P5CR) in the cytosol. In the last case, the combined action of ProDH and P5CR activates the Pro/P5C cycle. In plants and animals, stimulation of this cycle is associated with cell death. We suspected that the Pro/P5C cycle operates in Arabidopsis tissues generating the Hypersensitive Response (HR) against pathogens. To verify this hypothesis we analyzed ProDH activity (*in vivo*, *in vitro*), amino acid content, cell death and reactive oxygen species (ROS) levels in naive and infected tissues. The studies were performed in wild type, *p5cdh* mutant and *P5CDH* over-expressing plants. Our results confirmed that Pro catabolism is activated in HR before the onset of cell death. In addition, they showed that P5CDH and P5CR display different activities along HR progression, supporting evidence for activation of the Pro/P5C cycle in these tissues. We speculate that this cycle potentiates hypersensitive cell death through ROS accumulation.

PL-P13.

ALTERATIONS OF TRANSCRIPTION LEVELS OF CENTROMERIC LOCI IN ARABIDOPSIS INFECTED PLANTS.

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In higher eukaryotes, chromatin is organized in highly condensed and relaxed domains (heterochromatin and euchromatin, respectively). Heterochromatin is characterized by transcriptional repressive marks (methylated DNA and methylated Histone H3 lysine 9) and contains few genes intercalated among thousands of short repetitive sequences. Recent studies revealed that chromatin marks are susceptible to be modified by stress. We found that in Arabidopsis, the infection with *Pseudomonas* triggers heterochromatin decondensation and DNA demethylation, with centromeric repeats being target of these responses. To evaluate if these alterations affect the transcription level of centromeric repeats, we quantified the abundance of transcripts of the centromeric locus *TSI* (Transcriptional Silenced Information) in naïve and infected plants. We included wild and mutant plants having alteration in heterochromatic epigenetic marks (*ddm1*, *mom1* and *vim1*). A reduction of *TSI* transcripts was observed in infected-wild type but not infected-mutant plants. In addition, mutants showed increased activation of defense genes and enhanced resistance to *Pseudomonas*. These results suggest that alteration of heterochromatin structure induced by bacterial infection may affect plant defenses.

PL-P14.

MONITORING OF CADMIUM-INDUCED OXIDATIVE STRESS IN *Glycine max* L. LEAVES

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Heavy metals induce oxidative stress in plants. Monitoring levels of nitric oxide (NO), chlorophyll and essential elements of the plant are good indicators of oxidative damage. The aim of this study was to quantify oxidative stress parameters in soybean leaves after exposure to abiotic factors such as cadmium. We worked with soybeans germinated and developed under controlled conditions. The fourth day of germination they were placed in hydroponic conditions with Hoagland nutrient solution. On the tenth day they were subjected to intoxication with Cd (40 µM) during 6, 24, 72 h and 6 days. After that time the seedlings were harvested and measurements were made on leaves. Levels of Cd, Cu, Fe, Mg, Zn and Mn were measure in biomass ICP-MS. NO and chlorophylls were determined via colorimetric assays. Cd accumulates in all treated groups. NO measured levels showed a significant increase at 24 and 72 h ($p<0.001$). Chlorophylls decreased over treatment with Cd. Mg content showed similar behavior. Antioxidant SOD enzyme-related metals, Cu, Fe, Zn and Mn increased after Cd exposure. In a preliminary work in *Vigna mungo* L the chlorophyll content decreased and SOD activity increased, in a time-dependent way after treatment with Cd. In this model, we observed that Cd intoxication produces oxidative stress and disturbs the redox status in plants.

PL-P15.

CHARACTERIZATION OF PEACH CULTIVARS WITH DIFFERENT SUSCEPTIBILITY TO CHILLING INJURY

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Refrigeration of peaches during marketing and shipping affects fruit quality causing physiological disorders collectively named chilling injury (CI). CI is genetically influenced and triggered by a combination of storage temperature and storage period. In this work, early-, mid- and late-season peach cultivars were examined at harvest, during ripening at 20ºC until reaching firmness values <2 kgf, after cold storage (3 weeks, 0ºC) and after cold storage plus ripening at 20ºC. Among the tested cultivars, and based on extractable juice measurements, Springlady and Flordaking fruits showed contrasting susceptibility to CI symptoms after cold storage. After 3 weeks at 0ºC plus ripening at 20ºC Springlady peaches had expressible juice similar to that of unstored fruit held for 5 days at 20ºC, while Flordaking fruits had significantly lower expressible juice, thus being more susceptible to woolliness. Quality attributes, ethylene production and the expression of genes encoding proteins involved in cell wall metabolism (determined by qRT-PCR) were analyzed in these two cultivars. Thus, our aim is to inspect for transcripts associated with cell wall metabolism that could be differentially accumulated in these two cultivars with contrast susceptibility to CI in order to identify the principal cell wall proteins involved in the alleviation of CI.

PL-P16.

IDENTIFICATION OF ANTIFUNGAL PROTEINS ASSOCIATED TO ASPERGILLUS RESISTANCE INFECTION IN PEANUT SEED

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Aspergillus flavus and parasiticus are aflatoxigenics fungi that infect peanut seeds both under field conditions and post harvest storage. We have previously found a significant association between aflatoxin contamination and fungal infection. Therefore, in order to identify the components that contribute to Aspergillus resistance the present work investigate the proteins involved in the defense response to infection in peanut seed. The pathosystem used in our approach is composed by two genotypes: PI337394 and Florman previously characterized as resistant and susceptible to Aspergillus infection and aflatoxin contamination. Seeds of both genotypes were disinfected and inoculated with *A. parasiticus* and proteins extract with antifungal activity were enriched by chromatography. Elution fractions with antifungal activity were analyzed by 2D-eletrophoresis and identified by HPLC-ESI-MS. Proteins with known antifungal activity such as quitinases and pathogenesis related proteins, and proteins that participate in plant defense such as superoxide dismutases, lectins and alergens were found overrepresented in 2D-electrophoresis gels from the resistant cultivar. These results suggest that antifungal proteins play an important role in the defense response to *A. parasiticus* infection in resistant peanut seed.

PL-P17.

VITAMIN E BIOSYNTHESIS IS TRANSCRIPTIONALLY REGULATED DETERMINING THE CELLULAR REDOX STATE IN TOMATO

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Tocopherols are a group amphiphilic antioxidants belonging to the vitamin E (VTE) family. Although tomato fruits are important sources of VTE, its biosynthesis has been scarcely studied. To understand the VTE metabolism in tomato, we analyzed the gene expression of 47 enzymes involved in the biosynthetic pathway of VTE by a dedicated qRT-PCR array of different tomato tissues. Results showed that whilst most of the genes encoding ezymes of the VTE core-pathway are differentially expressed, only few of the MEP and Shikimate feeding routes showed differences in their expressions.
Silencing of *vte3(1)* in tomato fruits by VIGS resulted in significant increases of ß- tocopherol and reduced levels of Y-tocopherol. On the other hand, *vte4* silencing resulted in reduced levels of - and - tocopherol and a accumulation of g-tocopherol. Moreover, detailed metabolite profiles of the silenced fruits showed that alterations in tocopherol levels correlated with massive increases in the photorespiratory intermediate glycine and the levels of leucine as well as a significant reduction of lipid accumulation. These changes were accompanied by increments in carotenes, lycopene and antioxidant capacity, suggesting that both tocopherol and carotenoid pathways are co-regulated resulting in the regulation of the cellular redox state of the tomato fruits.

PL-P18.

ASR1 IS INVOLVED IN SUGAR SIGNALLING IN TOBACCO PLANTS

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Asr (ABA, stress, ripening) genes belong to the group 7 of LEA proteins. Grape ASR1 protein has been proposed to regulate the transcription of the hexose transporter VvHt. Silenced Asr1 tobacco plants were characterized in detail at vegetative phase. Transgenic lines showed higher glucose levels in leaves, diminished CO_2 assimilation, higher sugar contents in phloem sap, altered total protein pattern and foliar necrosis. This phenotype mimics tobacco leaf senescence. In agreement with this observation, the relative amount of ASR1 protein increases along with leaf age in wild-type plants. These results might indicate that ASR1 is involved in foliar senescence. Moreover, alterations in ABA and gibberellin levels and in sensitivity to GA3 were detected in the silenced lines, probably as a consequence of the interactions between sugar and hormone signalling. Expression analyses of a hexose transporter, NtHt, and a sucrose transporter-like, NtSut2, showed that their levels are reduced. These results suggest that Asr1 is involved in sugar signalling by a mechanism involving the regulation of sugar transporters gene expression and that the same mechanism contributes to modulate foliar senescence.

PL-P19.

EVALUATION OF NUTRACEUTICAL PROPERTIES IN LOCAL TOMATO POPULATIONS

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Tomato is one of the most important source of nutraceutical compounds to the western diet. In this work, we assessed the antioxidant metabolites composition in mature tomatoes from local populations and their biological activity, in order to identify the active components responsible of their nutraceutical properties. Antioxidant metabolites from tomato hydrophilic extracts were determined by HPLC-DAD-MS/MS. *In vitro* antioxidant capacity (AC) was determined by TEAC and FRAP methods. Biological activity was evaluated using a Saccharomyces cerevisiae model exposed to oxidative stress with H_2O_2. Three out of seventeen cultivars showed the highest AC and biological activity. Multivariate analyses showed that chlorogenic acid, vitamin C and rutin contents are strongly associated to AC and biological activity. These activities are dependent also of antioxidant metabolite composition, which showed significant difference between cultivars. Based on these results, we conclude that chlorogenic acid, vitamin C and rutin are the most important bioactive components which contribute to the tomatoes AC. So far, our current results point out the importance of analyzing the entire metabolite profile, in addition to the use of in vivo assays to understand differences in AC of tomatoes, which should be extensive to other food products.

PL-P20.

DECIPHERING AUXIN-REGULATED MECHANISMS DURING PLANT ACCLIMATION TO SALINITY

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The phytohormone auxin regulates gene expression through direct physical interaction with TIR1/AFB receptor proteins, which in turn remove the Aux/IAA family of transcriptional repressors. We have previously demonstrated that the double mutant, *tir1 afb2* displays increased tolerance against salinity. Thus, we hypothesized that down-regulation of auxin signaling could be part of acclimation to salinity in *Arabidopsis thaliana* plants. In this work, we demonstrated that NaCl-mediated salt stress inhibited GUS expression in a dose response manner in the Arabidopsis auxin-signaling reporter line, BA3::GUS. In addition, NaCl triggered repression of TIR1 receptor and stabilization of Aux/IAA repressors in the transgenic lines, TIR1::TIR1-GUS and HS::AXR3NT-GUS respectively, preventing the activation of auxin signaling in salt stressed plants. *TIR1* repression by NaCl was partially compromised in *ago1*-1 mutant suggesting that a miRNA directed pathway may also contribute to down-regulation of TIR1-mediated auxin signaling during salinity. All these evidence allowed us to propose a hypothetical model on the participation of auxin regulatory mechanisms during the adaptative response to salinity in Arabidopsis plants.
Supported by UNMdP, ANPCyT, CONICET.

PL-P21.
EXTRACELLULAR ATP INDUCES ADVENTITIOUS ROOT FORMATION IN CUCUMBER EXPLANTS
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In this study, we have investigated the regulation of adventitious root (AR) formation by extracellular ATP (eATP) in cucumber (*Cucumis sativus*) explants. Cutting seedlings responded in a dose-dependent manner to eATP as evidenced by a higher number and length of ARs compared with controls. The eATP-induced AR formation was mimicked by a very low dose (20.0 pM) of the non-hydrolysable ATP analogue, S- ATP. Adenosine, as other signal molecule, modulated S- ATP-mediated AR formation at concentrations that did not produce a significant response when applied separately to the cutting seedlings. Furthermore, the antagonist of purinogenic receptor, PPADS partially blocked the eATP-mediated response. In addition, the scavenger of nitric oxide (NO), cPTIO inhibited the eATP-mediated signaling indicating that endogenous NO may act downstream of eATP signal during AR formation in cucumber seedlings. In conclusion, these findings allowed us to suggest that an eATP-mediated perception and downstream signaling pathway may regulate AR formation in cucumber explants. In addition, adenosine can modulate eATP signaling by a fine-tuning mechanism. Thus, eATP appears as a novel component of the molecular mechanisms controlling AR formation.
Supported by UNMdP, ANPCyT, CONICET.

PL-P22.
RAPID MAIZE LEAF AND IMMATURE EAR RESPONSES TO UV-B RADIATION
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Plants have evolved adaptations to environmental factors, including solar radiation. In addition to acting as a developmental and physiological signal, UV-B photons also cause cellular damage. Elevated UV-B radiation has pleiotropic effects on plant development, morphology, and physiology, but the regulation of systemic responses is not well-understood. To gain a better understanding of the initial events in UV-B acclimation, we have analyzed a 10min to 1h time course of transcriptome responses in irradiated and shielded leaves, and immature maize ears to unravel the systemic physiological and developmental responses in exposed and shielded organs. To identify metabolites as possible signaling molecules, we looked for compounds that increased within 5-90 min in both irradiated and shielded leaves, to explain the kinetics of profound transcript changes within 1h. We found that myoinositol is one such candidate metabolite, and it also has support from RNA profiling: after 1h UV-B, transcripts for myoinositol-1-phosphate synthase, are decreased in both irradiated and shielded leaves suggesting down-regulation of biogenesis. We also demonstrate that if 0.1mM myoinositol is applied to leaves of greenhouse maize, some metabolites that are changed by UV-B are also changed similarly by the chemical treatment. Therefore, this metabolite can partially mimic UV irradiation.

PL-P23.
SCREENING OF GERMINATION INHIBITORS FROM PLANT EXTRACTS
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Several plant species release germination and/or growth inhibitors as a strategy to colonize and invade grounds occupied by other plants. As a result, these plants populate successfully the bare ground or displace other native plant communities. It is known that the production of these substances increases if plants grow in a harsh environment under stressful conditions. We have collected plant species that exhibited an invasive behavior in regions of Argentina with hostile geography and harsh environments. We have also harvested those plants popularly known for their medicinal use. Using parts and soil of these plants, aqueous extracts were obtained (8g material/70mL water). These extracts were tested for their inhibition activity in germination assays of lettuce. Extracts that produced inhibition were then tested on the germination of seeds of agronomic interest. 804 extracts were prepared and 184 of them inhibited lettuce germination at 100%: 97 were derived from Chaco and Santiago del Estero plants, 32 from Cordoba and San Luis and 53 from medicinal species. Regarding the distribution of extracts with inhibition activity, most of them were obtained from leaves (52%), followed by stems (14%) and flowers (12%). Among the extracts used in the germination of agriculturally important species, 24 were noted for their selectivity or aggressiveness in general.

PL-P24.
STUDY OF THE INTERACTION OF ADAPTOR PROTEINS WITH HSP100 CHAPERONES FROM *Arabidopsis thaliana*
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The chloroplastic proteolytic system ClpPR from *Arabidopsis thaliana* participates in the organelle protein quality control. It is composed by proteins ClpP1/3-6 and ClpR1-4, which self-assemble to delimitate a proteolytic chamber. Substrate presentation to the cavity is carried out by the Hsp100 chaperones ClpC1/2 and ClpD. They are in charge of selecting and unfolding proteins destined for degradation and translocate them into the chamber. Three recently discovered proteins, ClpT1/2 and ClpS, are thought to be adaptors and/or regulators of the Clp system. ClpT1/2 may regulate the binding of the Hsp100 chaperones to the ClpPR core and may aid in the assembly of the ClpPR complex. ClpS is proposed to modulate ClpC1/2 substrate selection and affinity, thus expanding the range of eligible targets. However, there is little *in vitro* evidence to support these functions. We studied the interaction of recombinant ClpT1/2 and ClpS with ClpC2 and ClpD by fast ultrafiltration analysis. The adaptor proteins were found to associate with the chaperones in the presence of ATP. Their oligomerization status was also determined. In addition, we have investigated the influence of ClpT1 in the binding affinity of ClpC2 to one of its substrates, the transit peptide of ferredoxin-NADP+ reductase. Our results provide new insights for understanding the regulation of protein homeostasis in chloroplast.

PL-P25.

EFFECT OF 1-MCP ON THE EXPRESSION OF CHLOROPHYLL DEGRADING GENES DURING SENESCENCE OF BROCCOLI

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Plant senescence involves several events that occur at the final stages of development, or are initiated in response to stress. The loss of green colour caused by chlorophyll degradation is the most characteristic symptom. Broccoli (*Brassica oleracea*) belongs to the family of Cruciferae. It is harvested when inflorescences are immature, which causes considerable stress and triggers the senescence. In this work, we studied by RT-PCR the effect of 1-MCP, an ethylene inhibitor agent, on the expression of genes associated to chlorophyll degradation pathway: Chlorophyllase 1 (BoCHL1), Chlorophyllase 2 (BoCHL2), Pheophytinase (BoPPH) and Pheophorbide a oxigenase (BoPaO). Broccoli heads were treated with 1-MCP and stored in dark at 22ºC for five days. The relative expression of BoCLH1 decreased continuously during senescence in both control and treated samples. In the case of BoCLH2, the expression increased after 72 h and then diminished after 120 h in controls, whereas in 1-MCP treated samples the expression was higher than in controls at 72 h without decreasing afterwards. The relative expression of BoPPH increased after 72 h and then diminished in controls. The treatment with 1-MCP induced the peak of BoPPH expression after 120 h. The expression of BoPaO was similar to that of BoPPH and BoCHL2 in controls. The treatment did not modify this pattern but the relative expression was lower.

PL-P26.

INFLUENCE OF THE CARBOHYDRATE-BINDING MODULE OF STRAWBERRY EXPANSIN2 ON *Arabidopsis thaliana* CELL WALL

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Expansins are proteins involved in plant cell wall modifications. It has been described three domains in expansins, a signal peptide which directs the protein to the secretory pathway, a central glycosyl hydrolase domain, and a C-terminal domain considered as a carbohydrate-binding module (CBM). The CBM would be anchoring the expansin to the cellulose surface, and the central domain would interact with hemicelluloses. In the present study, we have targeted the expression of the strawberry expansin2 CBM (CBM-FAEXP2) to *A. thaliana* cell walls, to test the hypothesis that the over-expression of a expansin CBM would bind cell wall sites and then interfere with cell wall degrading enzymes. *A. thaliana* plants were transformed and three homozygous lines were selected. An increase in the total amount of cell wall was observed, mainly due to an increase of pectin fraction, while cellulose and hemicelluloses fractions were not affected. Regarding the enzyme activities, there was a significant decrease in polygalacturonase, ß-galactosidase and xylosidase activities in transgenic lines. A tensile analysis of the shoot showed that transgenic lines were more extensible than wild type. Our results support the hypothesis that CBM-FAEXP2 over-expression would block the action of hydrolytic enzymes, leading to lower pectin degradation and then producing a more extensible cell wall.

PL-P27.

ISOFORMS OF GLUTAMINE SYNTHETASE DURING BARLEY FLAG LEAF SENESCENCE UNDER FIELD CONDITIONS

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In senescing leaves, a large amount of ammonium is produced as a result of protein hydrolysis. Most of the ammonium is re-assimilated into amino acids for export from the senescing leaf, whereas a minor part is evaporated as ammonia from the leaf canopy. Glutamine synthetase (GS) is a key enzyme involved in this process. According to their subcellular localization cytosolic GS (GS1) and chloroplastic GS (GS2) are distinguished. While GS2 is encoded by one gene, GS1 is encoded by 3–5 genes depending on the species. The roles of the different isoforms of GS1 are still unresolved. During leaf senescence cytosolic GS1 fulfills a key function in the assimilation and recycling of ammonia. This role is particularly important during grain development and filling in cereals, when nitrogen is remobilised. We analysed ammonium acumulation in barley flag leaf senescence under two N fertilization conditions. The total chlorophyll and protein content decreased, while ammonium content increased during senescence. A significant decrease of GS activity was observed. Symptoms of senescence appeared latter in plants grown with high N. We have also investigated the expression of senescence associated GS genes by real-time PCR. We have identified at least three GS1 barley genes; one of them increased during senescence and was down-regulated by N.

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PL-P28.

MOLECULAR BASES OF THE POST-ZYGOTIC BARRIERS IN INTERSPECIFIC CROSSES BETWEEN WILD POTATO SPECIES

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To investigate the molecular bases of post-zygotic hybridization barriers in tuber-bearing Solanums, two wild potato species, *Solanum commersonii* and *S. acaule* were crossed in intra- and interspecific genotypic combinations, and the transcriptome of inmature seeds were analyzed by using the cDNA-AFLP technique. From a total of 423 analyzed cDNA fragments, 107 were differentially regulated between the intra and interspecific combinations. Twenty one fragments were sequenced and five of the cDNA-AFLP differential patterns were validated using RT-PCR analysis. One of the selected fragments was used as probe for *in situ* hybridization experiments. Finally, the amount of DNA in endosperm nuclei of seeds was estimated based on the intensity of the Hoechst staining in relation to the ploidy of the endosperm, using ImageJ. Sequence analysis suggested a role for the differentially expressed sequences in the vesicle transport, cytokinesis, cell cycle and others. In-situ hybridization experiments with a fragment encoding a vesicle transport protein revealed expression of this gene in embryo and endosperm. The amount of DNA was 1.5 times greater in interspecific cross compared to intraspecific cross.

The results suggest and support the fact that the collapse of the embryo and endosperm in interespecific crosses may be related to the alteration in the vesicle transport machinery and cytokinesis.

PL-P29.

MALATE IN TOMATO: NOVEL BIOCHEMICAL FOR UNREVEALED FUNCTIONS?

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The C4 acid malate is present at high levels in leaves and fruits of tomato plants being implicated in numerous physiological processes. However, the current understanding of malate metabolism in tomato is still limited. Tomato genome contains seven genes encoding NAD(P)-malic enzymes (NAD(P)-ME); three are supposed to be cytosolic (NADP-ME1 to 3), two are predicted to be mitochondrial (NAD-MEm1 and -2) and two plastidic (NADP-MEpl1 and 2). This ME composition implies that tomato possesses an additional plastidic isoform in comparison to all C3 species characterized so far. Expression analysis based on qRT-PCR revealed that all ME are expressed with different pattern of expression in organs as well as during fruit development. In this regard, whilst both *NAD-MEm* transcripts are more abundant in green fruit stages, *NADP-ME1* and *NADP-MEpl2* are highly expressed during green-red transition. Enzymatic activity determination and western blot assays confirm these differences in the ME expression during fruit maturation. On the other hand, *NADP-MEpl1*, but mostly *-2*, transcripts are present at high levels in cells surrounding vascular tissue of leaves. Moreover, their abundance in leaf increases with the development of the fruit. Finally, the kinetics and regulatory properties showed by recombinant forms of these proteins suggest divergent functions of the two plastidic NADP-MEs in tomato

PL-P30.

METABOLIC AND PHENOTYPIC CHARACTERIZATION OF PEACH VARIETIES FROM INTA SAN PEDRO

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Fruits are a major source of micronutrients and phytonutrients in human diet. Among fruits, rosaceous fruit displays a great variety of flavours and textures, as well as high-content of metabolites exhibiting nutrition-related health properties. Peach [*Prunus persica* (L.) Batsch] is one of the most economically important rosaceous fruit that has become a model plant in genomic studies of fruit trees. In the present work, 10 varieties from INTA San Pedro, were selected for phenotypic and metabolic characterization. The varieties were selected based on their different origins and divided in early-, mid- and late-season varieties. Productivity and flowering time were compared among them. Fruits from the 10-varieties were harvested at physiological maturity (50N of firmness) and allowed to ripen at 20ºC until optimal firmness for consuming. Samples for GC–MS-based metabolic profiling were taken at harvest and after ripening. The ripening process, colour and soluble solid content were also compared among the varieties. Although these peach varieties are genetically similar, they are highly variable in fruit quality and postharvest performance. Therefore, their metabolic profile during the ripening process will be a helpful tool for the identification of metabolites involved in different traits and for the analysis of the processes related to fruit nutrient content, ripening and softening

PL-P31.

ALTERED OSMOTIC STRESS RESPONSE IN *Arabidopsis thaliana* LINES WITH MODIFIED LEVELS OF MALIC ENZYME

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A. thaliana is an organic acid hyperaccumulator plant species. Among the enzymes that metabolize these compounds one of the most important is malic enzyme (ME), which is present as a multigene family in this model species. In the present work, mutant and over-expressing lines in cytosolic AtNADP-ME2, the isoform that mostly contributes to the ME activity in all organs of the plant, were characterized. Despite the variations in the levels of ME activity, all genetically modified lines exhibited a normal vegetative and reproductive development under typical growth conditions. However, after exposure to different stress conditions that modify the water potential, the plants with increased ME activity were significantly more sensitive to PEG treatment. This differential response was not observed in the treatments of drought stress or high salt concentrations. This over-expressing line presented a higher NAD(P)/NAD(P)H ratio respect to the wild-type and a lower pyridine nucleotide total content, specially at the end of the night. This imbalance in redox status caused by increased ME2 levels could have a deep impact on the defense responses to stress. Thus, ME2 participates in the cellular redox homeostasis, contributing to oxidation and/or reduction of these compounds, given the reversibility of the reaction it catalyzes

PL-P32.

ANALYSIS OF NADP-MALIC ENZYME FROM PEACH DURING DEVELOPMENT, RIPENING AND AFTER A HEAT TREATMENT

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The predominant organic acids in peach fruit are malic and citric acids, which are important attributes of the fruit flavor and whose proportions vary depending on the cultivar. Malic enzymes (MEs) catalyze the oxidative decarboxylation of L-malate, producing pyruvate, CO_2, and NADPH in the presence of a divalent cation. This enzyme is widely distributed in nature due to the participation of the reaction products in a large number of metabolic pathways. The aim of this study was to characterize the different isoforms of NADP-ME from *Prunus persica*. Three different enzymes were identified. *In silico* studies indicated that two of them might be cytosolic (EMP1: ppa003214; EMP3: ppa003210) and EMP2: ppa002714 would be targeted to plastids. Real time RT-PCR studies and activity assays showed that NADP-MEs from peach are differentially expressed during development and ripening of the fruit. On the other hand, the effect on MEs of a postharvest heat treatment (HT, 39ºC for 3 days, which was proved to be effective in protecting peach fruit against chilling injury) was also analyzed, showing that the expression and activity of MEs was diminished as a consequence of the HT. Additionally, EMP1, was expressed in *Escherichia coli*, purified and its kinetic parameters analyzed, displaying particular properties in relation to other already characterized Mes

PL-P33.

VIRAL GENE SILENCING BY AN ARTIFICIAL TRANS-ACTING SMALL INTERFERING RNA IN PLANTS

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Trans-acting small interfering RNAs (ta-siRNA) are a special class of siRNAs that occurs endogenously in higher plants, with a similar mode of action as microRNAs, performing cleavage in trans of mRNA targets with complementary sequences. TAS1c is a long non-coding transcript with a miR173 target site in *Arabidopsis*. When miR173 recognizes this site, the TAS1c RNA is cleaved, protected by SGS3, converted into dsRNA by RDR6 and followed by a unique phased processing by DCL4 into 21nt tasi-RNAs.
In this work we designed a synthetic artificial RNA based on the TAS1c gene, replacing the ta-siRNAs encoded with sequences of a conserved region of the Nucleocapsid gene of TSWV, a *Tospovirus* which affects several crops. This construct would eventually be targeted by miR173, which we cloned in a different cassette in this construct, recruit SGS3, RDR6 and DCL4, to be phased processed and form ta-siRNA targeting TSWV.
In *Agrobacterium* transient assays conducted in *N. benthamiana* were this engineered TAS1c locus was co-delivered with fusion protein constructs of GFP:TSWV-N gene and two viral related species, total and consistent gene silencing was observed and confirmed by confocal microscopy, qReal Time PCR, and DAS-ELISA.
This is the first report of the application of this technology to target a viral gene; the implications of this novel system in transgenic virus resistance are discussed.

PL-P34.

O-GLYCOSYLATED CELL WALL PROTEINS ARE ESSENTIAL IN ROOT HAIR GROWTH

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Plant cell walls contain abundant hydroxyproline glycoproteins, which include Extensins (EXTs) and Arabinogalactan-proteins (AGPs). These proteins undergo posttranslational modifications such as firstly the addition of an hidroxyl group on proline residues, carried out by Prolyl 4-hydroxylases (P4Hs) and secondly, the addition of arabinoses and/or galactoses, done by glycosyltransferases, finally these modified proteins form a covalent network. We focused on root hairs were we found 3 P4Hs mutants with distinct short root hair phenotype: p4h2, p4h5 and p4h13. Through GFP-tagged P4Hs we were able to determine an ER and partially Golgi apparatus sub-cellular localization. This same root hair phenotype was mimicked when roots were treated with either ethyl-3,4-dihydroxybenzoate (binds to the active site of P4Hs), or , -dipyridyl (chelates the cofactor Fe2+). The Wt phenotype in these p4hs mutants was restored with P4Hs driven by either their own promoter or the strong 35SCaMV promoter. The p4h2-p4h5 double mutant had similar root hairs than that of the single mutant p4h5, suggesting a functional overlap. On the contrary, the double mutant p4h2-p4h13 had much shorter root hairs suggesting differences in specificity. We also identified several EXTs mutants with short root hair phenotype. Our results help to better understand the importance of O-glycosylation in polarized growth.

PL-P35.

PROLINE HYDROXYLATION AND O-GLYCOSYLATION IN PLANT GLYCOPROTEINS IS REQUIRED FOR CELL EXPANSION

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The hypocotyls elongation exclusively depends of cell expansion, which is promoted by the turgor force that causes changes in the primary cell wall extensibility. The plant primary cell wall consists on a network of cellulose microfibrils, xyloglucans, pectins, and hydroxyproline-rich glycoproteins (HRGPs). Some proline units are converted to 4-hydroxyproline in HRGPs by the action of prolyl 4-hydroxylase (P4Hs). In order to study in detail how plant cells expand, we choose to work with dark grown hypocotyls that have a 100-fold enhanced cell expansion when compared with other plant cell types. We have identified T-DNA P4Hs and HRGPs mutant lines that have a deficient hypocotyls elongation when compared to Wt-Columbia 0 (Wt Col-0). In addition, we have obtained the same deficient phenotype in Wt-Col 0 treated with P4Hs chemical inhibitor (2.2 '-dipyridyl), suggesting that this deficient phenotype is a consequence of the lack proline conversion to 4-hydroxyproline in HRGPs. Finally, to find new genes associated to O-glycosylation and cell expansion, we are performing a hypocotyl phenotype screening using an Activation Tagging (AT) library in the presence of P4Hs chemical inhibitors. Based on all these results, it becoming clear that proline hydroxylation and correct O-glycosylation on cell wall associated AGPs seems to be crucial for hypocotyls cell expansion.

PL-P36.

EXPRESSION ANALYSIS OF GENES POTENTIALLY INVOLVED IN INDUCED RESISTANCE MEDIATED BY PHOSPHITE IN POTATO

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Phosphite (Phi) compounds have the ability to protect plants against different pathogens. Treatments with Phi could be used in an integrated crop management program as an alternative that would reduce the use of fungicides during the crop cycle. We have shown through microarray analysis, that in potato sprouts treated with potassium phophite (KPhi), 26 genes were upregulated compared with non treated ones. Within those we have selected 3 genes (Cullin1, ERD15 & BCYA) known to be involved in induced resistance (IR) and we validated them by qRT PCR. The aim of this work was to analyse their expression in leaves treated or not with KPhi, infected or not with *Phytophtora infestans (Pi)*. Semi quantitative RT PCR showed that the 3 genes were expressed only in KPhi treated leaves. To analyze if *Pi* infection acts sinergically with KPhi, we quantify their expression 24 and 48 h after infection, in KPhi treated or non treated leaves. After 24 h, BCYA and Cullin 1 increased their expression in infected leaves, with respect to the non infected ones, however KPhi treatment did not act sinergically, moreover it was reduced for Cullin 1. ERD15 expression did not change with respect to the non infected leaves, but it increased when KPhi was applied. Their expression decreased after 48 h treatments. These results could help to understand the battery of responses involved in IR mediated by KPhi

PL-P37.
EFFECT OF WATER DEFICIT ON WHEAT SEEDLINGS: OSMOTIC ADJUSTMENT AND GROWTH REDUCTION
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Plants are often exposed to environmental stress challenges. In this sense, plant growth reduction is the main visible symptom of abiotic stress. The aim of the present study was to examine how water deficit modified the proliferation and expansion of root cells in wheat, a C3 species that usually grows in temperate climate regions, including drought prone-environment. Polyethylene glycol (PEG6000) was used to adjust the osmotic potential in the culture system. A decrease in the growth rate was observed with the increase in PEG concentrations, and from 38% PEG in the solution inhibited seed germination. After 3 days, coleoptile and root length were reduced by 53% and 35%, respectively, by 22% PEG, respect to controls. Seedlings treated with 22% PEG showed an osmotic adjustment, measured as an increase in tissue total conductance, osmolarity, proline and protein content. Evans blue staining, relative osmolarity and conductance showed a conserved integrity of the plasma membrane in treated seedlings, without evidence of cell death. Despite cell length in root apical meristem was similar in C and treated seedlings, a different transcript accumulation of genes involved in cell cycle (RDR, PCNA, MCM) and cell expansion (TaEXPB3, TaEXPB5) were observed. More results are necessary to understand the relationship between drought-induced osmotic adjustment and growth in wheat seedlings.

PL-P38.
S63-RNASE IS A NOVEL NON-FUNCTIONAL S-RNASE INDUCED BY PHOSPHATE STARVATION IN *Nicotiana*
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T2 ribonucleases (RNases) are secretory enzymes that catalyze the cleavage of single-strand RNA. In plants T2 RNases have different structural traits and biological roles and were classified in two subfamilies, a) S-RNases, involved in pollen recognition and rejection in plants with S-RNase-based self-incompatibility (SI) system and b) S-like-RNases, widely distributed in the plant kingdom and generally involved in the response to different biotic and abiotic stress scenarios.
We have cloned a style cDNA from *Nicotiana alata* that exhibited the typical sequence features of an *S-RNase* gene, but it was non-functional in the SI system and therefore, it was denoted as non-functional *S63-RNase*. Surprisingly, this gene was induced when plants were hydroponically grown without Pi, a typical response of the *S-like-RNases*, but not reported yet in *S-RNases* or non-functional *S-RNases*. The induction was detected 7 days after Pi deprivation and increased until day 22, roughly paralleling the induction of a well-characterized *S-like-RNase* of *Nicotiana alata*. A 15 amino acid peptide of S63-RNase was used to develop a rabbit antibody. The antibody recognized a protein of ~31 kDa in crude extracts of roots coming from plants grown under Pi starvation conditions only. We are now identifying the 31 kDa protein. So far, no function has been previously assigned to non-S-RNases.

PL-P39.
CHARACTERIZATION OF THE INTERACTION OF GAPC AND SINAL7 IN PLANTS
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The glycolytic enzyme Glyceraldehyde-3P dehydrogenase (GAPC), has been characterized in a wide range of organisms. This enzyme was found in the nucleus, cytoplasm and associated to the mitochondrial external membrane in plants. It has been postulated that GAPC may have an important role in apoptosis, gene transcription in neurons and DNA reparation. However, little is known about the possible functions of this protein in addition to its participation in glycolysis, mainly in higher plants. It was reported that human and murine GAPC binds a E3-ubiquitin-ligase and translocated to the nucleus. Besides, it has been informed that GAPC interacts with Siah1, an E3-ubiquitin-ligase by two hybrid assay. We recently identified SINA-L7, a plant homolog of Siah1. We found that SINA-L7 is induced in GAPC-1 knock out plants. Bioinformatic analysis predicts the existence of a RING finger domain in SINA-L7 and preliminary results showed that SINA-L7 has a E3 ubiquitin ligase activity. In addition, far western blotting experiments showed that SINA-L7 physically interacts with both GAPC isoforms from *A. thaliana*. Our results suggest that GAPC isoforms and SINA-L7 may participate in a retrograde signaling mechanism between the nucleus and mitochondria in plant cells

PL-P40.
AMINOACID RESIDUES IN SBD INVOLVED IN THE MODULATION OF THE ACTIVITY OF STARCH SYNTHASE III
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Starch synthase III (SSIII) from *Arabidopsis thaliana* contains an N-terminus region, including three in-tandem starch-binding domains (SBD, named D123), followed by a C-terminal catalytic domain (CD). We reported that SBDs are involved in the regulation of SSIII function and recently, we demonstrated protein interaction between both domains, showing that the amino acids in 316-344 and 495-535 regions in D2 and D3 domains respectively, but not the individual SBDs, are involved in interaction with CD. We performed bioinformatic analysis, alanine-scanning assays, pull down and enzyme activity measurements in order to determine which residues of the D23 region are involved in the interaction with the CD. We generated different Ala-modified D23 proteins by SDM between aminoacids 316-344, and evaluated the interaction with the CD using pull down assays. Results showed that S328-E330 and N338-W340 residues lost the ability to interact with the CD. When these residues were changed by Ala in the full length SSIII protein, the kinetic parameters were similar to that obtained for the D3-CD truncated enzyme. The results presented here indicate that the interaction of the N-terminal SBDs, particularly the 316-344 and 495-535 loop regions, with the catalytic domains, are important in the modulation of SSIII activity, and suggest that the S328-E330 and N338-W340 residues are involved in this process

PL-P41.
NEW INSIGHTS INTO THE ACTION MECHANISMS OF GENES FROM THE OXIDATION-RESISTANCE FAMILY IN PLANTS
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We studied the molecular mechanisms of action of two genes (AtOXR2 and AtOXR4) belonging to the eukaryotic oxidation resistance (OXR) family from *Arabidopsis thaliana*. Proteins from this family are implicated in protection to oxidative stress in animals and fungi. Using GFP fusions we demonstrated a nuclear and cytoplasmic localization for AtOXR2 and AtOXR4, respectively. To assess the function of these proteins in *Arabidopsis*, we studied T-DNA insertional mutants in both genes. Under normal growth conditions, AtOXR4 knockout plants showed no phenotypic differences with wild-type. In contrast, AtOXR2 mutant plants had reduced biomass, accelerated water loss and differences in photosynthetic responses under high irradiance conditions. Plants overexpressing AtOXR2 showed opposite characteristics and had higher expression levels of APX1, ASO, CAT3 and other genes related with defense against oxidative stress. In addition, AtOXR2 improved the capacity of yeast cells mutant in the ScOXR gene to grow in the presence of H_2O_2. According to our results, we propose that members of the OXR family have different roles that may in part be related to their subcellular localization and the interaction with other proteins. Particularly for AtOXR2, the pleitropic phenotypes and the nuclear localization of this protein suggest its participation as a modulator of developmental events in plants.

PL-P42.
THE ARABIDOPSIS TCP TRANSCRIPTION FACTOR ATTCP16 REGULATES THE EXPRESSION OF MERISTEM-SPECIFIC GENES
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TCP proteins constitute a family of transcription factors found only in plants and are generally involved in the regulation of cell growth and proliferation. In order to analyze the role of the class I TCP transcription factor AtTCP16, we transformed *Arabidopsis* plants with a construct that expresses this protein fused to the EAR repressor domain under the control of the 35SCaMV promoter. Lines expressing AtTCP16-EAR showed altered development of leaves and flowers. In addition, about 30% of the lines developed adventitious meristems (AM) on the adaxial surface of cotyledons. These AM expressed meristem-specific genes like STM, KNAT1 and CLV3, and promoted the formation of rosettes, inflorescences and seeds. Expression of TCP16-EAR in an stm mutant background diminished the appearance of AM to 7% of the lines, demonstrating that STM ectopic activation is necessary for AM formation. These observations suggest that AtTCP16 is involved in the fine regulation of the spatio-temporal control of meristem formation through the regulation of meristem-specific genes.

PL-P43.
MITOCHONDRIAL COPPER CHAPERONES COX17 AND COX 19: ROLES BEYOND COX BIOGENESIS
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Copper is an essential factor for cytochrome c oxidase (COX) activity. Copper is also required for other cellular functions and is highly toxic when present in excess due to its redox properties. In this work, we studied the function of Arabidopsis COX17-1, COX17-2 and COX19, homologues of yeast copper chaperones involved in COX assembly, using artificial miRNAs to silence their expression in plants. COX2 levels and total dark respiration were similar to wild-type in silenced plants, that did not show morphological or developmental alterations when grown under control conditions but were more sensitive to high salt concentrations and high light. Enhanced stress sensitivity was accompanied by accumulation of reactive oxygen species, higher lipid peroxidation, altered expression of stress-responsive genes, including some of the alternative respiratory pathway (AOX1a and NDB2), and lower transcript levels of antioxidant enzymes (CAT, APX, ASO). In addition, copper content was increased in these plants, that also showed repression of the copper-responsive miR398 and induction of Cu/ZnSOD. Thus, diminished levels of COX17-1, COX17-2 and COX19 are associated with altered copper homeostasis and increased oxidative stress without pleiotropic effects on respiration. These results suggest the existence of a link between mitochondrial function and the response to environmental cues in plants.

PL-P44.
ATCOX10, IMPLICATED IN HEME A BIOSYNTHESIS FOR COX ASSEMBLY, IS ESSENTIAL FOR PLANT EMBRYOGENESIS
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Cytochrome c oxidase (COX) biogenesis requires more than 20 accessory proteins implicated, among other processes, in copper and heme a insertion. COX10, a farnesyltransferase that catalyzes the conversion of heme b to heme o, is the limiting factor in heme a biosynthesis and would be essential for its insertion in the COX1 subunit in yeast. In order to elucidate the function of COX10 in plants, we have studied Arabidopsis mutant plants with a T-DNA insertion in the coding region of the AtCOX10 gene. It was not possible to obtain AtCOX10 knockout plants and segregation analysis of heterozygote mutants suggested that AtCOX10 function is required during embryogenesis. Siliques of these plants contained 25% abnormal seeds with embryos arrested at early stages of development. In addition, heterozygote mutants entered senescence before wild-type plants, suggesting a function of AtCOX10 during this process. We analyzed the expression of AtCOX10 by using promoter fusions to the GUS reporter. Supporting the hypothesis of a coordinated expression of genes required for COX biogenesis, we observed high expression of the reporter in anthers. As we observed previously for other COX assembly factors, the expression of AtCOX10 is essential during early stages of plant development, indicating the requirement of active COX biogenesis and respiratory activity during embryogenesis.

PL-P45.

A ROLE FOR GEMIN2 IN THE REGULATION OF PRE-mRNA SPLICING AND CIRCADIAN RHYTHMS IN *Arabidopsis*

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Circadian rhythms allow organisms to time biological processes to the most appropriate phases of the day. Defects in PRMT5, which transfers methyl groups to arginine residues present in histones and Sm spliceosomal proteins, impair circadian rhythms in *Arabidopsis*. This phenotype is caused, at least in part, by a strong alteration in alternative splicing of the clock gene PRR9. In order to test if this alteration is due to the non-methylation of Sm proteins we explored the effect of mutations that could disrupt the assembly of Sm proteins in small nuclear ribonucleopreteins (snRNPs) in a similar manner. In vertebrates, assembly of spliceosomal snRNPs is mediated by the SMN complex, composed of the proteins SMN and Gemins2–8. *Arabidopsis* contains orthologs of SMN and Gemin2 only. Interestingly, both of them are essential in animal cells but not in *Arabidopsis*. Mutations in SMN and GEMIN2 caused several developmental defects, including alteration of leaf shape, short petioles and early flowering. In addition, the gemin2 mutants displayed altered photomorphogenic responses and circadian rhythms. Moreover, genome-wide studies show that SMN and GEMIN2 contribute to the regulation of a subset of pre-messenger-RNA splicing events. The circadian phenotype of gemin2 mutants is consistent with observed changes in alternative splicing of the clock gene TOC1.

PL-P46.

ARE SnRK1 PLANT PROTEIN KINASES DISTINCTIVELY REGULATED BY METABOLITES UNDER LIGHT/DARK CONDITIONS?

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SnRK1s are plant protein kinases related to yeast SNF1 and mammalian AMPK. These kinases are hetero-oligomeric proteins of catalytic ( ) and non-catalytic (and) subunits that would have roles in regulation and targeting. SnRK1s play key roles as major integrators of energy signaling, growth, and carbon and nitrogen metabolism. Previous works have shown that SnRK1 activity could be regulated by Glc6P, Tre6P and Rib5P depending on the plant and tissue involved. We wondered if regulation of SnRK1 by different metabolites could be related with the trophic characteristics and/or the metabolic state of the plant tissue. In this work we purified SnRK1 kinases from *Arabidopsis* seedlings grown under light or dark conditions and we characterized its regulation by different metabolites. Rib5P and Rul5P behaved as strong inhibitors of the SnRK1 purified from seedlings grown under light but not under dark conditions. Characterization of SnRK1 inhibition suggests that Rib5P ($I_{0.5}$ ~2 mM) and Rul5P ($I_{0.5}$ ~100 µM) could act as competitive inhibitors respect to ATP. Several lines of evidence suggest that inhibition by Rib5P is not the result of ATP consumption, but the potential for this artifact remains to be unequivocally ruled out. We hypothesize that differential regulation could be related with changes in SnRK1 subunit gene expression patterns of the several coding genes.

PL-P47.

FUNCTIONALLY TESTING THE LOCALIZATION AND OLIGOMERIZATION OF ASR1, A STRESS-INDUCIBLE PROTEIN

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The ASR protein family is present in numerous economically relevant crops but absent in *Arabidopsis*. We explored two independent *in vivo* features of ASR1, one of its members, a small 14-kDa polypeptide, by means of transient expression in *Nicotiana benthamiana*.

1) ASR1 from tomato has a putative nuclear localization signal (NLS). However, it was detected not only in nucleus-enriched fractions but also in cytosolic fractions of tomato leaves and roots. This prompted us to test the functionality of its NLS by fusing ASR1 constructs to either the rather small GFP (green fluorescent protein) or the larger GUS-GFP chimera. Whereas the ASR-GFP fusion proteins readily entered the nucleus, GUS-GFP did not, even when attaching the NLS from ASR1. Therefore, nuclear localization of native ASR1 cannot be attributed to that amino acid tract but rather to simple diffusion. As a control, a proven viral NLS was able to target most of the GUS-GFP fluorescence to the nucleus. We thus conclude that the previously so-called "NLS" of ASR1 is not such.

2) Recombinant ASR1 can form homo-dimers *in vitro*. Its ability for *in vivo* oligomerization was tested by means of Bimolecular Fluorescence Complementation (BiFC) using split non-fluorescent variants of EYFP, each fused to ASR1. Oligomerization was inferred since fluorescence was evident only when both ASR1-fused halves were present at the same time.

PL-P48.

ALTERNATIVE SPLICING REGULATION BY LIGHT/DARK TRANSITIONS IN *Arabidopsis*

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Light is not only a source of energy for green plants, it is also a source of information for the regulation of their growth, development and gene expression. The alternative splicing (AS) process is another layer of gene expression's regulation that also contributes to the adjustment of an organism to its environment. Although, up to 90 % of human genes have at least two AS isoforms, this percentage is about 40 % for *Arabidopsis thaliana*. With the aim of understanding the biological relevance of AS isoforms, as well as deciphering the molecular mechanism regulating this process in plants, we analyzed about 100 AS events with a high-resolution RT-PCR panel and found that ~ 40 % of them respond to light/dark transitions. We chose RSp31 transcriptional unit as a model to go further. Light exposure, increases the proportion of the functional mRNA isoform and a functional chloroplast with active photosynthetic electron transport is needed. Both H2O2 and sucrose mimic the effects of light on RSp31's AS. This "light effect" is also observed in roots, but only in those that have not been dissected from shoots before light exposure, suggesting that the signal is able to travel. Future work will be to evaluate whether this model of light signaling for AS regulation applies for the rest of the light responsive events under study and to gain insight into the signal's nature.

PL-P49.

NITRIC OXIDE AND AUXIN REGULATES ASCORBATE PEROXIDASE 1 ACTIVITY THROUGH S-NITROSYLATION

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Nitric oxide (NO) is a signal molecule that functions in concert with auxin to regulate root developmental processes. One of the mechanisms of NO action is through S-nitrosylation of Cys residues in proteins. The thioredoxin-thioredoxin reductase (Trx-TrxR) system denitrosylates Cys residues and contribute to the regulation of S-nitrosylated proteins. We studied the participation of Trx-TrxR in auxin and NO-mediated root development. The treatment of Arabidopsis plants with the auxin NAA or the NO donor SNP induces TrxR activity in roots. NAA-treated roots present less S-nitrosylated proteins than control, suggesting that NAA induces a denitrosylating activity. Biotin switch and 2D-PAGE were conducted to identify denitrosylated proteins in NAA-treated roots. Ascorbate peroxidase 1 (APX1) was one of the proteins identified as target for S-nitrosylation. APX1 activity is inhibited by NAA treatment and this inhibition could be blocked by the TrxR inhibitor auranofin. APX1 was cloned and expressed in *E. coli*. Bacteria expressing APX1 are more tolerant to H_2O_2 and NaCl. Recombinant APX1 activity is inhibited by NO donors and DTT. It is postulated that a counterbalance effect of NO and TrxR activity is required for a fine-tuning modulation of APX1 activity during auxin signaling in roots.

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PL-P50.

NITRIC OXIDE IS REQUIRED FOR THE BRASSINOSTEROID REGULATION OF ROOT GROWTH AND STOMATAL APERTURE

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Genetic and pharmacological evidence supporting nitric oxide (NO) involvement in brassinosteroids (BR) signaling is presented. *Arabidopsis* seedlings treated with BR 24-epibrassinolide (BL) show increased lateral roots (LR) density, primary root (PR) inhibition and NO accumulation. BL-induced responses were abolished by specific NO scavenger c-PTIO, confirming NO participation in BL signaling. The nitrate reductase (NR) mutant *nia1-2* is partially insensitive to BL and NO synthase (NOS) inhibitor L-NAME addition abolished BL effect over root morphology. The lack of BL response in BR-receptor mutant *bri1-1* is reverted by NO donor GSNO addition, indicating that NO acts downstream BR. The distribution of the auxin efflux carriers PIN1 and PIN2 is affected by BL in a NO-independent pathway, suggesting that NO acts downstream of auxins. NR and NOS-like activities are required for BL-dependent NO accumulation in guard cells and full BL-induced response of stomatal closure. Stomata from *aba3-1* (deficient in ABA synthesis) were insensitive to BL, indicating that ABA is required for BL-induced NO production. This demonstrates that: (i) BL increases the NO concentration in roots and guard cells in a BR receptor, NR- and NOS-like-dependent pathway and (ii) NO is required for BR-induced changes leading to altered root architecture and stomatal closure.

PL-P51.

ASCORBATE AND GLUTATHIONE PROTECTS *Arabidopsis* PLANTS GROWING IN IRON DEFICIENT CONDITIONS

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Iron is an essential micronutrient required for a wide variety of cellular functions in plant growth and development. Chlorosis is the first visible symptom in iron deficient plants. As a consequence, cellular redox homeostasis is disturbed due mainly to the diminished photosynthetic activity. Both ascorbate (Asc) and glutathione (GSH) are multifunctional metabolites that are important in redox balancing. We were interested in studying the effect of Asc and GSH during iron deficiency in *Arabidopsis* plants. Either, Asc or GSH prevented the chlorosis in iron deficient plants without involving changes in hydrogen peroxide content, a ROS suspected to increase during iron deficiency stress. In addition, the content of the iron-sulfur protein ferredoxin 2 (Fd2) and the activity of the heme protein ascorbate peroxidase (APX), were diminished in leaves of iron deficient plants. Interestingly, Asc or GSH treatments were able to preserve the Fd2 levels and APX activity at the same level found in iron sufficient plants. Works are in progress to see if the protection mediated by Asc and GSH is due to improving internal iron availability increasing the cellular labile iron pool.

Supported by CONICET, ANPCyT, UNMdP.

PL-P52.

GENOMIC AND BIOCHEMICAL CHARACTERIZATION OF ANTHOCYANIN PROFILE VARIATION IN MALBEC GRAPEVINE CLONES

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The deep study of the variations observed within grapevine clones, for different phenotypic, agronomic and oenological traits may allow determining the particular potential of those clones in a collection or vineyard. Moreover, that information may lead to the identification of the molecular and genetic basis of the observed variation. In order to differentiate each of the 134 Malbec clones present in the studied collection we determined their anthocyanin profile by means of HPLC analysis. For each clone we determined the concentrations of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Po) and malvidin (Mv) for their glycosylated, acetylated and cumarylated forms: Dp3Gl, Cy3Gl, Pt3Gl, Po3Gl, Mv3Gl, Dp3acGl, Cy3acGl, Pt3acGl, Po3acGl, Mv3acGl, Dp3cumGl, Cy3cumGl, Pt3cumGl, Po3cumGl y Mv3cumGl. Clonal identity of the samples was verified by analyzing nine microsatellite markers. The obtained profiles allowed clustering the clones in 10 contrasted groups. Principal Component Analysis (PCA) showed that variation in Mv and Po concentration explained about 90% of the variation within the clones: Mv 63.5% & Po 25.5%. Based on this information we selected two contrasted groups of clones in order to perform different transcriptomic, genetic and epigenetic analysis in order to determine the molecular mechanisms underlining the phenotypic variation within the clones.

PL-P53.

EFFECT OF SOYBEAN (*G. max*) INTRASPECIFIC COMPETITION ON INDUCED DEFENCES BY *A. Gemmatalis* ATTACK

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Plants respond to insect attack with an increase in the production of toxic compounds which decrease or stop the attacks. Jasmonic acid plays an important role in the induction of defensive responses and its biosynthesis is regulated by the enzyme lipoxigenase (LOX). However, production of defenses against insect attack can be diminished as a consequence of intraspecific competition. Increase in plant density reduce red / far red ratio detected by phytochrome resulting in the promotion of a less capacity of plants to defend theirselves against insect. One of the most anti herbivore defenses studied are proteinse inhibitors. The aim of this work was analyze the effects of intraespecific competition in the production of insect-induced defenses. The commercial soy genotype DM 4670 was sown with three different distancing between plants: 20, 40 and 60 cm. Leafs attacked and no attacked by *Anticarsia gemmatalis* H. were harvested at 24 and 72 hours to study the expression of Lox8 and proteinase inhibitors *N2, L1, R1* y *Bowman-Birk I (BB1)* by RT-PCR. *Lox8, N2, L1, R1* y *BB1* were induced by the attacks of *A. gemmatalis* suggesting that *Lox8* regulates the level of expression of *N2*. It was identified a high rate of responses in plants sown under low density in contrast with the high level of expression observed in plants sown under high density.

PL-P54.

DIRECT DEFENSES IN DEVELOPING SEEDS OF SOYBEAN AGAINST GREEN STINK BUGS (*Nezara viridula*)

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Insect attack increases the production of defenses, which are regulated by the phytohormone jasmonic acid (JA). Southern green stink bug (*Nezara viridula*) is one of the main pests in soybean and affects both yield and seed quality. However, little is known about soybean response to stink bug attack on developing seeds. Developing seeds of soybean contain only lipoxygenases 1, 2, and 3, which probably regulates JA accumulation in these organs. Our aim was to determine the role of JA in the regulation of induced defenses, such as proteinase inhibitors (PI), when soybean seeds are attacked by stink bugs. We used the Williams mutant BRM 926600 genotype, without expression of *lox*1, 2 and 3; and Williams *wt* as a control. Four treatments were applied on seed pods: i) without damage; ii) mechanical damage; iii) damage produced by stink bugs attack and; iv) application of methyl jasmonate (MeJA). The transcriptional expression of *lox* genes and pi were estimated by the RT-PCR reaction. Stink bug attack and mechanical damage induced gene expression of *lox*1, 2 and 3, and pi in control. *Lox*3 expression explained better the variation in the expression of inhibitors than *lox*2 and 3. Although, no expression of either *lox*1, 2, 3 or pi were found in the mutant BRM, application of MeJA recuperated the expression of these genes, suggesting that JA regulate PI en developing seeds of soybeans.

PL-P55.

EXPRESSION OF BASI DURING BARLEY GERMINATION AND ITS RELATION WITH NITROGEN CONTENT

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Plant seeds have a large number of proteinaceous inhibitors which act on -amylase and proteases. Among Kunitz-type inhibitors there is one of endogenous -amylase in barley named BASI (Barley -Amylase Subtilisn Inhibitor). BASI is synthesized during grain filling and is an abundant protein of the mature seed. Thioredoxin *h* (Trx *h*), a ubiquitous protein containing a redox-active disulfide group, reduce storage proteins and inactivate various inhibitor proteins. The aim of this work is to determine the response to nitrogen content in Trx *h* and BASI expression during germination of barley. To this end, an experiment of nitrogen fertilization was conducted in barley. The resulting seeds were germinated and were measured both expression of Trx and BASI. The complete sequence of BASI was sequenced in cDNA synthesized from RNA of 48-hour-germination seeds. It was confirmed that the inhibitor is expressed in germinating seeds. Both Trx and inhibitor were greater in seeds with high nitrogen content than in seeds with lower nitrogen content. The understanding of these biochemical relationships will help to understand the relation between grain composition and the mechanisms of starch degradation during germination.

PL-P56.

OIWA REGULATES ROS HOMEOSTASIS DURING EMBRYO SAC DEVELOPMENT IN *Arabidopsis thaliana*

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Differentiation of gametes is a crucial step in the life cycle of all organisms with sexual reproduction. In the model plant *Arabidopsis thaliana*, the development of the female gametophyte involves three events of programmed cell death whose molecular bases are still poorly understood. In this work we present the study of the gametophytic mutant *oiwa*, which carries an insertion in a gene coding for a mitochondrial Mn-superoxide dismutase (Mn-SOD). While transient, localized oxidative bursts were observed associated with cell death events in WT embryo sacs -followed by a rapid restoration of the redox state-, mutant embryo sacs seem not able to reduce ROS levels after cell death, as observed by the use of specific probes at different stages of development both by confocal and DIC microscopy.

To determine the specific stage of development affected in *oiwa*, a comprehensive phenotypic analysis was performed. Mutant embryo sacs appear to have difficulties attracting pollen tubes, as a high percentage of ovules are not fertilized. Also, in those fertilized embryo sacs, embryogenesis is arrested at a zygotic stage, suggesting a maternal effect.

Our results indicate that tight regulation of ROS homeostasis is crucial for fertilization and embryogenesis and that Mn-SOD plays a critical role in its regulation during female gametophyte development.

PL-P57.

DEVELOPMENT RESPONSES AND TRANSCRIPTOMIC REPROGRAMMING IN *Lotus japonicus* UNDER ALKALINE STRESS

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Plant growth is affected in alkaline soils (associated with high concentrations of carbonates) due to a diminution of nutrient availability. It has been shown that many agronomic important legumes, as some species from the genus Lotus, are able to cope with this stress and grow in affected regions. A thorough understanding of the tolerance determinants is crucial for the generation of more resistant cultivars. However, the mechanisms involved in alkaline tolerance in these species remain still unknown. In order to add more light to our current knowledge on this field, we evaluated the effect of alkalinity in the development and molecular responses of two ecotypes of the model legume *Lotus japonicus*. Interestingly, we found that the ecotype MG-20 was completely tolerant to the stress conditions imposed in our assays, with no evident symptoms and all the plants reaching the reproductive stage. On the other hand, the ecotype Gifu was severely affected by alkalinity leading to leaf chlorosis and high rate of plant death. Global gene expression in leaf and root samples identified several genes with probable roles in stress tolerance, many of them being different transcription factors and protein kinases that might regulate and coordinate cell responses. The importance of these genes and the similarities with previously published reports are discussed.

PL-P58.

ARABIDOPSIS MITOCHONDRIAL ALKALINE/NEUTRAL INVERTASE C IS IMPORTANT FOR GERMINATION AND FLOWERING

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Invertases (Invs) catalyze the irreversible hydrolysis of sucrose into hexoses and play a key role when a demand of carbon and energy occurs. It has become evident that sucrose and its hydrolysis products are important metabolic signals that modulate gene expression and regulate plant development. Alkaline-Neutral invertases (A/N-Invs) have maximum activity at characteristic pHs between 6.5–8.0. Their physiological functions are not clear yet. A/N-Invs are mainly located in the cytosol but recently different subcellular locations were unraveled, although their roles are still unknown. In this work, we focused on the mitochondrial Arabidopsis *At-A/N-InvC*. Transcripts of *At-A/N-InvC* were detected in every tissue analyzed. Its biochemical identity was characterized by expression in *E. coli* cells. To explore its physiological function, *invc* knockout mutant plants were analyzed and compared with recently characterized *inva* mutant plants. Lack of *At-A/N-InvC* expression in homozygous mutant plants was verified by RT-PCR assays. A detailed phenotypic analysis showed that mutant plants present a developmental alteration relative to Col-0 plants, including delayed germination and flowering time, emphasizing the role of mitochondrial sucrose hydrolysis in development processes.

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PL-P59.

MUTANT RHT-1 ALTERED FUNCTION ALLELES INFLUENCE THE RESPONSE OF WHEAT PLANTS TO K^+ DEPRIVATION

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Potassium (K^+) is an essential nutrient for plant growth and development. The pathway by which higher plants restrict their growth under K^+ deprivation conditions is not well understood. In bread wheat (Triticum aestivum), DELLA proteins are encoded by the Rht-1B and Rht-1D genes: the allelic variants Rht-1Ba/Rht-1Da, determine a wild type (WT) phenotype, while the presence of the Rht-B1b /Rht-D1b altered function alleles determine a dwarf phenotype (DD). In this work, we evaluated the role of those alleles during the acclimation of plants to K^+ deprivation by using two set of near isogenic lines (NILs) of Maringa and April Bearded cultivars. Chlorophyll content of WT NILs sharply declined after a 16 d period of K^+-deprivationm while in DD NILs chlorophyll content only marginally declined. This difference was companied in Maringa, but not in April Bearded, by a positive impact on plant growth. In turn, no differences between WT and DD NILs were observed for indicators of oxidative damage oxidative. However, DD NILs of both cultivars exhibited an increased induction of ascorbate peroxidase and superoxide dismutase activities, as well as changes in the ionic composition (K^+, Na^+, Ca^{2+}) of plants grown without K^+-addition. Our data indicate that the acclimation response to K^+-starvation is strongly influenced by the presence of altered function alleles.

PL-P60.

StABF1, A B-ZIP TRANSCRIPTION FACTOR INVOLVED IN ABIOTIC STRESS RESPONSE AND TUBERIZATION

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ABF/AREB bZIP transcription factors mediate plant abiotic stress responses by regulating the expression of stress-related genes. These proteins bind to the abscisic acid (ABA)-responsive element (ABRE), which is the major cis-acting regulatory sequence in ABA-dependent gene expression. We have already cloned and characterized an ABF/AREB transcription factor from potato (*Solanum tuberosum* L), named StABF1, which is able to bind to the ABRE *in vitro*. StABF1 is induced by ABA, osmotic stress and cold, suggesting that it might be a key regulator of ABA-dependent stress response in potato.

Since a stimulatory effect of ABA in tuberization has been reported, we determined if StABF1 expression is regulated during the tuber formation process using a semi-quantitative RT-PCR approach. StABF1 expression increased during tuber development and then decreased in the last stage (S4), when the tuber is already formed. High sucrose:nitrogen ratio (*in vitro* tuber-inducing condition) increased StABF1 mRNA levels in leaves. As ABF/AREB activity is regulated by phosphorylation, in-gel kinase assays were performed to determine the phosphorylation status of StABF1 under tuber-inducing or tuberization inhibitory conditions. Interestingly, changes in phosphorylation were detected. These results suggest that StABF1, besides acting in stress response, might be involved in tuber development.

PL-P61.

BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT CDPK1 FROM *Solanum tuberosum*

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Calcium-dependent protein kinases (CDPKs) are essential calcium sensors in plants. In potato, several CDPK isoforms are present. Particularly, StCDPK1 is expressed during tuber transition and could also participate in hormone transduction pathways. In this work, the biochemical characterization of the recombinant polyhistidine-tagged StCDPK1 (StCDPK1::Hisx6) is presented. Enzymatic activity was assayed using a synthetic peptide (Syntide-2) as substrate. CDPKs typical phosphorylation of histone H1 was also observed. The enzymatic activity was enhanced ten fold in the presence of 1 µM calcium and was inhibited by the calmodulin inhibitor chlorpromazine (CPZ) and the kinase inhibitor staurosporine (ST). The kinetic parameters differed significantly from those exhibited by other isoforms. This is consistent with the fact that CDPKs are involved in various signal transduction pathways and are differentially expressed among tissues. The recombinant enzyme was capable of calcium-dependent autophosphorylation. This post-translational modification is broadly used by CDPKs as a means of modulating their activity. In silico analysis revealed several phosphorylation sites in the protein sequence. In an attempt to determine the functional autophosphorylation sites and understand the enzyme modulation, 2D PAGE analysis of StCDPK1 is being performed.

PL-P62.

ANALYSIS OF POTATO TRANSGENIC LINES CONTAINING THE StCDPK3 PROMOTER FUSED TO THE REPORTER GENE GUS

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Ca^{2+} sensors function to regulate diverse downstream targets leading to a stimulus-appropriate shift in physiology or developmental patterning. Calcium dependent protein kinases (CDPKs) are important components in plant signaling, capable of directly transducing a signal via catalytic activity. In potato, StCDPK1 is expressed in tuberizing potato stolons and in sprouting tubers (Gargantini *et al.*, 2009), while StCDPK2 is highly expressed in leaves and sprouts (Giammaria *et al.*, 2010). In an attempt to study a third isoform and its expression pattern, transgenic lines were generated carrying the StCDPK3 promoter region fused to a GUS reporter gene. Fourteen shoots were obtained that were able to grow and root in kanamycin selection media. Four of them presented GUS activity in a fluorometric assay but only two stained positively for GUS. The expression pattern was analyzed using *in vitro* potato plants and minitubers. The promoter was active in leaves, roots, early stages of tuber formation, in minitubers eyes and in the tip of minituber sprouts during primordial leaf development. Our results show that StCDPK3 could play a role in underground tissues (roots) and in aerial photosynthetic tissues (leaves and shoots). The fact that the promoter is active in minitubers eyes and sprouts is indicative of a role in developmental stages, leading to plant growth and survival.

PL-P63.

DEFINIG THE FLAVOUR OF TOMATO FRUITS FROM METABOLITE PROFILES AND SENSORY ANALYSES

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Most of the commercial cultivated tomatoes in Argentina are imported hybrids. They have been mainly selected for long shelf life and pests resistances, but taste and flavor are not well accepted by consumers. Tomato populations cultivated by peasants from Argentine Andean Valleys were analyzed in this work. The goal is to contribute to incorporate these varieties with better consumer preferences to the local and regional breeding programs. Populations were screened for their agronomical and biochemical traits by ^{1}H-NMR and GC-MS and in parallel by semi-trained panels. Primary and secondary metabolites contents and agronomical attributes were analyzed by a neuronal network approach which allowed discrimination of a discrete number of cultivars which grouped into few neurons from others interspersed into many neurons exposing the environmental variance affecting the measured characters. Moreover, hidden novel relations between the analyzed traits were found, for example, one variety negatively evaluated by sensory panels shows highly significant levels of pyruvic acid, aspartate, benzoate, 1,4-pentadiene and hexanal. On the other hand, it shows a significant low amount of terpinolene, linalool and camphor, all volatiles that contribute to accepted flavor. Overall, these data indicate that metabolic profiles could be used as good indicators of the flavor properties of tomato fruits.

PL-P64.

SILENCING OF MITOCHONDRIAL GAMA-CAS USING SMALL INTERFERENCE RNA AND ARTIFICIAL MICRORNAS

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The gene family of putative gamma-class Carbonic Anhydrases (CA) in Arabidopsis genome includes five members. Three of these genes (CA1, CA2 and CA3), share high homology with the highly characterized gamma-CAs from the archaea M. thermophila, and two CA-Like genes (CAL1 and CAL2) showing a minor degree of homology. CA and CAL proteins are located into mitochondria and bonded to mitochondrial Complex I, forming an extra domain exclusive of photosynthetic organisms. However, the physiological function of this plant specific extra domain is largely unknown. Single mutants do not show a visible phenotype although ca2 null mutant shows 90% reduction of mitochondrial complex I. Thus, partial redundancy might exists. In order to gain insight about this redundancy, several approaches to silence multiple genes encoding proteins of the CA domain were undertaken. Using a microRNA designer tool, 35S::amiRCAs and 35S::amiRCALs were constructed and introduced into Arabidopsis thaliana, var Col-0. On the other hand, a specific 200 bp to perform siRNA against CAs or CALs using pHANNIBAL or pOpOff2 (dexamethasone inducible) vectors were independently introduced into Col-0. Physiological parameters such as oxygen consumption, carbon dioxide fixation and preliminary mitochondrial proteomic analyses were compared with those of single and double null mutants.

PL-P65.

ACETOACETYL-CoA tHIOLASE REGULATES THE MEVALONATE PATHWAY DURING ABIOTIC STRESS ADAPTATION

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Acetoacetyl-CoA thiolase, also called thiolase II, condenses two molecules of acetyl-CoA to give acetoacetyl-CoA. This is the first enzymatic step in the biosynthesis of isoprenoids via mevalonate (MVA).Thiolase II from alfalfa (MsAACT1) was identified and cloned. The enzymatic activity was experimentally demonstrated in planta and in heterologous systems. The condensation reaction by MsAACT1 was proved to be inhibited by CoA suggesting a negative feedback regulation of isoprenoid production. Real-time RT-PCR analysis indicated that MsAACT1 expression is highly increased in roots and leaves under cold and salinity stress. Treatment with mevastatin, a specific inhibitor of the MVA pathway, resulted in a decrease in squalene production, antioxidant activity, and the survival of stressed plants. The addition of vitamin C suppressed the sensitive phenotype of plants challenged with mevastatin, suggesting a critical function of the MVA pathway in abiotic stress-inducible antioxidant defence. MsAACT1 over-expressing transgenic plants showed salinity tolerance comparable with empty vector transformed plants and enhanced production of squalene. Thus, acetoacetyl-CoA thiolase is a regulatory enzyme in isoprenoid biosynthesis involved in abiotic stress adaptation.

PL-P66.

EFFECT OF THE IMAZETHAPYR HERBICIDE (VEROSIL®) ON THE MICROALGAE *Scenedesmus vacuolatus* (CHLOROPHYTA)

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The aim of this work is to analyze the participation of oxidative stress in the phytotoxicity of Verosil® (imazethapyr), an herbicide of the imidazolinone family, in *S. vacuolatus*. This green microalgae was exposed to 0-8 mg imazethapyr /L, added to the medium, at 24±1ºC, under continuous light and agitation. After 96 hr, were evaluated cellular growth (EC50); gluthation S-transferase activity (GST, a metabolic parameter), pigment and TBARS (a lipid peroxidation parameter) content, antioxidant defenses (catalase -CAT- and superoxide dismutase -SOD- activities). When the toxic concentration increased the cell number decreased ($p<0.01$), with a EC50 of 3.2 mg/L. TBARS levels increased significantly ($p<0.01$) with 4 mg/L (196±70%). The carotene/chlorophyll ratio had a significant increase with 6mg/L (63±22%, $p<0.01$), while the chlorophyll *a*/chlorophyll *b* ratio remained unchanged. At 6 mg/L CAT activity showed a significant increase (4.5-fold, $p<0.05$) as well as GST activity (11.8-fold, $p<0.01$), while SOD had no significant variation compared to the control. The formulation mix without imazethaphyr resulted in a decreased cell number (43±5%, $p<0.01$) and increased MDA levels (195±56%, $p<0.01$). Although studies with the active principle alone remain to be done, these preliminary results indicate that not only the formulation but also the coadjuvant *per se* has a toxic effect on *S. vacuolatus*.

SB-P01.

A NOVEL HETEROBIFUNCTIONAL PROBE BINDS AN ALLOSTERIC SITE ON THE NICOTINIC ACETYLCHOLINE RECEPTOR

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The probe (AC4-ASA) was developed as a tool for the study of cholinergic receptor binding sites. Acetylcholine was derivatized at its alkyl end and, through a short spacer, with a photoactivatable aryl-azide group susceptible to radioiodination. The probe can interact specifically with the muscle nicotinic receptor and has a considerable selectivity for its / binding site.
The ligand showed the capability of modyfing the affinity of [^{3}H](-)-nicotine by the muscle-type receptor. Competition experiments between AC4-ASA and [^{3}H](-)-nicotine revealed that the ligand could perform its modulating activity through at least one new allosteric binding site, different from the typical orthosteric binding sites.
With the aim of delineating this site, the *Torpedo californica* receptor was modified with the ligand and submitted to SDS-PAGE. All subunits were digested with trypsin and peptide mixtures analyzed by MALDI-TOF-TOF mass spectrometry. Analysis of the spectrum from the alpha subunit allowed us to find a 1600.8 Da molecular mass - absent in the non-modified receptor subunit - and corresponding to the mass of the tryptic peptide WNPADYGGIKK plus that of the ligand. Moreover, such peptide is not involved in the orthosteric receptor binding sites.
New photolabelling experiments are in progress to define the location of other ligand binding determinants at the receptor.

SB-P02.

GUANINE QUADRUPLEXES IN *Danio rerio*: EXISTENCE, RELATIVE ABUNDANCE, AND PUTATIVE FUNCTION

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Guanine quadruplexes (G4) are secondary structures of single-stranded DNA or RNA formed by the stacking of planar layers of four guanines interacting by Hoogsteen hydrogen bonds. G4 were characterized in several species and related to specific biological functions, such as gene expression regulation and chromatin structure modification. Genome-wide studies of these structures in the model species *Danio rerio* have not been performed yet. Here, zebrafish genomic, gene, and promoter sequences were downloaded from Ensembl (version ZV9; www.ensembl.org) and searched for the existence G4 structures using the Quadparser software (www.quadruplex.org). We found 55,109 G4 in the zebrafish genome. Among them, 25,602 corresponded to 9,517 genes and 2% (1,093 G4) were found in mRNAs synthesized from 674 genes. In addition, we found 1,023 G4 (1.9%) in 918 gene promoters. Gene ontology categories enriched in G4 containing sequences were measured with the Cytoscape software (www.cytoscape.org). Interestingly, developmental categories were significatively enriched among the identified sequences. This study presents a first draft of the distribution and potential function of G4 sequences in zebrafish genome and establishes a starting point concerning their role in development and disease.

SB-P03.

INSIGHTS INTO THE STRUCTURE OF BCY1, THE REGULATORY SUBUNIT OF PKA FROM *Saccharomyces cerevisiae*

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Yeast is the first non-metazoan organism for which some structural information of PKA is available. Our group has obtained the crystal structure of the cAMP binding domains of Bcy1, the regulatory subunit of PKA. We now show results that contribute to understand the structure of the whole Bcy1 dimer. The solution structure of 140-Bcy1, as well as of whole Bcy1 have been solved by SAXS (small angle X-ray scattering). The R_g and D_{max} of the two structures were determined. The structure of 140-Bcy1 was modeled *ab initio* by using de DAMMIN program. The resulting structure compares very favorably with the crystal structure, thus confirming the particular relative orientation of A and B cAMP domains. The structure of whole Bcy1 seems to be more compact than the one of RI and RII. The amino termini of R subunits constitute the so called D/D domain, responsible for the dimerization of the subunits and for the interaction with anchoring proteins (AKAPs). Chemical crosslinking experiments confirm that Bcy1 exists as a dimer, via its N-terminus. The N terminus of Bcy1 was modeled by homology using Swiss Model and further minimization with GROMACS. The preliminary model shows that Bcy1 shares with its counterparts the residues important for dimerization; however the exposed surface, that should interact with anchoring proteins (not described yet in yeast) have characteristics of its own.

SB-P04.

RATIONAL DESIGN TO CHANGE PRODUCT SPECIFICITY IN CYCLODEXTRIN GLUCANOTRANSFERASE

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Cyclodextrin glucanotransferase (CGTase), a member of the GH13 family, catalyses starch conversion into cyclodextrins (CDs), an important industrial process. CDs are cyclic oligosaccharides with 6 ( ), 7 ( ) or 8 ( ) glycosil units. CGTases produce CDs mixtures difficult to separate; because of that, researchers are interested on producing only one type of CD. We performed a multiple sequence alignment of all CGTases and the positions with residues only presents in -CGTases were identified and localized into a molecular model of the CGTase from *B. circulans* DF 9R. All type of interactions with substrate binding sites and catalytic cleft were examined. Four residues, V137, V144, A280 and I329, considered as possible -specificity markers were selected and single, double, tripled and quadruple mutants were done on CGTase gene from *B. circulans* DF 9R. Here, we analyzed the singles mutants A137V, A144V, L280A and M329I. Proteins were expressed in *E. coli* BL21, purified and characterized. The CD production only decreased a 50 % for A137V respect to wild type. The CDs ratio was not affected by singles mutations. All enzymes showed the same K_m value while the V_{max} decreased 1.5 times for A144V, L280A and M329I mutants but 10 times for A137V. This work plus the other mutants analysis will contribute to knowledge of the relationship between structure and product specificity of this enzyme.

BT-P01.
PRODUCTION OF EXTRACELLULAR EMULSIFYING AGENT BY *Streptomyces* SP. MC1

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Emulsifiers of microbial origin have significant implications in the biological treatment of pollutants from environmental. They are employed in mechanisms for hydrocarbon and inorganic pollutants bioremediation, including heavy metals. *Streptomyces* sp. MC1 isolated from sugar cane, showed significant capacity to reduction of Cr(VI) to Cr(III) in minimal medium supplemented with sulphate or phosphate. The aim of this work is to evaluate now the effect of sulphate and phosphate ions, either in presence and absence of Cr(VI) on emulsifier production from this strain. Cells of *Streptomyces* sp. MC1 were grown in liquid minimal medium either with or without 20 mg/L of $K_2Cr_2O_7$ as Cr(VI) source, and different concentrations of Na_2SO_4 or K_2HPO_4 (5 to 20 mM). Cultures were incubated at 30ºC in orbital shaker at 170 rpm. Emulsifiers production was monitored using kerosene as water-immiscible substrate. After 48 h of cultivation, only in presence of phosphate ions it was detected the emulsifier production in supernatant of this strain, with a highly-stable emulsion. However, supplementation of Cr(VI) to the minimal medium inhibited the emulsifier production. Results presented here may have important implications in bioremediation of hydrocarbon and inorganic pollutants. Therefore, optimization of studies for emulsifier production could be required for future applications.

BT-P02.
STABILITY OF BUDDED VIRUS OF BACULOVIRUS IN SERUM-FREE MEDIUM

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Biotechnology applications of baculoviruses are constantly expanding. Any of these applications, from biopesticides to vectors in gene therapy, requires the proper production and preservation of high-titer stocks of budded virus (BVs) in serum-free culture medium. It has been described that frozen baculovirus storage in serum-free media is less efficient than in culture media added with serum, but the causes are unknown. The stability of BVs of the baculovirus AgMNPV was evaluated in a serum-free medium under different conditions of lipid supplementation, freezing and thawing, and exposure time to the production temperature (27°C), employing a 2^3full factorial design in duplicate. The time of exposure to 27°C, as wells as the freezing and thawing of samples, did not affect significantly the stability of BVs. However, it was strongly altered by the presence of lipids in the culture medium: the mean titer of samples preserved in medium supplemented with lipids was almost 4 times lower than the mean titer of samples stored in lipids-free medium. The deleterious effect of the lipids was magnified when BV samples were frozen and thawed: more than 90% of the mean viral infectivity was lost when samples supplemented with lipids were frozen and thawed. These results strongly suggest that the reduced stability of baculovirus BVs in serum-free media is associated to the presence of lipids.

BT-P03.
REMAZOL BLACK DECOLORATION BY *Burkholderia cepacia* IMMOBILIZED IN SOL-GEL SILICA MATRICES

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Among many pollutants, textile industry effluents are the major source of environmental pollution. Synthetic dyes are widely used in the textile, pharmaceutical, cosmetic, and food industries. These effluents are a serious concern because of their adverse effects on many organisms and ecosystem as a whole. Even small concentrations of dye are highly visible and can be toxic to aquatic organisms. Azo dyes are the largest and most versatile class of dye but due to their chemical properties, they are not easily degradable under natural conditions and thus, are not thoroughly removed from water by conventional wastewater treatment systems. In this work we propose the use of sol-gel silica matrices obtained from sodium silicate to immobilize *Burkholderia cepacia*, a Remazol Black (RB) decolorizing bacteria. The advantages of bacteria immobilization are the possibility to recycle the beads, protect bacteria and reduce their presence in the resulting fluid. Bacteria were cultured aerobically at 35ºC in aqueous solutions of RB at concentrations from 1% to 0.005%. RB was measured directly by absorption at 597 nm. Immobilized *B. cepacia* was resistant to 2% of RB. It also reduced more than 80% of RB within 24 hs when 0.005% was added. Further studies are being performed to estimate the reutilization and half-life of the immobilized bacteria.

BT-P04.
INTERACTION OF SILICA AND BETAMETHASONE LOADED SILICA NANOPARTICLES WITH MONOCYTES

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Silica nanoparticles (SiNP) were investigated for their potential use as a novel drug carrier for targeted and controlled release of drugs. Betamethasone is a potent glucocorticoid steroid with anti-inflammatory and immunosuppressive properties. In this work betamethasone was encapsulated into monodisperse dense amorphous silica nanoparticles, to be used as a drug delivery system for the treatment of inflammatory and autoimmune diseases. SiNP were prepared via base-catalyzed hydrolysis and polymerization of tetraethyl orthosilicate under sterile conditions using ammonia (Stöber process), dialyzed and characterized before use. SiNP sizes were determined by dynamic light scattering (310 nm). In vitro studies were conducted on monocyte cells cultures for 24 and 48 h with culture media, free drug (20 µg/ml), betamethasone loaded SiNP (1 µg/ml) or SiNP without drug as controls. SiNP did not induce any morphological change in monocytes after exposure. Cell proliferation (evaluated with the MTT test of tetrazolium reduction) is not affected in the presence of free betamethasone, while SiNP significantly stimulate monocytes proliferation. This stimulation was effectively inhibited by betamethasone loaded SiNP. These results suggest that SiNP could be used as drug carrier, although several factors such as size and charge of the SiNP must be further analyzed.

BT-P05.
SOL-GEL NANOSTRUCTURED ANTIBIOFILM GLASS SURFACES

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Various types of surfaces in clinical or industrial settings are prone to unwanted biofilm generation. In order to prevent the large doses of antibiotics that are required to eradicate them, superhydrophobic surfaces are being developed as alternative. These surfaces are shown to prevent contact between a bacterium and surface attachment points and they are generally made from a low energy material with a highly developed microstructure, some incorporating two different length scales in this roughness.
In this work, we describe a simple method to fabricate superhydrophobic sol-gel nanostructured glass coatings to control bacterial adhesion. These surfaces were manufactured by coating glass slides with a mixture of Aminopropyl trietoxysilane and Tetraetoxysilane in order to functionalize the surface with amino groups. Then, two different sizes of silica particles: nanoparticles (NPs: 27 nm) and microparticles (MPs: 2 µm) were also functionalized with NH_2 groups and attached to the surface by glutarahaldeyde crosslinking. The effect of different proportions of the particles was studied (0:100, 25:75, 50:50 Mps:NPs). Finally, the surfaces were treated with dodecenyl succinic anhydride in anhydrous ethanol (1/10, 1/100, 1/1000). Surfaces hydrophilic/hydrophobic behavior was analyzed by means of contact angle and inhibition of biofilm formation by exposing the surfaces to *Pseudomonas* spp.

BT-P06.
SCLEROGLUCAN PRODUCTION BY *Sclerotium rolfsii* ATCC 201126: INFLUENCE OF THE CARBON SOURCE

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Scleroglucan is a ß-D-(1,3)-(1,6)-glucan produced by *Sclerotium rolfsii* ATCC 201126. This neutral water-soluble exopolysaccharide (EPS) was produced by submerged culture in MP_{20} liquid medium (sucrose: 20 g/L; initial pH: 4.5). The influence of 9 different simple and complex carbon sources on scleroglucan, biomass and oxalic acid production was evaluated. Batch fermentation processes were carried out in Erlenmeyer flasks at 30ºC and 250 rpm during 72 h. Higher EPS production (7 g/L) was obtained with sucrose and maltose as C-source. Biomass was significantly increased with maltose (~9 g/L) and soluble starch (9.6 g/L). Variable concentrations of oxalic acid were observed depending on the C-source used. The possibility to select appropriated culture conditions for *S. rolfsii* ATCC 201126 according to the product of interest was demonstrated. Additionally, biomass and EPS production obtained with molasses as C-source would allow to propose the use of low-cost substrates for the production of high-added value metabolites.

BT-P07.
***Sclerotium rolfsii* SCLEROGLUCAN: EFFECTS OF THERMAL, ALKALINE AND ULTRASONIC DEGRADATION**

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Scleroglucan is a neutral water-soluble ß-1,3-ß-1,6-glucan produced by *Sclerotium rolfsii* ATCC 201126 whose triple helical conformation can be turned into single coiled chains at high pH or temperature and/or in DMSO. Effects of thermal (65, 95 and 150ºC), alkaline (0.01-0.2 N NaOH) and ultrasonic (1, 5 and 10 min with 20% amplitude) treatments onto the solution properties of *S. rolfsii* scleroglucans (EPS I, EPS II and EPSi at 0.2% w/v) were comparatively evaluated vs. commercial scleroglucan (LSCL). The more drastic assayed conditions (150ºC, 0.2 N NaOH and 10-min ultrasonication) significantly modified the rheological behavior of scleroglucan solutions, with an abrupt decline in apparent viscosity and the loss of pseudoplastic behavior. EPS I was identified as the less sensitive polymer to thermal degradation, whilst EPSi and LSCL were particularly susceptible to alkaline treatment but the more stable ones against ultrasonication. Scleroglucan samples were all denatured (triple helix —› single brand transition) at 150°C and 0.2 N NaOH. Additionally, size exclusion chromatographic (SEC) profiles of control and treated polymer samples revealed aggregates, single chains and triple helices distinctive zones in accordance with the applied treatment.

BT-P08.
CONFORMATION AND STRUCTURAL COMPACTION OF SCLEROGLUCAN BIOPOLYMERS AS WITNESSED BY FRET SPECTROSCOPY

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Scleroglucans from *Sclerotium rolsfii* ATCC 201126 produced at fermenter scale and ethanol recovered at different times (EPS I: 48 h and EPS II: 72 h) did not exhibit significant variations in MW, degree of polymerization or branching, and adopted a triple helical semirigid structure in aqueous neutral solution. However, they showed certain differences in rheological behaviour, anti-syneresis, emulsifier and suspending properties, hydrogel microstructure and their ability to activate LAL and Glucatell coagulation tests. That may be related to different conformational features such as the triplex expansion degree, which tried to be assessed by fluorescence resonance energy transfer (FRET) spectroscopy. Alkali denaturation of EPSs and subsequent renaturation events over time were evaluated by FRET. EPSs double-labelled with a donor/acceptor fluorophores pair (AP/FITC) were treated with increasing NaOH concentrations (0.015-1.0 M) and a gradual partial opening of the triple-helix rather than a complete strands separation was observed. An isopropanol-processed scleroglucan, EPSi (recovered at 72 h), and a commercial scleroglucan LSCL, also exhibited a similar behaviour. FRET results suggested a decreasing triplex compaction degree as follows: LSCL > EPS I > EPS II > EPSi. Renaturalization showed that even without neutralization the triple-helix conformation could be restored after 96 h.

BT-P09.
OPTIMIZED LOVASTATIN PRODUCTION BY SOLID-STATE FERMENTATION USING AGRO-INDUSTRIAL DERIVED SUBSTRATES

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Lovastatin is a drug that can inhibit the *de novo* synthesis of cholesterol by competitive inhibition of HMG-CoA reductase, which catalyzes the rate limiting step of cholesterol biosynthesis. It can be then effectively used for the hypercholesterolemia treatment. Nowadays, interest has focused in searching new statins and/or developing low-cost production strategies. This work aimed at optimizing lovastatin production by solid state fermentation (SSF) with *Aspergillus terreus* MEC, looking for low-cost and readily available substrates. Cultures consisted in textured deffated soy bean flour (HSDT) embedded with A. terreus-inoculated LP (whey protein concentrate) or SQ (whey) liquid media. Different initial pHs (4.3, 5.3, 6.3, 7.3), incubation temperatures (23, 25, 28, 30°C), moisture contents (CHI: 36, 46, 56, 66, 76%) and bed heights (1, 1.5, 2, 2.5, 3, 4 cm) were evaluated. Extracted lovastatin was analyzed by RP-HPLC with a Diode Array Detector and fungal growth was indirectly determined by ergosterol quantification. A lovastatin production of 1017.55 µg per g of dry weight was reached by using optimized SSF consisting in SQ liquid medium adsorbed on HSDT at an incubation temperature of 25°C, pH = 6.3, CHI = 46-56% and a bed height of 1.5-2.5 cm. These results highlight the relevance of SSF strategies for the production of high-added-value secondary metabolites.

BT-P10.
SUCCESSFUL FIBRINOLYTIC ENZYMES PRODUCTION BY SOLID STATE FERMENTATION

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Fibrin accumulation in blood vessels increases thrombosis risks promoting myocardial infarction and cardiovascular diseases. *Bionectria* sp. LY 4.1, a wild fungi isolated from Las Yungas Pedemontana rainforest, was recently reported by our group as a novel fibrinolytic enzyme source. In the present study we attempted the production of fibrinolytic enzymes by solid state fermentation (SSF) with *Bionectria* sp. LY 4.1 looking for low-cost substrates and simplified operational conditions. The different solid substrates (SS) evaluated (polyurethane foam - PUF, bagasse, textured soy flour, soy flour pellets and wheat bran), in adequate amounts were inoculated with fresh production medium containing 72 h-old *Bionectria* sp. culture. The effect of different conditions of moisture (9, 11, 13, 15, 17%), bed height (1, 2, 4, 6, 8 cm) and particle size (0.25; 0.5; 1 and 2 cm^2) was studied. Fibrinolytic activity was determined by the fibrin plate technique and protein concentration by the BCA method. The obtained results confirmed the possibility to produce fibrinolytic enzymes by SSF with *Bionectria* sp. LY 4.1. Furthermore, assays demonstrated the convenience of using PUF as SS under specific operating conditions over the other substrates tested. The findings herein presented offer an interesting alternative for the production of a high added-value pharmacological product.

BT-P11.
MALTOOLIGOSACCHARIDES PRODUCTION USING CYCLODEXTRIN GLUCANOTRANSFERASE FROM *Bacillus circulans* DF 9R

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Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) catalyzes the starch conversion into cyclodextrins (CD), linear maltooligosaccharides (MOS) and limit dextrins. Many investigations are aimed at increasing the performance of CD production processes but there is little information regarding the use of CGTases on MOS production. The aim of this work was to study the optimal conditions to convert soluble potato starch into MOS by the action of CGTase from *Bacillus circulans* DF 9R. To study the reaction products obtained by CGTase activity on substrate in presence of carbohydrate acceptors, flasks containing 5 % soluble potato starch were incubated with 15 U of purified enzyme per gram of starch in a phosphate buffer 25 mM pH 6.4 at 56 ºC and 100 rpm. The effect of the addition of glucose or maltose as acceptors to reaction mixtures was evaluated in concentrations from 1.25 to 5.0% and the incubation time from 4 to 24 h. Products were analyzed by colorimetric reactions, paper chromatography and HPLC. When glucose or maltose concentrations increased, MOS production also increased. The best results were obtained using 5% soluble potato starch and 5% glucose. After 10 h of incubation, a highest yield of MOS and negligible CD concentration were obtained. This process might apply to MOS industrial production that nowadays is carried out using at least three different enzymes.

BT-P12.
ENGINEERING *Streptomyces coelicolor* FOR THE PRODUCTION OF FREE FATTY ACIDS

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Due to their high energy density and low water solubility, fatty acids are arguably the most appropriate biofuel precursors in a cell. Here we describe the design and construction of a *S. coelicolor* host strain suitable for the production of free fatty acids. To achieve this end, we engineered the cell by deleting genes of competitive pathways and inserted novel enzymatic activities, thus redirecting the malonyl-CoA flux to the desired product. We employed the CH999 strain, a widely used host for *in vivo* production of engineered natural products, and a mutant strain deficient in triglyceride biosynthesis. In this genetic background, we overexpressed the *S. coelicolor* Acetyl-CoA Carboxylase complex and the thioesterase TesA from *Escherichia coli*. The expressions of these genes were achieved using *ad hoc* customized vectors that consist in modular-combinable DNA features with BioBricks format. Using this approach, we constructed several plasmids carrying the genes coding for target enzymes under different endogenous *Streptomyces* promoters, active in late exponential or in stationary phase of growth. Under these conditions we were able to exert a temporal control of gene expression in order to minimize the interference with cell chassis and optimize the timing of gene induction accordingly to the metabolic state of the cell; finally improving the levels of production of free fatty acids.

BT-P13.

IMMOBILIZED *Nocardioides nitrophenolicus* NSP41T STRAIN AS A STRATEGY FOR ATRAZINE BIOREMEDIATION

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Atrazine (ATZ) is a triazinic herbicide widely used in agriculture. The potential toxicity of its residues motivates continuous bioremediation-directed research. Beside the pollution generated by intensive agricultural practices, final disposal of herbicides containers generate small highly contaminated spots, often in farmer houses vicinity. The characteristics of these spots make them an important issue for *in situ* bioremediation strategies. The aim of this work is to design a strategy for *in situ* bioremediate the residues that remain in disposed ATZ containers. In this way, ATZ degrader *Nocardioides nitrophenolicus* NSP41T strain was cultured in a low cost medium and immobilized in calcium alginate beads. Biodegradation experiment was performing in Erlemneyer flasks containing minimal culture medium at 26ºC and 200 rpm. Alginate beads were cultured at 250 and 500 ppm of ATZ. After extraction and HPLC analysis, 99% of herbicide degradation was found after 5 days in all treatments. Beads were efficiently reused after 1 month of being incubated in ATZ presence. Results indicate that alginate immobilized *N.nitrophenolicus* NSP41T strain is a promissory strategy to bioremediate high concentration ATZ residues as a consequence of its regular agricultural handling. In addition, considering the degradation performance, this technology could be extended to effluent treatment.

BT-P14.

HEMAGGLUTINATING ACTIVITY AND STRUCTURAL STABILITY OF A SNAIL EGG-PROTEIN

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Many glycan binding proteins have been isolated from mollusks, where they function as recognition and immune molecules. Here we report the hemagglutinating activity of scalarin (SC), an egg protein from the aquatic snail *Pomacea scalaris*, and characterize this activity in the context of the structural stability of the protein.
Two biological functions, found in proteins of a related species, were assayed: trypsin inhibition and hemagglutinating activity. SC did not inhibit trypsin but showed high agglutinating activity against rabbit red blood cells. Hemagglutination was not altered by the presence of divalent cations and it was strongly inhibited by glucosamine, galactosamine and GalNAc. SC was resistant to pepsin and trypsin digestion. Temperature and pH stability, analyzed by fluorescence and visible spectroscopy, indicated that it was stable up to 60°C; in coincidence with a loss of hemagglutinating activity above this temperature. While SC did not show structural perturbations between pH 2.0 and 10.0, a loss in activity was observed below pH 4.0 and above pH 8.0.
These results strongly suggest that SC has the potential of becoming a biotechnologically useful lectin, due to its wide pH and temperature stability range. This is the first report of a protease-resistant glycan binding protein in a *Pomacea* snail, a genus where novel egg-defense systems were recently described.

BT-P15.

HIGH IFN– 2B PRODUCTIVITY OBTAINED FROM LENTIVIRUS–TRANSDUCED CHO CELLS

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Interferons are cytokines that can interfere viral activity, inhibit cell proliferation and modulate the immune system. Human IFN– 2 produced in bacteria is employed in the treatment of cancer and chronic viral diseases. Due to its short half-life, the use of high and repeated doses is necessary, leading to several side effects. In our lab, we have obtained a hyperglycosylated rhIFN– 2b mutein (IFN–4N) with improved pharmacokinetics with respect to the native protein.
Considering that low productivity is the major problem of the production of recombinant proteins in mammalian cells, we designed a new strategy to generate stable cell lines with high IFN–4N expression. Lentiviral vectors containing the cDNA of IFN–4N in their genome were assembled into HEK–293 cells and employed for transduction of CHO.K1 cells. Cells were then subjected to a selection process using increasing concentrations of puromycin. Successive increases in specific productivity of IFN–4N were observed after each selection step. Thus, the cell line resistant to the highest concentration of the antibiotic exhibited a 10–fold higher specific productivity compared to the one obtained after the first selection event. Furthermore, its productivity was 10 times higher than that obtained using conventional plasmidic vectors, representing an attractive alternative for high yield production of the cytokine.

BT-P16.

CONSTRUCTION OF A HIV–1 BASED LENTIVIRAL VECTOR PSEUDOTYPED WITH THE RABIES VIRUS GLYCOPROTEIN

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During the last decades lentivirus vectors (LV) have been widely used for gene transfer protocols. An important feature of these vectors is their tropism. It can be modified specifically by changing the membrane glycoprotein that is part of the viral structure. The goal of this work was to assemble a lentiviral vector using the rabies virus glycoprotein (RV–G) as the membrane protein and evaluate its ability to transduce cells *in vitro*.
Adherent HEK293 cells were used to generate Green Fluorescent Protein (GFP) producing LV, transfecting third generation plasmids: two vectors coding packaging proteins (pGag/Pol and pREV), a transfer plasmid (pLV GFP) and a plasmid encoding the membrane RV glycoprotein (pZC–GlycoG). As a positive control we assembled LV with the VSV glycoprotein (VSV–G) and as negative control the transfection was done without glycoprotein. For both, the RV–G and the VSV–G pseudotyped LV, we achieved concentrations in the order of 10^6 TU/ml. Contrarily, for the negative control we could not detect the presence of LV. After that, we used the produced LV to transduce HEK293 cells and transduction efficiency was assessed by flow cytometry, obtaining values of 77% and 64% of fluorescent cells for RV–G and VSV–G pseudotyped LV, respectively. Therefore, we can say that the obtained RV–G pseudotyped LV were efficiently assembled and were able to transduce animal cells.

BT-P17.

TYPE I IFNS: NEW REPORTER CELL LINES TO DETERMINE THEIR BIOLOGICAL ACTIVITY

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The potency of interferons (IFN) must be correctly identified because they are used as biopharmaceuticals since they have an important role in the immune defence. For this purpose, 4 new human reporter cell lines derived from different tissues (A549, Hela, Wish and HEp–2) were developed using the eGFP gene under the control of type I IFN–inducible Mx2 promoter.

The Mx2 promoter responded specifically and quantitatively to type I IFNs (IFN– and –ß) showing a good correlation between the eGFP expression and the IFNs added.

Comparing the cell lines responsiveness to IFN– subtypes, no differences in detection limit were observed among them. Contrarily, differences were observed when comparing the activity of IFN–ß subtypes in HEp–2 cell lines. Therefore, the standardization of several assays to measure the potency of IFNs might be carried out using the cell lines herein documented.

These systems have many advantages with respect to already existing assays: they are very fast, they can determine the potency of type I IFNs using only one cell line and have the specificity of the Mx promoter. They are sensitive and safe, showing reproducible responses in a dose dependent manner. Outstandingly, the main contribution of this work was the development of alternative reporter systems as suitable candidates to evaluate the way that IFNs induce their activity in different human tissues.

BT-P18.

DEVELOPMENT OF A PRODUCTION SYSTEM FOR Z VLPS IN INSECT CELLS

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The assembly of proteins as virus-like particles (VLPs) using protein expression technology in yeast, insect and mammalian cells is a highly effective type of subunit vaccine, already tested and approved. The Arenavirus´s Z protein is involved in the assembly and budding of virions from the cell membrane. It also has been reported that the only expression of Z is sufficient for the release of Z-containing particles of Lassa Fever Virus. The generation of this VLPs from Z expression could have different biotechnological applications, such as drug and antigen delivery. Because of this is necessary the development of a safe, efficient and easy to scale up VLPs production system. Insect cells are a suitable candidate, since they express large amounts of correctly folded recombinant proteins and have been used for previously approved vaccines, as Cervarix.

In order to determine if Junin virus Z protein maintains its budding capacity on insect cells, the recombinant Z-EGFP protein was expressed on Sf9 insect cells using the Bac-to-Bac system. We analyzed the culture supernatant of infected cells expressing Z-EGFP and we detected its presence in particles obtained by ultracentrifugation. Also these particles were preserved from degradation of proteinase K by a lipid membrane. These results suggest the presence of Z-EGFP in particles obtained from the culture supernatant of infected cells.

BT-P19.

RESISTANCE TO CANKER DISEASE IN TRANSGENIC PINEAPPLE SWEET ORANGE PLANTS BY DERMASEPTIN EXPRESSION

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Orange production has significant contribution to regional economies of Argentina. *Citrus canker disease*, caused by the bacterium *Xanthomonas axonopodis pv. citri (Xac)* is the most important disease of citrus and causes important economic losses worldwide. No natural resistance was found in commercial orange cultivars or feedstock plants. Disease control is limited to agronomical practices and/or eradication of infected individuals. We developed a biotechnological strategy based on the expression of the dermaseptin antimicrobial peptide in transgenic Pineapple sweet orange plants.

Pineapple sweet orange and *N. benthamiana* plants were transformed via the *A. tumefaciens* protocol to express constitutively a dermaseptin. Bacterial growth inhibition assays were performed *in vitro* using extracts from transgenic *N. benthamiana* plants. These assays showed a strong inhibition of Xac growth with every extract tested. In planta assays performed with two selected transgenic Pineapple sweet orange plants showed similar results. A bacterial suspension was sprayed on the surface of punctured leaves (6 punctures per leaf) and canker development was evaluated up to 35 days under a dissecting microscope. Analysis of 70 to 100 punctures per/plant consistently showed lower frequencies of severe canker symptoms in transgenic orange leaves as compared to non-transformed controls.

BT-P20.

CLONING AND EXPRESSION OF THE LACCASES FROM THE BASIDIOMYCETE *Trametes trogii* BAFC 463 IN *Pichia pastoris*

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Fungal laccases are polyphenoloxidases that show many industrial applications as in delignification, textile dye or stain decolorization, water or soil bioremediation.

Lignin degradation activity in basidiomycetes is due to the combined action of different enzymes, including laccases, Mn peroxidases, and lignin peroxidases. The white-rot fungus *Trametes trogii* BAFC 463 has shown an outstanding laccase activity (148,6 U/ml) and dye bleaching ability. This activity is due to at least 2 different isoenzymes: lcc1 and lcc2. In order to clone the lcc1 and lcc2 genes and look for other potential laccase isoenzymes in *T. trogii*, we design degenerated oligonucleotides corresponding to the conserved copper binding regions. Four different laccase coding genes were amplified from total cDNA, and complete coding sequence obtained by 5´RACE and 3´RACE techniques. Two of these cDNAs correspond to the lcc1 and lcc2 genes previously reported for *T. trogii* 201 (GenBank CAC13040 and CAL23367), and the other 2 correspond to novel isoenzymes (named lcc3 and lcc4), related to lcc1 from *Coriolopsis gallica* (97% similarity) and lcc2 from *Trametes pubescens* (86% similarity), respectively.

All the four laccase coding genes were expressed in the methylotrophic yeast *Pichia pastoris* in order to obtain a simple source of laccase and to perform the biochemical and kinetic characterization for each isoenzyme.

BT-P21.
IMPROVEMENT OF THE STABILITY OF A BAEYER-VILLIGER MONOOXYGENASE
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Baeyer-Villiger monooxygenases (BVMOs) catalyze the oxidation of cyclic or linear ketones to lactones or esters, respectively. The enzymatic oxidation of ketones avoids the use of peroxides and strong oxidizing acids frequently employed in organic synthesis to carry out this conversion. This enzymatic reaction is very valuable for synthetic applications and it is an environmentally friendly process. However, limitations of these monooxygenases are mainly related to low protein stability and cofactor recycling. In this work, we address the first problem aiming at developing BVMO variants with increased stability and with no substantial changes in substrate preference and selectivity. Mutations predicted by a structure-guided consensus approach were incorporated in the sequence of cyclohexanone monooxygenase from *Acinetobacter* NCIMB 9871 and selected for an increase in thermal stability. Advantageous mutations were combined and variants with an increased stability were selected. Here, the structure-guided consensus approach was successfully applied for the first time to the thermal stabilization of a flavin monooxygenase.

BT-P22.
BIOSYNTHESIS OF NEW ANTIMALARIC MACROLIDES
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Previously we have synthesized macrolide derivatives meg-azi and meg-ery through biotransformation experiments using a heterologous host and demonstrated substrate flexibility of the sugar transferase for the macrolide substrate. Now new macrolide derivatives were synthesized by expression of alternative TDP-aminosugar operons. These results demonstrate also substrate flexibility towards the TDP-aminosugar recognized by the sugar tranferases. Antibacterial and antiparasitic activities were determined for these derivatives. Antibacterial activity of these new derivatives did not change notably compared to original molecules. Meg-azi and meg-ery derivatives were tested *in vitro* against a standard laboratory and a mefloquine resistant strain of Plasmodium falciparum. These compounds showed a ten-fold increase in effectiveness after a short-term exposure and retained the similar effectiveness to azithromycin after longer exposures. The new compound also remains active against azithromycin resistant strains of *P. falciparum*. These results encourage us to synthesize new modified forms of these compounds to further improve the short term activity and evaluating the antibacterial and antiparasitic activity of these new compounds.

BT-P23.
EFFECT OF CYCLODEXTRINS AND ELICITATION ON ANTHRAQUINONE PRODUCTION IN *Rubia tinctorum* CELL CULTURES
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Anthraquinones (AQs) are secondary metabolites produced by *Rubia tinctorum* that exhibit interesting biological properties. Since the production of these and other secondary metabolites is often low in plant *in vitro* cultures, different strategies have been applied to overcome this limitation. In the present work, we combined the addition of cyclodextrins with two elicitors: methyl jasmonate (MeJ) and a fungal extract (FE), in cell cultures of *R. tinctorum*. We performed an experiment combining methyl-ß-cyclodextrin (MCD) with MeJ and a fungal extract (FE). After 3 days of elicitation, MeJ increased AQ intracellular content by 262%, whereas FE addition resulted in a lower increase (77%). When MCD and MeJ were added together, the intracellular AQ production increased by 450%. On the other hand, FE and MCD resulted in a mild increase in the intracellular content of AQs (31%). However, the proportion of the AQs released to the culture media after this treatment was the highest (37%). We also evaluated AQ production after the addition of hydroxypropyl-ß-cyclodextrin (HPCD) and MeJ. The combination of them enhanced AQ accumulation by 160%, after 3 and 5 days of elicitation. These results provide new evidence of the synergistic effect of cyclodextrins and MeJ on the accumulation of secondary metabolites and set the basis for a promising strategy to enhance AQ production.

BT-P24.
PHYTOFERMENTATION PROCESSES IN STIRRED TANK BIOREACTORS BY *Rubia tinctorum* SUSPENSION CULTURES
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Considering the complexity in the chemical synthesis of some secondary products, plant cell cultures represent an alternative for industrial application. However, an impediment of the practice of large-scale culture in stirred tanks is the shear stress. *Rubia tinctorum* suspension cultures produce anthraquinones (AQs) which are important in the pharmaceutical and food industries. The aim of this work was to study the performance of *R. tinctorum* suspension cultures in a stirred tank bioreactor including the evaluation of shear stress effects. A 1.5 L stirred tank bioreactor agitated at 150, 450 and 800 rpm was used. Controls were carried out in 100 mL shake flasks agitated at 100 rpm. Cell growth and AQs production were evaluated. Specific growth rate (μ) attained in shake flasks cultures was 27% and 42% higher than those obtained in the bioreactor cultures at 450 and 150 rpm, respectively. Cells cultured at 800 rpm were not able to grow into the bioreactor. Cell cultures were mechanically elicitated by shear stress at 450 and 800 rpm increasing AQs biosynthesis by 230% and 243%, respectively. AQs volumetric productivity (Q_p) at 450 was 167% higher than that achieved in shake flasks. *R. tinctorum* cells showed moderately sensitivity in phytofermentative processes. The metabolic capabilities showed by these plant cells could be used as a potential strategy for industrial AQs production.

BT-P25.

EXPRESSION OF A DENGUE VIRUS ANTIGEN AND HEPATITIS B CORE FUSION PROTEIN IN *Nicotiana benthamiana* PLANTS

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Hepatitis B core antigen (HBcore) can self-assemble into VLP (virus-like particles) when it is produced in plants and it has shown to be an attractive carrier for presentation of foreign epitopes for the development of subunit vaccines. The small and critical dengue virus domain III of envelope protein (DVd3) contains multiple serotype-specific neutralizing epitopes. However, previous work showed that mice immunized with plant-made DVd3 protein without adjuvant elicited low immune responses. In order to produce a candidate for subunit vaccines and to avoid low immune responses in the corresponding pre-clinical studies, DVd3 was fused to HBcore and introduced into tobacco plant cells using deconstructed viral vectors. DVd3 and HBcore antigens were linked by a flexible linker peptide and co-infiltrated in *Nicotiana benthamiana* plants with 5' Module and Integrase Module. HBcore-DVd3 was produced successfully in *N. benthamiana* plants (0.43 mg/g fresh weight or 2.23% of total soluble protein) and the fusion protein obtained was reactive with anti-E and anti-HBcore antibodies. Preliminary sedimentation analysis on sucrose gradient showed a particulate nature of the plant-produced fusion protein. Our study demonstrated the potential use of plant-produced HBcore as a carrier protein for immunogenic presentation of dengue epitopes. Electron microscopy studies are currently being done.

BT-P26.

PRODUCTION OF 5-HALOGENATED NUCLEOSIDES BY SUSTAINABLE BIOPROCESS

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Nucleoside analogues are powerful chemotherapeutic agents. Halogenated derivatives are widely used in cancer treatment and they have the ability to be incorporated into nucleic acids for inhibiting their synthesis. Fluorinated nucleosides have shown their effect on several kinds of cancers.

Enzyme-catalyzed reactions have high catalytic efficiency and selectivity, and can take place under very mild conditions offering environmentally clean technologies. The efficiency of transglycosylation process for the synthesis of purine nucleoside analogues has been widely studied but there are few reports about biocatalytic obtaining halogenated pyrimidine nucleosides.

The aim of this study was to obtain 5-halogenated nucleoside analogues with potential antitumoral activity using a smoother, cheaper and environmentally friendly methodology.

A taxonomic screening was performed using different bacterial genus. The standard reaction contained 5-fluorouracil and different nucleosides as sugar donor. The supernatants were assayed by HPLC and product identifications were realized by HPLC/MS. In this work we describe an efficient, simple and one-pot biotransformation to obtain 5-halogenated compounds. Gram negative microorganisms have shown the highest activity in short reaction times. Yield of 32% (1h) and 94% (3h) of 5-halogenated ribo-and 2'-deoxyriboside were obtained, respectively.

LI-P01.

FUNCTIONAL CHARACTERIZATION OF *Bacillus cereus* ACYL-LIPID DESATURASES

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Fatty acid desaturases are responsible for the insertion of double bonds into fatty acids in reactions that require O2 and reducing equivalents. They play an important role in Bacilli cells to maintain membrane fluidity in response to a decrease in growth temperature. After cloning and functional characterization of two identified genes (*BC2983 and BC0400*) in *B. cereus* we concluded that they have 5 and 10 desaturase activity. Using different mutants well characterized in our lab we found that phospholipids are obligate intermediates for both desaturases since unsaturated fatty acids (UFAs) did not accumulated in mutants in the synthesis of phospholipids, and that ferredoxin and flavodoxins are part of the electron transport system associated with these desaturases. Previous results show that *B. cereus* UFAs proportion increases as the growth temperature decreases at 25ºC. This response was suppressed by addition of chloramphenicol or rifampicin for 5-Des while 10 was not affected. Surprisingly for 5-Des the levels of UFAs were increased in presence of both antibiotics. The response to temperature was analyzed with P*des-lacZ* fusions and -galactosidase activity measurements in order to identify a promoter region involved in expression. The dissection of this molecular mechanism in these bacteria will provide important insights into the basic question on the desaturation reaction.

LI-P02.

LIPID-INDUCED ACROSOMAL EXOCYTOSIS ANALYZED *IN VIVO* BY TIRFM

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During fertilization, the spermatozoon penetrates the zona pellucida to reach the oolema. Only sperm that have completed the acrosome reaction (AR) can accomplish this task. The AR is a calcium-regulated exocytosis where the membrane of the acrosome fuses to the plasma membrane. Sphingosine 1-phosphate, diacylglicerol and phorbol esters trigger the AR. The aim of this research was to analyze *in vivo* the dynamics of exocytosis in individual live sperm using TIRF, confocal microscopy and calcium variations in real time during exocytosis. Lysotracker allowed to localize the field in TIRFM and PSA-FITC stains the acrosome. We tested the response to different lipids in permeabilized and non-permeabilized cells (intact). Kinetics of acrosomal content losing between both groups varied greatly. In intact sperm AR started at the cell apex followed by a slow dispersal of acrosomal content. Permeabilized sperm initiated the AR at the ecuatorial segment and it finished in seconds. High resolution of TIRFM allowed the observation of different intensity spots representing fusion pores. Measurements of intracellular calcium using Fluo-3 AM yielded an immediate calcium increase after stimuli in intact cells and the emptying of the acrosomal store in permeabilized sperm. Our data provide a real-time view of lipid-activated exocytosis in a cell in which this fusion is a single and irreversible step.

LI-P03.

THE ANTIOXIDANT EFFECT OF SOME PINEAL INDOLES AGAINST LIPID PEROXIDATION DEPENDS ON THE ASSAY SYSTEM

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There is no general agreement yet on the antioxidant effect of pineal indoles against lipid peroxidation. Accordingly, it was the main goal of the present work to study the antioxidant activity of melatonin (MLT), N-acetylserotonin (NAS), 5-HO-tryptophan (5HO-TRP) and 5-methoxytryptamine (5MTP) in two different lipid systems with high content of polyunsaturated fatty acids (PUFAs): triglycerides (20:5 n3, C22:6 n3) dissolved in chloroform and sonicated liposomes (C22:6 n3) made of retinal lipids. In the triglyceride-chloroform-system the peroxidation reaction was initiated by cumen hydroperoxide whereas liposomes were peroxidated with Fe2+. The techniques employed at the present work were: 1) chemiluminescence, 2) TBARS production, 3) DPPH assay, 4) determination of conjugated dienes production and 5) analysis of fatty acid profile by CG-MS. Butilated hydroxitoluene (BHT) was employed as a reference because of its well known antioxidant capacity. Our results showed that MLT and 5MTP were unable to protect PUFAs against lipid peroxidation in both systems, whereas NAS and 5HO-TRP were better antioxidants that BHT in the triglyceride-system but ineffective in the liposome-system. We conclude that the antioxidant behaviour of pineal indoles depends not only on their functional groups but also on the assay system.

LI-P04.

***St*Asp-PSI AFFECTS THE STRUCTURE OF PHOSPHOLIPIDS DURING ITS INTERACTION WITH BIOMEMBRANES**

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We have reported the ability of the swaposin domain of potato aspartic proteases (*St*Asp-PSI) to produce leakage in vesicle membranes containing anionic phospholipids. However, the mode that *St*Asp-PSI perturbs the structure of lipidic bilayers is not well understood. Here, we examined the effect of *St*Asp-PSI on the structural and thermotropic properties of the major phospholipid types of bacterial and animal cells. Results obtained from the steady-state fluorescence anisotropy of the probe DPH showed that *St*Asp-PSI was able to perturb the lipid acyl chains in anionic membranes, composed of DMPG, DMPS and DMPA. The protein also caused a change in the anisotropy on DMPA vesicles using TMA-DPH probe, suggesting a perturbation of the lipid headgroups. Results obtained from differential scanning calorimetry showed that *St*Asp-PSI was able to disturb bilayers composed of DMPG and DMPS. The main thermal transition of the lipids was associated with two peaks, which should be due to mixed phases, indicating that one of them would be enriched in *St*Asp-PSI whereas the other one would be impoverished in it. The results suggest that the difference in charge between the lipid headgroups affects the *St*Asp-PSI incorporation into the lipid bilayer, and therefore it could be suggested that the protein should be primarily located at the lipid-water interface, influencing the fluidity of the phospholipids

LI-P05.
MOLECULAR MECHANISMS INVOLVED IN COX-2 EXPRESSION UNDER HYPERTONIC STRESS

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Renal medullary cells are exposed to variable and high concentrations of NaCl as part of the urinary concentrating system. Despite such adverse conditions, renal cells still survive and function by activating the transcription of various osmoprotective genes, among them, cyclooxygenase 2 (COX2). It was reported that PI3K and ERK1/2 are signaling pathways involved in cell survival and activated by osmotic stress. Therefore, in the present work we studied their role in the induction of COX2 expression reported as cytoprotective protein. With this purpose cultures of the renal cell line MDCK were grown during 5, 15, 30, 60 min and 1.5, 3, 6, 12 and 24 h in isotonic (298 mOsm/Kg H_2O) and NaCl-hypertonic (500 mOsm/Kg H2O) conditions, in the absence or presence of different specific inhibitors (LY294002, U0126). After the treatments cells were collected and submitted to westernblot for COX2 analysis. Hypertonic medium induced COX2 expression after 12 h of NaCl treatment. Surprisingly, LY294002, a PI3K inhibitor, did not prevent NaCl-induced COX2 expression but caused an over-expression of the protein from 6 h of treatment. ERK1/2 inhibitor U0126 did not affect COX2 protein in 60 min of treatment but a slight decrease in COX2 was observed in long-term treatment. Our data show that PI3K and ERK1/2 signaling pathways counterbalance NaCl-induced COX2 expression contributing to renal cell survival.

LI-P06.
OSMOTIC REGULATION OF LIPID METABOLISM IN RENAL EPITHELIAL CELLS

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Due to the urine concentration mechanism, the renal papillary interstitium has the highest osmolality of the body. To survive and work in such harmful environment, renal cells have protective mechanisms. We showed that papillary cells possess the highest phospholipid (PL) synthesis and turnover of the kidney, which contribute to preserve membrane homeostasis and cell viability. We also showed that renal phospholipid metabolism is regulated by osmolarity. Considering that PL synthesis require an adequate supply of fatty acids, in this work we characterized the relationship between PL and triacylglycerol (TG) in renal cells.The levels of PL and TG were determined in the different zones of the kidney and in cultures of the renal cell line MDCK grown during 24, 48, 72 and 96 h in media with osmolalities from 298 to 570 mOsm/kgH2O. Renal cortex (isosmolarity) showed the highest PL content but the lowest TG content and PL synthesis. In opposite, papilla showed the lowest PL content but the highest TG content and PL synthesis. In MDCK cells, we found that hyperosmolarity significantly increases PL and TG content. Such increase is dependent on the time of incubation and the osmolarity of the medium. These results demonstrate the relationship between renal PL synthesis and TG content and the role of environmental osmolarity as a distinctive regulatory factor of renal lipid metabolism.

LI-P07.
ETHER-LINKED TRIGLYCERIDES OF RAT EPIDIDYMIS

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Long-chain n-9 polyunsaturated fatty acids (PUFA), particularly 22:4n-9, are normal components of the plasmenylcholines of rat epididymal spermatozoa. Since these uncommon PUFA are absent from lipids of testicular cells, their accretion is expected to occur during the passage of the gametes through the epididymis. In this work we focused on the neutral lipids that are present in the epididymal caput, corpus, and cauda regions. In addition to cholesteryl esters, the three areas contained ether-linked triglycerides (1-O-alkyl-, and 1-alk-1´enyl- diacylglycerols, ADG). Interestingly, the latter were characterized by high proportions of n-9 PUFA. The amounts of these ADG gradually increased during development: they were detected in rat epididymis on day 14 after birth (P14), their amounts increased markedly at P49, in association with the complete differentiation of all epididymal cells, while reaching maximum levels at P55, the time at which the first spermatozoa appeared in the epididymal lumen. In adult rats, the smallest but metabolically most active portion of the epididymis, the corpus, had relatively more 22:4n-9-rich ADG than the other two regions. Our data suggest that epididymal ADG may be involved as lipid intermediates during the active membrane remodeling that spermatozoa are known to undergo during their extra-testicular maturation

LI-P08.
GERANIOL REGULATES HMG COA REDUCTASE AT A TRANSCRIPTIONAL LEVEL IN HepG2

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Plant isoprenoids, a broad class of plant products derived from the mevalonate pathway are widely distributed in fruits and vegetables. It has been suggested that these compounds suppress the growth of cancer cells and development through multiple effects on mevalonate pathway. We have reported that geraniol (G), a natural monoterpene, inhibits cholesterol synthesis and cell growth at concentration greater than 10 µM and 100 µM respectively in HepG2 cells. We also reported that 50 µM G inhibit the conversion of lanosterol into cholesterol. The aim of this study was to analyze the mechanism through which G exerts these effects on the mevalonate pathway in human liver cells. We studied the expression of the HMGCoA reductase and SREBP2 genes in cells incubated at different times with 50µM and 200µM G. HMGCoA reductase levels were determined by western blot and enzyme activity was measured. At 4 h G treatment, real time PCR revealed that the expression of genes was reduced in a dose dependent manner. Consequently, a decrease in quantity and activity of the enzyme and a reduction in cholesterol levels were observed at 24 h. This induces an increase in the enzyme expression. These results suggest that geraniol decreases cholesterol synthesis by transcriptional inhibition of HMGCoA reductase in a direct or indirectly way, and by inhibiting the conversion of lanosterol to cholesterol.

LI-P09.

CHARACTERIZATION OF MANDARIN ESSENTIAL OIL (MO). EFFECT ON CHOLESTEROL METABOLISM IN HepG2

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Essential oils are fragrant volatile oils found in specialized plant cells or structures. Among their components, monoterpenes are phytochemicals that could have antitumor activity because of their multiple effects on mevalonate pathway. We have shown that MO (*Citrus reticulata* Blanco) and limonene (LM) exert antiproliferative activity against HepG2 cells with an IC50 = 0.063 µl/ml and 0.15 µl/ml respectively. The aim of this work was to determine the volatile organic compounds (VOCs) of MO and to study the effect of MO and LM on cholesterol homeostasis in HepG2 cells. VOCs of MO were determined by capillary gas chromatography coupled to mass spectrometry. Cholesterogenesis, cholesterol content, export and import were determined using radioactive precursors and biochemical methods. More than 89% of MO volatile fraction corresponds to limonene. Linalool, n-decanal, perill aldehyde, citronellol and dodecanal are minor components. Endogenous synthesis and incorporation of exogenous cholesterol are decreased in both treatments with a cholesterol content maintained or slightly increased. Cholesterol export diminished only in LM treated cells. Our data suggest that the antiproliferative activity of MO and LM in HepG2 cells would not be related to depletion of endogenous cholesterol pool and the effect on cholesterogenesis would be the consequence of the inhibition of cell growth.

LI-P10.

LINALOOL AFFECTS MEVALONATE PATHWAY AND CHOLESTEROL HOMEOSTASIS IN HepG2 AND A549 CELLS

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Monoterpenes including geraniol, limonene, linalool (LN), menthol, are non-nutritive components of diet present in essential oils of citrus, rosemary, basil, mint and other herbs. These 10-carbon isoprenoids are derived from the mevalonate pathway (MP) in plants but are not produced by mammals and fungi. In previous studies we demonstrated that LN inhibits cell proliferation (CP) in HepG2 (IC50 = 1550 uM) and A549 (IC 50 = 1100 uM) cells, phenomenon attributed to the multiple pharmacological effects of monoterpenes on the MP. The aim of this work was to study the effect of LN on the MP and cholesterol (Cho) homeostasis. Cells were treated with increasing concentrations of LN that do not inhibit significantly CP in HepG2 (0-800 uM) and A549 (0-700 uM) cells. Incorporation of *14*C-acetate into MP intermediates separated by TLC was determined. Acording to the results we chose an LN concentration (200 uM) that inhibits cholesterol synthesis (ChS) in both cell lines to assess Cho import (ChI), Cho export (ChE) and its cellular content (ChC). Our data show that ChS decreases (30 and 24%), ChI increases (19% and 49%) and ChC decreases (18%) in HepG2 and A549 cells, respectively. We conclude that in conditions where the cell proliferation was not affected, linalool significantly altered the choleterogenesis and its cholesterol homeostasis.

LI-P11.

SIMVASTATIN AND GERANIOL INHIBIT TUMOR GROWTH OF A549 CELLS IMPLANTED IN NUDE MICE

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Plant isoprenoids and statins inhibit HMGCoA reductase and mevalonate pathway by different mechanisms. We have previously reported that both classes of compounds affect cholesterol synthesis and cell proliferation "*in vitro*". To investigate the impact of dietary geraniol (GOH), a natural monoterpene, simvastatin (S), a statin, and their combination on lipid metabolism and growth of tumor cell "*in vivo*", we implanted A549 cell line in nude mice. Animals were fed a control or experimental diet (50mmol.GOH/Kg diet; 50 ug/g body weight/day; 50mmol.GOH/Kg diet + 50 ug/g body weight/day) for 21 days after tumor implant. Tumors were measured twice a week. Three hours before sacrifice, animals were injected with 25uCi of ^{14}C-acetate. Cholesterolemia, incorporation of radioactivity into tumor and liver lipids were determined. Tumor caspase activity and percent of apoptotic cells were measured. Tumor growth was significantly reduced by all treatments. Although serum, hepatic and tumor cholesterol did not present any differences, the incorporation of ^{14}C-acetate into nonsaponifiable lipids was significantly decreased in GOH + S group. Moreover tumor apoptosis was induced by treatments. Our data contribute to a better understanding of the action of a common monoterpene targeting a complex metabolic pathway which would improve the use of statins in the fight against cancer

LI-P12.

ANTIPROLIFERATIVE EFFICACY OF DIVERSE *Lippia alba* CHEMOTYPES. EFFECTS ON THE MEVALONATE PATHWAY

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Lippia alba (Mill.) N. E. Brown is an aromatic shrub widely used as folk medicine in America. There are many chemotypes (CHM) of *L. alba* essential oil (EO) mainly due to its wide geographical extension. Each EO has a different composition of monoterpenes qualitatively and quantitatively. Several monoterpenes have an antiproliferative activity and synergistic effect, affecting the mevalonate pathway at multiple stages. The aim of this study is to evaluate the antiproliferative capacity of EOs of four CHMs with marked composition differences. HepG2 and A549 cells were treated with increasing concentrations of EOs from citral(C), piperitone(P), tagetenone(T) and carvone(Ca) CHMs. Cellular proliferation (CP) was determined by MTT assay. C and T CHMs that are composed predominantly of acyclic monoterpenes present an IC50 ten times lower than P and Ca CHMs whose composition is mainly of cyclic ones. This would indicate that acyclic monoterpenes have a higher efficacy in the CP inhibition. To analyze the effect of T CHM at non inhibitory CP doses in the mevalonate pathway, the incorporation of ^{14}C acetate in intermediates and final products were determinated. A dose dependent decrease in the cholesterol synthesis and increase of ubiquinone and some intermediaries were observed, suggesting an inhibition of cholesterogenesis at a latter phase from the ramification point of the pathway.

LI-P13.

AEROBIC PATHWAY FOR UNSATURATED FATTY ACID SYNTHESIS IN PEANUT NODULATING RHIZOBIA

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In *Bradyrhizobium* sp.TAL1000 and *Ensinfer meliloti* 1021, unsaturated fatty acids (UFA) play an important role in maintaining the fluidity of the membrane against a change in environmental conditions.

In certain bacteria the synthesis of UFA occurs by an aerobic mechanism, mediated by desaturase enzymes (Shanklin *et al*., 1998). In the present work, we have succeeded in cloning a gene encoding a putative desaturase from *B.* TAL1000 and *E. meliloti* 1021 and have determined its function by expression in *E. coli*. The putative desaturase genes from *B.* TAL1000 and *E. meliloti* 1021 were amplified by PCR and cloned into plasmid pET17b. These construct were used to transform *E. coli*. After addition of IPTG, the cultures of transformed *E. coli* strains were collected and their FA compositions were analyzed by GC. Desaturase activity was analyzed by addition of radioactive FA to the culture. The levels of 16:0 and 18:0 decreased, while 16:1 9 and 18:1 (11+ 9) levels were enhanced in both transformed strains compared to the control. *E.coli* strains overexpressing each of the putative desaturase genes were able to desaturate [1-^{14}C] 16:0 and [1-^{14}C] 18:0 to monounsaturated FA. These results indicate that *Bradyrhizobium* sp.TAL1000 and *Ensinfer meliloti* 1021 contain in their genomes a gene of a single sequence encoding a FA desaturase.

LI-P14.

IMPORTANCE OF FATTY ACIDS IN THE IDENTIFICATION AND CHARACTERIZATION OF NATIVE STRAINS FROM PEANUT

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Due to the importance of biochemical tests in bacterial identification and characterization, this work studied three native strains isolated from peanut. The strains were grown at 28ºC. Cells were harvested, total lipids extracted and fatty acids (FA) methyl esters prepared using BF_3 in methanol. FA analysis was performed using GC. Phospholipids (PL) and FA biosynthesis were studied by adding [1-^{14}C]sodium acetate at time 0 of culture and then using TLC plates. L115 and L5N23 showed similar FA profiles: 18:1 >50% and 18:0 >19%, unlike L3N23: 17:0 40%, 15:0 29%. In L115 and L5N23 strains, the greater radioactivity incorporation was into monounsaturated FA fraction, while in L3N23 was into saturated FA fraction. The main PL radioactive fraction in L115 and L5N23 was phosphatidylcholine and in L3N23 was phosphatidylethanolamine. The identification by 16S rDNA gene sequence indicated: L115, genus *Ochrobactrum*; L5N23, *Phyllobacterium*; L3N23, *Paenibacillus*. FA composition from the three strains is consistent with the genetic identification, but the proportion of some components differs from that in the bibliography. The FA composition in L115 and L5N23 resembles to that described for other rhizobia, while FA composition in L3N23 does not.

LI-P15.

K107: A NATURAL VARIANT OF APOLIPOPROTEIN A-I PROMOTES DIFFERENTIAL CELLULAR RESPONSES

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Apolipoprotein A-I (apoA-I) is the major protein in the high density lipoproteins (HDL), and it plays a relevant role in the antiatherogenic reverse cholesterol transport. ApoA-I have two repeats with a particular type Y charge distribution, which have been proposed as key role players in the apoA-I mediated cell lipid efflux. Patients carrying an apoA-I variant with a single deletion at the central type Y helical region (K107) have an impaired HDL metabolism and increased atherogenic risk. To know if the cellular responses to K107 are altered we have compared the behavior of K107 with wild type apoA-I and another variant with a deleted Lysine (K226) in the C-terminal type Y helical repeat. Macrophages RAW 264.7 stimulated with the different proteins were used for comparing the expression and intracellular cholesterol (Chol) pool available for esterification by acyl CoA cholesterol acyl transferase (ACAT). Also we have evaluated the efflux of choline-phospholipids (PL) as well as sphingomyelin (SM) and Phosphatidylcholine (PC) intracellular content. We have detected that K107, but not K226, increased ACAT protein level in different Chol loading conditions (0, 10 or 50, µg/ml Chol). However, only a slight ACAT activity increase is produced by k107 with low Chol load condition. All the proteins were active in promoting PL efflux.

LI-P16.

EFFECT OF *Amaranthus hypochondriacus* ON THE FATTY ACIDS METABOLISM IN RATS INTOXICATED WITH ETHANOL

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The aim of this study was to evaluate the possible protective role of the seed of *Amaranthus hypochondriacus* (Ah) on expression of gene involved in the regulation of fatty acids: acetyl-CoA carboxylase (ACC), peroxisome proliferator-activated receptor (PPAR) and carnitine palmitoyl-transferase I (CPT-I), and the content of triglycerides, in liver of Wistar male rats treated with ethanol.

We worked with four groups of six rats each; two of these groups were fed with diet AIN-93M and the other two with AIN-93M containing Ah as protein source. One of each group received 20% ethanol in the drinking water, being: AhC (Ah control) and CC (casein control), AhE (Ah ethanol) and CE (casein ethanol). The experiment was performed for 4 weeks. The mRNA levels were estimated by RT-PCR.

In AhE group, the ACC mRNA levels decreased, compared to CE and AhC groups ($p<0.01$ and $p<0.05$, respectively). The groups fed with amaranthus did not exhibit differences in the expression of CPT-I, PPARa and triglyceride content. CE group showed an increase of triglycerides ($p<0.01$), PPARa ($p<0.01$), and trend of increase of CPT-I, compared with CC. We conclude that *Amaranthus hypochondriacus* would contribute to reduce fatty acid synthesis caused by ethanol.

LI-P17.

TEMPORAL REGULATION OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN SYNCHRONIZED CULTURES OF FIBROBLASTS

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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 (Marquez *et al*., 2004). However, little is known about the temporal regulation of phosphatidylcholine (PC) metabolism. Here, we examined the daily variations in PC levels and expression of PC synthesizing enzymes in quiescent NIH 3T3 cells synchronized by serum shock (SS). PC is mainly synthesized through the Kennedy's pathway and catalyzed by choline kinase (CK), choline cytidylyltransferase (CT) and and cytidine phosphotransferase (CPT). We found a higher content of labeled PC at 6.5 h after SS. We also found detectable mRNA levels for all CT isoforms, with higher expression of CTa at all times tested (0-36 h). Both CTa1 transcript and protein peaked at 3 h post stimulation, while CTb2 mRNA was higher at 9-12 h. We also detected increased levels of CTb isoform proteins during 9-18 h by WB. Preliminary results showed that CKa transcript assessed by qPCR is elevated during first 18 h post-synchronization while CKb mRNA remains constant during 36 h examined. Results suggest that synchronized fibroblasts exhibit temporal variations in PC content which could be due, at least in part, to changes in the expression of CK and CT enzymes.

LI-P18.

CIRCADIAN VARIATION IN PHOSPHATIDYLCHOLINE CONTENT AND SYNTHESIZING ENZYME EXPRESSION IN MOUSE LIVER

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Previous studies showed a circadian regulation in the biosynthesis of phospholipids in the vertebrate nervous system as well as in an immortalized cell line (Guido *et al*., 2001; Marquez *et al*., 2004). The circadian control of phospholipid synthesis and specifically of phosphatidylcholine (PC), the most abundant lipid in all eukaryotic cells, is slightly known. In the present work, we investigated the temporal variation in the membrane phospholipid composition, mainly focused in PC as well as in the expression of its synthesizing enzymes choline kinase (CK) and cytidylyltransferase (CCT) in mammalian peripheral tissues. For this, C57BL/6J mice were exposed to a 12:12 h light dark (LD) cycle or kept in constant dark (DD), killed at different times while livers and testes were dissected out and collected for further assays. mRNAs levels were determined by real-time qPCR using the clock gene Bmal1 as a circadian marker. In testes, we did not observe any temporal variation in lipid composition, expression of PC synthesis enzymes or Bmal1 mRNA, under any illumination condition tested. However, in the liver, we found a significant change in levels of CK mRNA, in content of PI, PC and in the ratio PC/PE in DD. These results strongly suggest that there is a close relationship between clock driven-mechanisms and phospholipid metabolism in peripheral tissues of synchronized mice.

LI-P19.

EFFECT OF WATER-SOLUBLE CRUDE OIL HYDROCARBON EXPOSURE ON PROTEIN EXPRESSION OF A FRESHWATER PRAWN

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Oil spills are common around the world and a major source of estuarine oil pollution. However, little is known of how it affects species in the community. In order to study the biological effect of a freshwater oil spill on organism and identify potential pollution biomarkers, we analyzed male and female subproteomes of the prawn *Macrobrachium borellii* exposed to the water-soluble fraction (WSF) of crude oil in a laboratory bioassay.

To this aim, protein mixtures were extracted from midgut gland prawns exposed for 7 days to a sublethal concentration of WSF (0.34 ppm), mostly a mixture of low-boiling aromatic hydrocarbons. Midgut gland proteins from non exposed prawns were used as control. Protein extracts were subsequently subject to 2D gel electrophoresis and spots from exposed and control gels compared. Differentially-expressed proteins were analyzed by ESI-TOF/TOF mass spectrometry and their identity assigned in a MASCOT search mined against all known proteins. The presence of several proteins over- and underexpressed was observed in both sexes.

The analysis of differentially expressed proteins in prawns exposed to hydrocarbons provided the identification of proteins which could be employed as potential biomarkers, highlighting the usefulness of 2DE-based proteomic analysis in pollution studies.

LI-P20.

EFFECT OF LDL-OX ON RAW MACROPHAGE SURVIVAL

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Isolated LDL fraction from human plasmas was in vitro peroxidized with Cu^{++} (5 µM) for 4 h (low, L), 8 h (medium, M) and 24 h (high, H) , dialyzed overnight to wash-out the copper ions, and assayed for 24 h at a final concentration of 100 µM in cultures Raw 264.7 murine macrophages to study the effect of LDL-Ox receptor activation on cell survival pathways. Percentage of dead cells -evaluated by trypan dye exclusion- compared to control flaks incubated with native LDL fraction was increased in the L, M and H assays. Lactate-dehydrogenase leakage was only significantly increased in cells treated with H-LDL. Specific activities of mili- and µ-calpaines picked in cultures treated with L fraction and returned to control values under M or H treatments. Caspase-3 activity increased consistently from L to H assays. These changes were observed concomitantly with increased activities of glutathione-peroxidase, -transferase and –reductase, catalase and superoxidedismutase. Total glutathione and the ratio GSH/GSSG were progressively decreased by these treatments (L to H). Results indicated that survival of macrophages exposed to oxidized LDL depends on the degree of peroxidation of the lipoprotein suggesting that the mechanism of damage is associated to alterations of the enzymatic antioxidant system and it involves different intracellular protease systems.

LI-P21.
COPPER-DEPENDENT CHANGES ON LIPID COMPOSITION OF VARIOUS RAT TISSUES
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Copper (Cu) levels are associated to the etiological mechanism(s) involved in neurodegeneration, and they strongly influence the cell antioxidant status. We studied the effect of Cu deficiency and overload on lipid and fatty acyl composition (FA) of plasma, brain, testis, heart, lungs and liver in Wistar rats fed a semi-synthetic diet Cu-deficient and with different doses of the metal (7 to 35 ppm) added to food and water, or injected intraperitoneally (5 weeks) in order to evaluate the impact of the administration route and the changes induced by the grade of overload. Changes observed were different depending on the dose, administration route and tissues analyzed. A common alteration in Cu deficiency was the elevation of cholesterol and triglyceride (TG) with no significant changes in the phospholipid (PL) subfraction. This effect decreased in the order liver>plasma>testis=brain=lungs>hearth. In TGs of Cu-deficient rats the ratio monoenoic/saturated FA (M/S) and the level of 20:4n-6 decreased while 22C-FAs decreased with the exception of 22:5n-3 content that was increased. In PL, 18:2n-6 increased while 20:4 n-6 decreased. Cu overload provoked an increase of M/S in TGs while 18:2n-6 decreased. Saturated FA increased in PL fraction concomitantly with a decrease of linoleic and PUFAs. Results demonstrated that intraperitoneal overload produced stronger changes than oral overload.

LI-P22.
COPPER HOMEOSTASIS AND ANTIOXIDANT DEFENSE SYSTEM IN RATS WITH COPPER DEFICIENCY OR OVERLOAD
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Copper (Cu) availability is involved in atherosclerosis and neurodegeneration through changes in the antioxidant defense system (AS). We studied the AS in plasma, brain, liver, testis, hearth, lungs and intestine of Wistar rats fed a semi-synthetic diet Cu-deficient and with different doses of the metal (7 to 35 ppm) added to food and water, or injected intraperitoneally for 5 weeks. Total glutathione (at expense of GSSG) increased in response to Cu overload in all the tissues. Nitric oxide and protein carbonyls were increased (especially in liver). Lipid peroxidation (TBARS) was important in brain, as well as in liver, testis and intestine. Metallothioneins were increased in intestine and liver -and to a lesser extent in the other tissues- while cerulloplasmin increased in all the tissues in a similar magnitude. Glutathione reductase, -peroxidase and catalase were activated by Cu overload in all the tissues being the effect more important in the intraperitoneally-injected rats than in the orally treated ones. Cu deficiency decreased glutathione peroxidase and superoxidodismutase activities. Ferric reducing ability of plasma and tocopherol levels was modified according with those damages observed in the AS. Results demonstrated that Cu overload caused more alterations than deficiency, and that the acquisition of the metal by parenteral route was more dangerous than the oral one.

LI-P23.
PUFA-RESTORATIVE EFFECT ON THE DIMETHOATE-INDUCED DAMAGE OF RAT LEIDYG CELL STEROIDOGENESIS
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Previous studies from our lab demonstrated that dimethoate (D) administered to rats produce a strong inhibition of the androgenic activity in testis. The mechanism involves the inhibition of the StAR protein, stimulation of COX-2 and inhibition of steroidogenic enzymes (HSD) via ROS overproduction. In this work we studied the possible reversion of the damage by co-incubation of D with various fatty acids in isolated Leydig cells. The level of expression of StAR protein was increased by 20:4n-6 (AA) and, to a lesser extent, by 20:3n-6 (ETA), while 22:4n-6 (DTA), 22:6n-3 (DHA) and 22:5n-3 (DPA) did not show any effect. AA and ETA increased COX-2 expression and the production of PGF2? and E2, TBARS, the content of cholesterol and AA in mitochondrial phospholipids, while they decreased the activities of 3? and 17?HSD. DTA increased TBARS production and decreased both HSD activities. DHA and DPA strongly inhibited the expression of COX-2 and the production of PGs, the level of TBARS and the phospholipase A2 activity. Both acids increased mitochondrial cholesterol and the activities of HSD enzymes. As a result of the balance between these variables, the decreased production of testosterone caused by D treatment was restored by AA, DHA and DPA. DTA evidenced no restorative effect and DTA not only failed to restore but also caused an ulterior decrease of the androgen biosynthesis.

LI-P24.
A *DES*A3 KNOCK-OUT *Mycobacterium smegmatis* MUTANT IS UNABLE TO SYNTHESIZE PALMITOLEIC ACID
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Fatty acid desaturases are enzymes that introduce double bonds into saturated fatty acids to yield unsaturated fatty acids (UFAs). In mycobacteria UFAs are essential for growth. Oleic (C18:1) and palmitoleic (C16:1) acids are present in membrane phospholipids, mycolic acids, lypoglycans and triglycerides. Despite the importance of UFAs in the mycobacterial physiology, little is known about the mechanisms of their synthesis and regulation. desA3 is the only stearoyl-desaturase from *Mycobacterium tuberculosis*, whereas at least five homologous genes are encoded in the genome of *M. smegmatis* (*MSMEG*_1886, *MSMEG*_1211, *MSMEG*_1743, *MSMEG*_6835 and *MSMEG*_3333). We generated knock-out mutants of *MSMEG*_1886 and *MSMEG*_1211 on the basis of their high homology to *des*A3: we evaluated the growth of *M. Smegmatis* 1886 in liquid and solid media and the lipid profile by TLC and GC/MS in presence/absence of oleic acid at 25, 37 and 42°C. The mutant grown in the absence of oleic acid displayed an altered colony morphology and could grow (albeit at slower rate) at 37 and 42°C, but was unable to grow at 25°C. Fatty acid analysis revealed that C18:1 was severely reduced and C16:1 was absent. These data suggest that *MSMEG*_1886 could be a fatty acid desaturase, with specificity for palmitic acid.

LI-P25.

ANALYSIS OF ORTHOLOGS OF STEROL METABOLISM FROM *Tetrahymena*

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Tetrahymena is a free living ciliate, that neither is auxotrophic nor synthesizes sterols *de novo*. Instead of sterols, it synthesizes tetrahymanol. However, the ciliate harbors orthologs of sterol metabolism, such as C-4 methyl oxidases (SMO), which are involved in C-4 demethyation process in sterol biosynthesis. *Tetrahymena* is able to perform sterol modifications when they are added to the culture media: C-5, C-7 and C-22 sterol desaturase and C-24 dealkylase.

In order to find out whether *Tetrahymena* has an active SMO (SMOTt), we assayed different 4-methylsterols. Wild type (WT) *Tetrahymena* cells were cultured with 4-methylsteros and their sterol profile was analized by GC-MS. Moreover, we have knocked-out (KO) four putative SMOTt genes and the sterol profile of the mutants was analyzed by HPLC.

The preliminary results obtained suggest that none of the substrates assayed are metabolized by WT cultures. Although phylogenetic analysis showed that orthologs from *Tetrhaymena* group in the same cluster that SMO, the sequence analysis showed the lack of some conserved motifs present in known SMOs. On the other hand, the sterol profile of the KO mutants cultured with cholesterol or stigmasterol showed no differences with WT, indicating that the genes are clearly not belonging to the sterol desaturases nor C-24 dealkylase. However, full characterization is still necessary.

LI-P26.

LIPID BIOCHEMICAL AND BIOPHYSICAL CHANGES AFTER RAT SPERM CAPACITATION AND ACROSOMAL REACTION

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Recent work showed that sperm capacitation and acrosomal exocytosis, when induced *in vitro* in rat spermatozoa, are accompanied by hydrolysis of specific lipid classes. Here, we aimed at studying how these reactions affect the biophysical properties of the sperm total lipid. In contrast to ejaculated sperm, in spermatozoa that are isolated from cauda epididymes a rapid uptake of extracellular calcium occurs that leads to many of them undergoing a premature acrosomal exocytosis. To avoid this "spontaneous" process, we included EDTA in all media used for sperm isolation from rat epididymis. This prevented the partial hydrolysis of sperm head-located sphingomyelin to ceramide and of main glycerophospholipids to lysophospholipids and free fatty acids or diglycerides. Significant increases in the generalized polarization of the fluorescent probe Laurdan were observed between liposomes prepared from the lipid of these controls, of gametes isolated with no EDTA, and of those incubated in conditions that promote their capacitation and acrosomal reaction. Interestingly, the hydrolysis of main glycerophospholipids evoked by the latter reactions affected mostly the 1,2-diacyl subclasses, the gametes thus becoming relatively enriched in plasmalogens. Our data suggest that the lipid changes that occur during these physiologically relevant processes encourage an increase in sperm membrane order.

LI-P27.

SECRETORY PHOSPHOLIPASE A_2, A RELATIONSHIP BETWEEN OXIDATIVE STRESS AND INFLAMMATORY RESPONSE?

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Iron accumulation and oxidative stress are hallmarks of retinas from patients with age-related macular degeneration (AMD). We previously characterized the involvement of phospholipase A_2 (PLA_2) isoforms in a model of AMD. Retinal iron exposure provoked an increase in lipid peroxidation levels, a decrease in mitochondrial function and stimulated ERK activation (SAIB 2010). We demonstrated that cytosolic PLA_2 ($cPLA_2$) participates in the generation of lipid peroxides whereas the calcium-independent isoform prevents lipid peroxidation. The purpose of this work was to demonstrate a connection between $cPLA_2$ activation and inflammatory responses in a model of AMD. We found that U0126 (MAPKK inhibitor) inhibited the iron-induced increase of arachidonic acid release in cytosolic fractions. The expression of secretory PLA_2 and its relation with cyclooxigenase-2 were also evaluated. Both enzymes were associated with microsomal membranes in an iron concentration-dependent manner. We also investigated NF- B state as one of main targets of inflammatory response. p65 (RelA) levels were increased in nuclear fractions from retinas exposed to iron. Our results demonstrate that PLA_2 isoforms are key players in this experimental model resembling AMD and we suggest that they are connectors between iron-induced oxidative stress signaling and inflammatory responses.

LI-P28.

THE CERAMIDE METABOLISM DURING MDCK CELL DIFFERENTIATION IS REGULATED BY HYPERTONICITY

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Recently, has been reported that stearic acid besides to enter the sphingolipid metabolic pathway as ceramide synthase (CerS) substrate can also be a substrate for serinepalmitoyl transferase (SPT). Hypertonicity is a condition of MDCK cell differentiation mediated by an increase of complex sphingolipids synthesis. Now we evaluate the ceramide (Cer) metabolism under iso or hypertonicity by submitting confluent MDCK cells to extracellular iso or hypertonic medium. Cer metabolism was determined using ^{14}C-palmitic (PA) or ^{14}C-stearic (SA) acids for 24h in the presence or absence of cicloserine (CS) or fumonisin B1 (FB1). Two bands of radioactive Cer were found in the TLC plate, a slower Cer1 (lower MW) and an upper Cer2 (higher MW). Hypertonicity induced an increase of both radioactive fatty acids in Cer1 and Cer2. Under isotonicity, both ^{14}C-SA Cer1 and Cer2 were inhibited by FB1 but not by CS, while under hypertonicity both agents evoked inhibition. FB1 and CS decreased ^{14}C-PA incorporation to Cer1 and Cer2 under isotonicity while CS had no effect under hypertonicity. The results demonstrated that ^{14}C-PA enters the sphingolipid metabolic pathway as substrate of SPT and CerS under isotonicity but it is not substrate of SPT under hypertonicity. By contrast, ^{14}C-SA enters the pathway only as substrate of CerS at basal conditions, but under hypertonicity can also be a substrate for SPT.

LI-P29.
NUCLEAR LIPID DROPLETS PROTEINS
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Nuclear lipids participate in signaling events and as ligands (FA) of transcription factors (PPARs and HNF 4). We have demonstrated that neutral lipids are organized within the nucleus as Nuclear Lipid Droplets (NLD), composed of TAG and CE in a hydrophobic core surrounded by a monolayer of polar lipids with C and associated proteins. NLD are lipid sources with different chemical composition, physical properties regulation and functions to that of nuclear membrane lipids. The aim of the present work was to study NLD proteins. With this purpose NLD were isolated by sucrose-gradient and analyzed by 1D electrophoresis and MALDI TOF/TOF. Individual spots from 2D gels were not detected due to NLD low sample amount. NLD protein profile was: 1) different to that of cytosolic LD (CLD) and nucleus (N); 2) resolved in 11 - bands (main: 3, 7, 11, 4, 2); 3) 1, 3 and a11 were not present in CLD; 4) 3 was enriched in NLD with respect to N. Isoforms of hnRNP (heterogeneous nuclear ribonucleoproteins) A2/B1, C1/C2, K and M were identified by proteomic analysis from main bands.
In conclusion, since hnRNP form actin nuclear complexes involved in nucleoplasmic transport, we can consider that hnRNP of NLD monolayer would participate in NLD movement within the nucleus to deliver lipids and proteins to different nuclear domains.

LI-P30.
RESVERATROL MODULATES ATPase ACTIVITY OF CHOLESTEROL TRANSPORTER ABCG1
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Atherosclerosis is an inflamatory disease of the blood vessels that constitutes the primary cause of cardiovascular disease.
High density lipoproteins have a pronunced protective effect against since they are involved in the transport of cholesterol from peripheral tissues to the liver. Stimulation of the activity of cholesterol transport proteins involved in this process was suggested as a promising strategy for the treatment and prevention of atherosclerosis.
ABCG1 is one of the key transporters involved in the formation of HDL. In this work, human ABCG1 gene was sub-cloned into the pCHH10m3C shuttle vector. Then two strains of *Saccharomyces cerevisiae* were transformed using a variation of the classic lithium acetate protocol.
The transporter was purified from the membrane fraction by affinity chromatography. Purified ABCG1 kept its ATPase activity in detergent micells as well as after reconstitution into liposomes. Finally, the modulation of such activity was evaluated upon addition of resveratrol; and it was concluded that this polyphenol was able to enhance ABCG1 ATPase activity by aproximately 50% at 0.1 mM.
This work provides the foundation for the future implementation of this strategy in the evaluation of compounds on ABCG1 activity, enabling a quick and reliable identification of potencially active substances by *in vitro* activity assays.

LI-P31.
EFFECTS OF URBAN DISCHARGE ON LIPID AND FATTY ACID COMPOSITION OF *Diplodon chilensis*
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The mussel *Diplodon chilensis* is abundant in lakes and rivers from Patagonia and it is considered as a potential bioremediation tool, reducing coliform bacteria from domiciliary wells. We evaluate changes in their lipid and fatty acid composition, by TLC and GC, on control and urban discharge polluted sites. Oxidative stress parameters were determined (antioxidant enzymes, GSH, lipid and protein oxidation) as well as glycogen and lipofuxin contents. The neutral/polar lipid ratio was higher in organisms come from the polluted area due to an increase in DAG and TAG and a decrease in PE. Polar lipids were the most affected, displaying higher amount of saturated FA (C18:0) and a decrease in the unsaturated FA, n3 and n6 (C16:1n7, C20:4n6, C21:5n3, C22:3n6). Lipid oxidation levels and lipofuxins were higher in bivalves from the polluted area, showing higher GSH levels and lower glycogen content. No changes were observed in antioxidant enzyme activities. These results demonstrate that a chronic exposure to urban effluent produces in *D. chilensis* an increase in GSH level, which is not enough to counteract the oxidative damage to lipids, especially to the polar ones and their PUFA. This last would indicate changes in the membrane permeability, being capable of maintaining storage lipid levels, probably at the expense of the glycogen consumption.

MI-P01.
IDENTIFICATION OF WHEAT PROTEINS INTERACTING WITH *Mal de Río Cuarto virus* VIROPLASM PROTEIN P9-1

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Mal de Río Cuarto virus (MRCV, *Fijivirus*, *Reoviridae*) causes the most important maize disease in Argentina. The virus infects grasses such as maize and wheat, where the infection is limited to the phloem, as well as planthoppers which transmit the disease. During its infection cycle, MRCV replicates in citoplasmic viral inclusion bodies termed viroplasms, composed mainly of the non-structural viral protein P9-1. In animal reovirus it has been shown that viroplasms also contain other viral proteins and host cellular components with unknown functions. In this study, we searched for possible interactions between P9-1 and wheat proteins by screening a cDNA library obtained from wheat leaves against the bait P9-1 by yeast two-hybrid (Y2H). We identified three wheat interacting proteins: a putative acyltransferase (act3 and/or acT4), a chlorophyll a/b-binding protein (Wcab) precursor and an unknown protein with aminoacid sequence homology to the spinach tylakoid soluble phosphoprotein (TSP9). Remarkably, Wcab and TSP9 are both chloroplastic, and it has been previously reported that MRCV infection alters the chloroplast´s shape and the organization of the thylakoid system of maize bundle sheath cells. Our results suggest that MRCV P9-1 could interfere with chloroplast components, yet the impact of such alteration in the infection cycle remains so far unknown.

MI-P02.
DEVELOPMENT OF HALOPHILIC BIOFILMS AS A TOOL FOR STUDYING ANTAGONIC INTERACTIONS

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Biofilms are the most frequent bacterial way of life in Nature. These multi-species communities grow in virtually every interface and are the focus of intense research all over the world. Notably, the present knowledge on biofilms composed by halophilic microorganisms is scarce. Aiming at analyzing the capacity of these microorganisms for biofilm formation, pure cultures of *Salinibacter ruber*, *Halorubrum* sp. and *Halobacterium* sp. were grown onto glass surfaces in flow through cells that allowed the direct observation by phase contrast microscopy. An structural study was performed by optic sectioning and digital image analysis. All the studied microorganisms were successfully formed biofilm that exhibit particular structural features and different surface coverage.
Once biofilm growth was proved, interactions between sensible and antagonist cultures analyzed by connecting the corresponding flow-through cells. The structure and viability of sensible biofilms were fatally altered by the presence of the antagonist demonstrating both the importance of antagonic interactions in sessile halophilic communities and the utility of the experimental strategy for their study.

MI-P03.
SCREENING OF MOLECULES DIRECTED TO THE HOST CELL NUCLEUS IN *Trypanosoma cruzi*

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Trypanosoma cruzi (Tc), the protozoan parasite causative of Chagas' disease, secretes abundant molecules in all developmental stages of its complex life cycle. One intriguing possibility is that intracellular trypomastigotes (T) and amastigotes (A), secrete molecules to the host cell nucleus to co-opt cellular processes. In this work, an in silico strategy was developed to search for candidate molecules potentially directed to the host cell nucleus during a Tc infection, using the bioinformatic tool TDR Targets. The genome of Tc was screened looking for molecules with defined features: a) Presence of a Signal Peptide (SP), b) Consensus for the addition of GPI, c) Presence of Nuclear Localization Signals (NLS), e) SP-independent secretion, and f) Nuclear features, among others. After manual curation of results, some candidates were selected for biochemical characterization, involving: a) Evaluation of predicted NLS functionality, b) Study of the expression and subcellular localization of candidates in Tc developmental forms. As a result of these studies, some interesting candidates emerged: a protein with homology to CEST (Chaperone for *E. coli* Secretion of Tir), a protein with a WD40 domain, an homologue of CAF-1B (Chromatin Assembly Factor 1 B), and a protein with unknown putative function. These candidates are being subjected to further biochemical characterization.

MI-P04.
FLAGELLA PARTICIPATES DURING *Yersinia enterocolitica* BIOFILM FORMATION

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Yersinia enterocolitica (Ye) is a food-borne Gram-negative bacterium that causes gastroenteritis. The ability to form biofilms protects bacteria from various stresses. It has been demonstrated the importance of flagella in biofilm formation in other bacteria. The objective of this study was to determine whether flagellar expression is involved in Ye biofilm formation. Two Ye strains were chosen: CLC001 (a high biofilm forming strain) and GFA003 (a weak biofilm forming strain). The biofilm ability was measured by crystal violet technique onto a 96 wells polystyrene plate. Strains were incubated in trypticase soy broth (TSB) added with 0.25% glucose at 24°C for 18, 24 and 48 h. Each time point, planktonic and sessile growth were measured and RNA was extracted and 2µg were used for RT-PCR of flagellar gene (*fliA*). At 24 h of culture, the strongest biofilm was formed; after that, it was weak and easy to remove; on the other hand, the planktonic growth was increasing and peaked at last time point. With CLC001 strain, the expression of *fliA* was higher at 24 h and it was not seen at 48 h. With GFA003 strain similar results were obtained but the expression of this gene was weaker than with CC001 strain. We concluded that Ye uses flagella during the earlier steps of biofilm formation. It is necessary more studies to know the mechanisms involved in fliA expression.

MI-P05.

A NEW SYSTEM TO ANALYZE RECOMBINATION IN GRAM-NEGATIVE BACTERIA: EXAMPLE *Pseudomonas aeruginosa*

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The DNA mismatch repair system (MRS) is an evolutionary conserved pathway that besides of its function in mutation avoidance, inhibits the recombination between divergent DNA sequences by interacting with mismatches in the recombination intermediates. The aim of this study was to construct a system to analyze recombination between identical (homologous) or partially divergent (homeologous) DNA sequences in Gram-negative bacteria. This system, constructed in a broad host-range plasmid, contained two non-functional 3´- and 5´- truncated *lacZ* genes sharing an overlapping region either 100% or 95% identical, followed by a gentamicin resistance gene. The two *lacZ* genes were separated by a spacer region which included a transcriptional terminator. A single recombination event re-constituted a functional *lacZ* gene and allowed the expression of the gentamicin resistance gene. To analyze the performance of this system, we transferred the homologous and homeologous recombination systems to the opportunistic pathogen *Pseudomonas aeruginosa*. DNA divergence between the two *lacZ* genes reduced the frequency of recombination by 1000-fold. In addition, inactivation of the MRS proteins MutS or MutL specifically increased the frequency of homeologous recombination. Finally, the recombination process was characterized by restriction and sequencing analysis of the recombinant products.

MI-P06.

MONITORING OF IN-PLATE BACTERIAL MUTAGENESIS USING A LUMINESCENT APPROACH

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Rifampicin resistance assay is the most used method to determine mutation rates in bacteria. Although it is a useful technique in a wide range of bacterial species, it has several limitations such as lack of representation of frameshift mutations and the difficulty to examine mutant emergence along with incubation time.

Here we present a novel strategy to assay mutation rate and spectrum based on a luminescent reporter adapted from the mini-Tn7 vector, which inserts specifically in the attTn7 chromosomal site of *P. aeruginosa*. In our construction, the promoter of P. aeruginosa MexCD-OprJ efflux pump (Pcd) was cloned upstream of lux operon in the pUC18-mini-Tn7-Gm-lux vector. Pcd promoter is endogenously repressed by NfxB protein, and we showed that many types of mutations occurring in this protein (including frameshifts) release the expression of the controlled operon. The expression of lux operon provides a strong luminescent phenotype which can be visualized on colonies directly from the plates and time-monitored.

We also describe an application of this reporter strategy by comparing the emergence of NfxB mutants on ciprofloxacin-containing plates from *P. aeruginosa* WT strain compared to the *recA*- and *lexA*- deficient strains, in order to investigate the participation of the SOS response and RecA-dependent recombination in the mutagenic effect of sub-lethal doses of ciprofloxacin.

MI-P07.

ANTIFUNGAL ACTIVITY AND SIDEROPHORE PRODUCTION OF CHITINOLYTIC BACTERIA AGAINST *Colletotrichum*

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Four chitinolytic *Bacillus* strains were previously isolated from bat guano (*Taradita brasilensis*). These bacteria showed antagonistic properties, *in vitro*, against the phytopatogenic fungus *Colletotrichum acutatum* and *C. fragariae*. *Bacillus* sp. PB1 showed the highest antifungal activity and was selected for further studies. The aim of this work was to study the stability of the fungal activity and its relationship with siderophore production. Selected bacterium was incubated in SPI-colloidal chitin, SPI-pupa and Standard nutrient (SN) liquid media, at 200 rpm 72h, at 28ºC. Supernatants were assayed against *C. cutatum* and *C. fragariae* on potato dextrose agar. Under all tested conditions, supernatants were able to inhibit fungal growth. Major activity was reached on SN medium. In order to evaluate the stability of the antifungal activity, supernatants were treated with proteinase K or heat-treated at 50ºC and 100ºC. Heat and proteinase K treated supernatants could were only able to modify mycelial morphology. *Bacillus* sp. PB 1 produced siderophores as evidenced by positive reaction in $FeCl_3$ test. The presence of hydroxamate type siderophores was also evidenced by standard the tetrazolium test. These results suggest that *Bacillus* sp. PB 1 siderophores could play a significant role in antifungal activity of selected chitinolytic bacteria

MI-P08.

NEW STEROL ANALOGUES WITH ANTIVIRAL ACTIVITY

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The threat of emerging viruses and the appearance of viral strains resistant to therapeutic agents lead to the continuous search for innovative antiviral compounds. Steroids are an attractive source for antiviral drug discovery since these compounds are known to possess several biological properties. Previously, we have reported a new synthetic procedure to obtain sterol analogues based on an Ugi four-component reaction. This approach was successfully applied to obtain a family of eight azasterols with a polyfunctionalized side chain at C-16. The new azasterols were evaluated for their cytotoxicity and activity towards HSV-1 and VSV in Vero cells. Furthermore, we examined the effect of the compounds on the transport of HSV-1 glycoproteins by immunofluorescence staining. The antiviral assay revealed that all tested compounds have antiviral effect against HSV-1 at subtoxic concentrations. However, no activity against VSV was observed for any of them. Moreover, the active compounds would be affecting the viral glycoproteins transport since accumulation of gD fluorescence at juxtanuclear sites was visualized. In conclusion, these sterol analogues were identified as novel anti-HSV-1 agents. Further studies are under way to decipher the detailed antiviral mechanism of action of these compounds.

MI-P09.

CHARACTERIZATION OF *Pseudomonas aeruginosa* MUTL ENDONUCLEASE TARGET SITES

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In *Escherichia coli* and closely related bacteria, MutH protein displays the endonuclease activity necessary to direct the postreplicative DNA mismatch repair system (MRS) to the newly synthesized DNA strand. However in the rest of bacteria and in eukaryotes, MutL homologues take on this role. Although MutH recognizes and nicks a transitory unmethylated GATC site in the newly synthesized DNA strand, the endonuclease activity of MutL homologues seems to be independent of any methylation process, and the sequence where they cut the DNA molecule has never been identified.

The aim of this work is to characterize the *Pseudomonas aeruginosa* MutL (PaMutL) endonuclease target sequence. Using plasmid DNA and different methodologies (e.g. second digestion with a restriction enzyme followed by native agarose gels electrophoresis and ethidium bromide staining, or denaturing polyacrylamide gels electrophoresis and silver staining) we analyzed PaMutL target DNA sites.

Our results showed that PaMutL is able to cut plasmid DNA at different sites, although no specific sequence seems to be recognized. This result represents a significant difference in the way the MRS functions among bacterial species in which the MRS is directed by MutH or by MutL, and could have implications for genome stability and consequently for adaptive and evolutionary processes.

MI-P10.

ISOLATION AND CHARACTERIZATION OF *Escherichia coli* MUTANT STRAINS

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Escherichia coli strains deficient in DNA adenine methyltransferase (Dam) are soft mutators but their mutation rate rapidly increase in the presence of mutator agents like the base analogue 2-aminopurine (2AP). Although this increment in mutation rate could represent an adaptive advantage under stress conditions, as Dam methylation is important for expression of a number of virulence factors, *dam* cells of several bacteria strains are attenuated.

Therefore, we screened a collection of human *E. coli* isolates searching for strains sensitive to 2AP but that retain their DNA methylation capacity. We found two strains with these characteristics.

Complementation assays showed that overexpression of the wild type *dam* gene reverts the sensitivity to 2AP of one of these strains. DNA sequencing of the endogenous *dam* gene let us identify a mutation in its coding sequence, as well as others in the 5´ region that could be affecting the promoter regulation. This suggested that a mutant, but still functional, Dam protein or an altered expression of it, could be responsible for the phenotype of these strains. We are currently analyzing the molecular mechanism responsible for this phenotype and the putative adaptive advantage of these strains.

MI-P11.

ANTIMICROBIAL ACTIVITY OF ENT35-MCCV AGAINST FOODBORNE PATHOGENS AND SPOILAGE BACTERIA IN FOODS

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Bacteriocins are ribosomally-synthesized antimicrobial peptides produced by bacteria with antibiotic spectra generally limited to phylogenetically related microbial strains. We carried out a transcriptional fusion between structural genes of enterocin CRL35 and colicin V in order to obtain the recombinant broad spectrum bacteriocin, namely Ent35-MccV. A partially purified preparation of Ent35-MccV was assayed against a number of Gram (-) and Gram (+) food pathogens and spoilage bacteria in vitro inhibition by the agar diffusion method.

On the other hand the antimicrobial action of Ent35-MccV on hamburger inoculated with 10^4 CFU/g of *Listeria monocytogenes* CECT 4032 and 10^4 CFU/g of *Escherichia coli* O157:H7 CECT 4622 and fresh mackerel (*Scomber scombrus*) previously inoculated with the altering food and histamine-producing strain *Enterobacter cloacae* was evaluated.

The treatment was able to decrease the viability of *Listeria* and slow the growth of *E. coli* in hamburger after a week of storage at 4ºC. However no significant difference was found between CFU/ml of control and bacteriocin treated samples of *S. scombrus*, after a week. These results demonstrated that Ent35-MccV can effectively inhibit the growth of some Gram (+) and Gram (-) pathogenic bacteria in foodstuffs and their antimicrobial efficiency is similar to that obtained in culture media.

MI-P12.

MCE2R CONTROLS THE EXPRESSION OF A REGULON INVOLVED IN LIPID METABOLISM IN *Mycobacterium tuberculosis*

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The *mce2* operon is one of the four *mce* operons present in *Mycobacterium tuberculosis* that encode exported proteins with a probable role in the virulence mechanisms of this bacterium. In a previous study we demonstrated that *Rv0586*, which encodes a putative GntR-like regulator, is part of the *mce2* operon. Moreover, we found that the product of this gene represses the expression of Mce2 proteins (Santangelo *et al*., 2009). For this reason, we have re- named the repressor protein *Mce2R*. In order to find other proteins regulated by Mce2R, in the present study we performed microarrays experiments comparing the expression profile of a *M. tuberculosis* mutant strain, in which the *Rv0586* gene was deleted, with that from the parental strain, H37Rv. The results obtained from this analysis were validated by real time PCR. We confirmed our previous findings indicating that Mce2R represses the expression of the *mce2* operon, and we also found other genes regulated by Mce2R. Some of those genes encode proteins likely involved in lipid metabolism. Therefore, these results support the hypothesis that mce genes are involved in lipid transport/metabolism. In addition, the comparative lipid profile of cell extract from the mutant, the complemented and the wild type strains showed that a glycolipid is absent in the mutant *Mce2R*.

MI-P13.

MALTOSE METABOLISM IN *Enterococcus faecalis*

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Enterococcus faecalis is one of the most controversial lactic acid bacteria. This microorganism is normally present in the microflora of the animal gut but also emerged as an opportunistic pathogen in the last decade. It can also be found in cheese where improves the quality of the fermented product. In clinical *E.faecalis* isolates biofilm formation play an important role being this phenotype associated to maltose metabolism. The maltose cluster is organized in a divergent fashion, *malPBMR* operon encodes putative maltose phosphate phosphorylase (*malP*), ß-phosphoglucomutase (*malB*), aldose-1-epimerase (*malM*) and transcriptional repressor (*malR*); and *malT* encoding for a PTS enzyme II.
[^{14}C]maltose uptake experiments in a strain with a polar interruption of *malP* indicated that uncharged compounds are accumulated suggesting that a specific phosphatase might still be active. Bioinformatic analysis allowed us to detect downstream from *malT* an ORF annotated as a phosphatase protein (*mapP*). A *mapP* deficient strain showed diminished growth in maltose supplemented media indicating a connection with the pathway. Uptake experiments in the *mapP*$^-$ strain revealed the accumulation of charged products (probably maltose-6P). In order to confirm their activity both enzymes were over-produced and purified from *E.coli*. The results presented contribute to understand the maltose metabolism of *E.faecalis*.

MI-P14.

METHICILLIN-RESISTANT *Staphylococcus aureus* BELONGING TO CC121 RESPONSIBLE FOR INVASIVE INFECTIONS

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Since 1990s methicillin resistant *Staphylococcus aureus* (MRSA)emerged as a community-associated pathogen (CAMRSA) worldwide. Most CAMRSA carry the staphylococcal cassette chromosome (SCCmec) type IV and the Panton-Valentine leukocidin (PVL) gene. Successful lineages of MSSA PVL+ would behave as a reservoir for emergence of CAMRSA PVL+ clones. Although the CC121 is one of most dominant MSSA lineages, its genetic background seems to be incompatible for stable integration of SCCmec. The aim of this work was the molecular and clinical analysis of two cases of CAMRSA infections caused by strains belonging to the lineage CC121 by molecular typing methods. These strains were isolated from children (11 and 14 years old) hospitalized in a tertiary care public hospital in Córdoba. They were affected by osteomielitis with torpid evolution; one of them was complicated with sepsis. Molecular analysis of the strains showed that both belong to CC121 characterized by: Pulsotype U-ST1210 (CC121), agr 4, SCCmecIVa-spat645. They harbor the following genes pvl, Enterotoxin B, cluster egc, adhesins for bone sialoprotein (bpp) and for collagen (cna). We demonstrated the emergence of two MRSA isolates PVL+ belonging to CC121, associated to SCCmecIVa. As far as we know, this genetic background has never been reported in MRSA, moreover this clone have all the genetic potential to becoming an epidemic CAMRSA.

MI-P15.

***Staphylococcus aureus* -TOXIN ACTIVATES MAPKS AND ALTERS C-JUN AND KLF6 PROTEIN LEVELS IN LUNG CELLS**

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The *Staphylococcus aureus* -toxin is a key virulence factor involved in the onset of diverse and often fatal infections, such us necrotizing pneumonia. However, its molecular mechanisms are not well understood. The biochemical response mediated by the main group of Mitogen-Activated-Protein Kinases (MAPKs) JNK, p38 and ERK in A549 lung epithelial cells intoxicated with -toxin was analyzed. Distribution of c-Jun and KLF6 transcription factors, which are key regulators of cell fate upon diverse stimuli, including microbial infections was additionally investigated. Western-Blot (WB) analyses of cells treated with *S. aureus* culture supernatants or with purified -toxin, showed the activation of the three MAPKs – JNK, p38 and ERK – phenomenon which was not observed in cells treated with supernatants from an isogenic -toxin-deficient srain. Pharmacological inhibition of MAPKs indicates that activation of the three pathways were important for cell survival. Furthermore, -toxin increase phosphorylation and decreased the c-Jun protein level. Finally, WB of nuclear and cytoplasmic fractions, as well as confocal microscopy, showed that -toxin provoked a fast decrease of KLF6 at the nucleus, while gaining a more cytoplasmic distribution. These results shed light on the role MAPKs pathways and the involvement of c-Jun and KLF6 in the complex cell response upon *S. aureus* -toxin intoxication.

MI-P16.

STRUCTURAL COMPARISON OF THE WILD TYPE AND RFB303 MUTANT LPSS FROM *Xanthomonas axonopodis* PV. *CITRI*

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Xanthomonas axonopodis pv. *citri* (*Xac*) causes citrus canker, provoking defoliation and premature fruit drop with concomitant economical damage. In plant pathogenic bacteria, lipopolysaccharides (LPSs) are important virulence factors, and they are being increasingly recognized as major PAMPs for plants. We have previously characterized the LPS extracted from the *Xac* wild type and a *wzt* mutant. In the present work the LPS obtained from *Xac-rfb303* defective in genes that codify for a glycosyltransferase involved in core synthesis was studied in order to get deeper in the role of *Xac* LPS in this disease. LPSs from harvested cells were extracted with a 50% phenol/ water mixture. Purified LPS was hydrolyzed with 1% acetic acid, the precipitated lipid A was recovered by ultracentrifugation and the solution containing the sugar moiety (called oligosaccharide) was separated. When the UV-MALDI-TOF ms analysis of the Lipid A obtained from *rb303* was performed, a spectra showing similar species to the one obtained for the wild type Lipid A was detected. However, penta-acylated species bearing two pyrophosphoethanolamine groups were predominant. Total hydrolysis of the oligosaccharide moiety and HPAEC-PAD analysis revealed that the same monosacharides than the wild type were present, but in a different relationship. These structural differences were confirmed by UV-MALDI-TOF ms analysis.

MI-P17.

IDENTIFICATION OF QAC GENES IN *Staphylococcus haemolyticus*. CORRELATION; PHENOTYPIC AND GENOTYPIC EXPRESSION

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Among the coagulase negative staphylococci *Staphylococcus haemolyticus* is frequently found associated with septicemias, peritonitis and otitis. The genes qacA/B, smr, qacG, qacH and qacJ from Gram positive cocci and qacEdelta1 from Gram negative species codify for resistance to quaternary ammonium compounds (QACs) through efflux pumps. The aims of this study were: 1) to determine the relationship between the phenotypic and genotypic expression in a clinical isolate of *S. haemolyticus* and 2) to determine the genomic organization of these genes. The QACs resistance genes were investigated using standard PCR. To investigate the presence of a mobile element, the transposase of Tn552 and IS256 were searched using PCR. The genomic organization was analysed by combination of primers PCR and gene expression by Reverse-Transcriptase PCR. The genes *qacA/B*, *qacG*, *qacH*, *qacJ*, *smr*, *qacEdelta1*, *Tn552* and *IS256* were detected in the isolate studied. The "combined primers" PCR showed that *Tn552* and *IS256* were upstream of *qacA/B*. The RT-PCR was negative for the genes *qacA/B*, *qacG*, *qacH*, *qacJ* and *smr*. These findings suggest that *qacEdelta1* could be responsible for the phenotypic expression observed and the presence of *Tn552* and *IS256* would indicate the existance of transposable elements that would explain the concomitant resistance to antiseptics and antibiotics.

MI-P18.

IMPAIRED EXPRESSION AND SECRETION OF SOPB, SOPD AND SOPE2 IN *Salmonella DAM* MUTANTS

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Dam methylation is an essential factor involved in the virulence of an increasing number of bacterial pathogens including *Salmonella* spp. *dam* mutants have a reduced capacity to colonize murine organs compared to the parental strain. This partial inability could be related to a reduced secretion of invasion proteins. Then, we investigated the involvement of Dam methylation on the expression and secretion of essential proteins for invasion, such as SopB, SopD and SopE2. These effectors are located outside of SPI-1 but are translocated in a SPI-1-dependent manner. *S.* Typhimurium *sopB*, *sopD* and *sopE2* tagged (3xFLAG) strains and their derivative *dam* were used during the experiments. The expression and secretion of these effectors were determined *in vitro*, under SPI-1 culture conditions, analyzing bacterial pellets and culture supernatants, respectively. Tagged proteins were examined by SDS-page and immunoblotting with anti-FLAG antibodies. Western blot results showed an impaired SopB, SopD and SopE2 expression and secretion in *dam* mutants with respect to the parental strain ($p < 0.05$). These results were further confirmed by QRT-PCR showing that *sopB*, *sopD* and *sopE2* genes transcription were two or three-fold lower in *dam* mutants compared to wild type strain. Taken together, our results demonstrate that Dam methylation modulates the expression and secretion of SopB, SopD and SopE2.

MI-P19.

PMRA AND RCSB EXPRESSION IS AFFECTED BY DAM METHYLATION IN *Salmonella* Enteritidis

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We reported earlier that *S.* Enteritidis *dam* mutant, has a partial defect in the O Ag chain length distribution, possibly due to a reduced expression of *rcsB*, one of the two regulators of *wzz*, together with *pmrA*. Here we show, not only that *pmrA* is also regulated by Dam methylation, but that a compensatory effect would exist between both *wzz* regulators. We investigated the PmrA protein expression in the *dam* mutant. PmrA was tagged adding a 3xFLAG. The expression was analysed by Western blot. Similar PmrA expression was found in *dam* mutant and in wt strain. However, the expression of *pmrA* studied by RT-PCR showed reduced levels of mRNA in the *dam* mutant. Next we studied the expression of *wzz* in *pmrA* and *rcsB* mutants. Results showed that in the absence of *rcsB*, the amount of *wzz* mRNA was reduced in 50% compared with the wt. Surprisingly, in *pmrA* mutant the amount of *wzz* mRNA was 30% higher than in the wt. In order to investigate the putative interaction between both *wzz* regulators, we determined the expression of *rcsB* in a *pmrA* mutant and *pmrA* expression in a *rcsB* mutant. We found that in *rcsB* mutant the expression of *pmrA* mRNA decreases 40%, whereas in the absence of *pmrA*, *rcsB* mRNA increases 10%, suggesting a compensatory effect. Thus, in *S.* Enteritidis Dam regulates Wzz through its two regulators, PmrA and RcsB; a compensatory mechanism would exist between both regulators.

MI-P20.

INTI1 INTEGRASE EFFICIENTLY MEDIATES RECOMBINATION INVOLVING THE *BLA*$_{GES-1}$ UNUSUAL CASSETTE

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Within a *Pseudomonas aeruginosa* colistin-only-sensitive isolate, was found a class 1 integron with the cassette array *orf126-bla*$_{GES-1}$-*aac(6')-IId*. The *bla*$_{GES-1}$ unusual cassette has the particularity that downstream the stop codon of the *bla*$_{GES-1}$ gene there is a sequence of 11 nucleotides with 100% identity to the *attI1*, instead of a typical *attC* recombination site. The aim of this study was to determine if the IntI1 integrase mediates site-specific recombination of the *orf126* cassette or the *bla*$_{GES-1}$ unusual cassette.

The *orf126-bla*$_{GES-1}$ array was cloned in order to use it for *in vivo* recombination assays. *E. coli* cells harboring the plasmid that served as substrate and another containing the *intI1* gene alone or also the *attI1*, were cultured in the presence of IPTG, and plasmid DNA was isolated. Specific PCR primers were used to identify plasmid species where excision or insertion has occurred.

We observed that the IntI1 excised the *attI1-orf126-attC-bla*$_{GES-1}$ (*attI1*) structure as a module as well as separated units and inserted them at the *attI1*.

We demonstrated that the IntI1 recognized the *orf126* cassette and the *bla*$_{GES-1}$ unusual cassette, showing that an unusual cassette harboring a *attI1* could be used by IntI1 as a substrate to mediate the specific not only excision but also insertion reactions, demonstrating its functionality.

MI-P21.
CORRELATION BETWEEN HIGH MBC VALUES AND QAC EFFLUX PUMPS IN *Staphylococcus haemolyticus* ISOLATES

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Hygienic handwashing using quaternary ammonium compounds (QACs) is a mechanism for removing microorganisms and prevent cross-infection in hospitalized patients. The aim of this study was to investigate the phenotypic profile to benzalkonium chloride (BC) and efflux pumps related genes, in multiple drugs resistant *S. haemolyticus* clinical isolates from catheter infections. Forty four *S. haemolyticus* clinical isolates were collected from 3 hospitals from Buenos Aires city. The Minimun Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to BC were determined according to the CLSI guidelines. To search the efflux pump genes that codify for resistance to QACs (qacA, smr, qacG, qacH and qacJ), standard PCR was performed. Nineteen of 44 isolates (43.18%) showed MBC values between 32 and 64 ug/mL. The PCR amplification demonstrated a remarkable association between smr and qacG genes in 10 of the 19 isolates (52.63%) with these characteristics. This association was found alone or acompanied by one of the other qac determinants. It can be concluded that regarding resistance to BC there would be a correlation between high MBC values and concomitant presence of smr and qacG which could imply the existance of a mobile element that harbor and spread both genes.

MI-P22.
PREVENTION OF ENTERITIS AND SYNOVITIS IN *Salmonella* Enterocolitis IN MICE FED WITH *Lactobacillus casei*

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We investigated inflammatory responses of intestine and knee joints in a mouse model for enterocolitis. Mice were inoculated orally with 20 mg of streptomycin and 24 hs later they received 10^3 CFU of a virulent strain of *Salmonella* Enteritidis by the same route. *Salmonella* was recovered from Peyer´s patches (PP) up to day 4 and from spleen up to day 14. Bacteria were neither recovered from knee joints nor from draining lymphnodes. Infected mice showed a significant increase in intestinal IL-17 and TNF- , determined by qPCR. 21 days post-infection animals presented synovitis in the knee joints, suggesting that this model of *Salmonella* enterocolitis is suitable for studying reactive arthritis. We then tested the effect of probiotics in preventing the joint sequelae. Mice were fed with commercially-available *Lactobacillus casei* fermented milk for 7 days before infection. PP and spleen of mice pretreated with probiotics were less colonized with *Salmonella*, and for a shorter period. Probiotic feeding previous to *Salmonella* infection resulted in a significant decrease of gut inflammatory cytokines, compared to infected animals not receiving *Lactobacillus*. Our results showed that the model for enterocolitis might be useful for studying the pathogenesis of *Salmonella*-induced synovitis and that these sequelae were prevented in mice pretreated with fermented milk containing *L. casei*.

MI-P23.
GALACTOSYLCERAMIDE TRANSFERASE OF *Trypanosoma cruzi* EPIMASTIGOTES BEAR SULFATED OLIGOSACCHARIDES

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Little experimental evidence exists up to date related to the mode of regulation of sphingolipids in parasites. With the aim of determining new targets for antiparasitic drugs, different enzymes of this pathway are being studied in *T. cruzi*. Cerebrosides are synthesized by a ceramide UDP-galactosyltransferase [CGalT] using UDP-galactose as donor and ceramide as acceptor. In the present work, we describe the identification and partial characterization of the CGalT purified from epimastigote forms of *T. cruzi*. After different chromatographic steps a fraction containing three bands with apparent molecular weight of 70-61 kDa in SDS/PAGE was determined. This fraction presented CGalT activity. A specific CGalT antibody recognized the two higher MW bands. Knowing that CGalT is a glycoprotein, each gel band was cut and digested with PNGase F. The glycan fraction was analyzed by high performance anion exchange chromatography (HPAE-PAD) and UV-MALDI-TOF mass spectrometry. As expected, neutral high-mannose type oligosaccharides were shown, however main signals correlate with sulfated oligosaccharides. This is the first report of the analysis of the sugar moiety of CGalT. On the other hand, these results show CGalT as the second sulfated glycoprotein described in epimastigote forms of *T. cruzi*.

MI-P24.
BIOINFORMATICS ANALYSIS OF THE RCSCDB SYSTEM REGULON IN *Salmonella* Typhimurium

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The Rcs phosphorelay system, involved in the control of *Salmonella* virulence, is a complex regulatory system that consists in three proteins: the RcsC sensor, the RcsB response regulator and the phosphorelation intermediary RcsD. Even when the signal remains unknown, several conditions have been characterized by the ability to activate it. The RcsCDB controls the expression of a wide range of genes, which can be divided according to their dependency on the RcsA co-activator. RcsA-dependent genes include the *cps* operon and the *rcsA* gene, while the RcsA-independent genes consist of different targets including the *ftsA* and *fhlDC* genes, and those involved in motility and chemotaxis. Here we analyzed a microarray assay carried out under RcsCDB system activation. We classified the genes according to the effect exerted by RcsB and RcsA, in up or down-regulated and RcsA-dependent or –independent clusters, respectively. We found that 143 of 304 analyzed genes were down-regulated by RcsB while 161 were up-regulated. In this assays only 29 genes were expressed in an RcsA-dependent pathway, all were included in the RcsB up-regulated cluster. In addition, the microarrays results were validated by the analysis of the *osmY* gene expression, involved in the stress response. To this end we cloned the promoter region in a promoter expression vector and we performed gel shift assay.

MI-P25.

INTERACTION BETWEEN IMMUNEMODULATORY MOUSE SIGLEC-E AND SULFATES FROM *Trypanosoma cruzi*

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Although binding of all Siglecs (sialic acid-specific lectins), mainly expressed on cells of the hematopoietic system, is critically dependent on sialic acid, a strong difference in the binding pattern of different human siglecs to *T. cruzi* glycoproteins has been found. Interestingly, siglec-9-Fc, expressed on human monocytes, avidly binds to *T. cruzi* structures. In addition, an enhanced affinity for its ligands was shown in the presence of sulfated structures. Knowing that sialylated structures from a *T. cruzi* pathogenic strain interact with mouse Siglec-E strongly than a less pathogenic one, we analyzed 12 additional *T. cruzi* strains. Moreover, we observed that the microsomal fraction of *T cruzi* lysates binds with the highest affinity to Siglec E among other fractions. On the other hand, in order to investigate if sulfated oligosaccharides are also directly involved in the modulatory effects on *T. cruzi*, several techniques using mouse Siglec-E-Fc fusion molecule and parasite sulfated glycoproteins were performed showing a significant binding decrease after desulfation treatment of these molecules. Our findings support the notion that the incidence of sulfates in the interaction of Siglec-E, an ortologue protein of human siglec-9, with sulfate containing-syalilated glycoproteins might modulate the immune response of the host, favoring parasitemia and persistence of the parasite.

MI-P26.

ROLE OF *Staphylococcus aureus* TWO COMPONENT SYSTEM SA1158-SA1159 IN MEMBRANE FLUIDITY PERCEPTION

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Staphylococcus aureus, a G+ bacterium, is a frequent component of human microbial flora that can turn into a dangerous pathogen. *S. aureus* methicilin-resistant (MRSA) strains are prevalent in hospitals, but recently, the emergence of community associated MRSA infections acquired by healthy people has led to a major concern of Public Health. The pathogenicity of *S. aureus* is caused by expression of a myriad of virulence factors. A sudden change in temperature may be a signal for the pathogen of being inside the host, inducing the expression of virulence-related genes. As two component systems (TCSs) allow bacteria to sense environmental temperature, in this work we analyzed two genes located in *S. aureus* N315 chromosome, SA1158-SA1159, as they showed homology with *B. subtilis* DesKR TCS, which regulates transcription of its sole desaturase in response to changes in membrane fluidity. By complementation tests we found that expression of SA1158 restores the ability of *B. subtilis desK* strains to activate transcription of *des* gene after cold shock induction. In addition, a mutant strain lacking DesK and DesR is also able to promote *des* transcription at low temperature when SA1158 and SA1159 are expressed under a conditional promoter. This suggest that the kinase of *S. aureus*, SA1158 is responding to the same signal sensed by DesK, and support a general role of these TCSs in G+ bacteria

MI-P27.

F0F1 H+ATPASE IS INVOLVED IN THE INVASION PROCESS OF *Streptococcus pneumoniae* INTO PNEUMOCYTES

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S. pneumoniae must overcome adverse conditions to cause human diseases, such as acidic stress in inflammatory niches, for which it has developed survival mechanisms such as Acid Tolerance Response (ATR). It was described that F0F1 H+ ATPase is a major contributor of bacterial ATR because of the tight regulation of intracellular concentration of protons. We have previously identified and characterized different point mutations in the *atpC* gene that encodes the C-subunit of the F0F1 H+-ATPase.
Here, we found that these *atpC*mutants lost the ATR mechanism, suggesting a restriction to survive intracellularly in acidic endosomes. To assess intracellular survival, we exposed A549 cells (cell line of type II pneumocytes) to *atpC*mutants, and we could not recover bacterial live cells inside the pneumocytes at different incubation times. By confocal microscopy, we observed that the *atpC*mutants failed to invade A549 cells, in contrast to the wild-type strain. By co-localization experiments, we found that *atpC*cells adhered to pneumocytes via PAF receptor as described, indicating that metabolic changes caused by *atp* mutations altered the pneumococcal ability to invade A549 cells, but not their adherence capacity. These results suggested that the F0F1 H^+-ATPase is involved in the invasion process of *S. pneumoniae* into pneumocytes.

MI-P28.

STKP CONTROLS STRESS-INDUCED AUTOLYSIS IN *Streptococcus pneumoniae* BY PHOSPHORYLATION OF ComE

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In pneumococcus, autolysis is triggered by acidic stress. This process requires ComE, a response regulator that normally controls competence development at alkaline pH, which is activated by its cognate histidin kinase ComD by phosphorylation. We demonstrated that acidic stress-induced lysis (ASIL) was not dependent on ComD, suggesting a cross-talk mechanism between ComE and other kinases. However, we showed that no other histidine kinases were involved in the control of ASIL by a cross-talk mechanism.
To study the possibility that phosphorylation of ComE by an alternative phosphodonor could be required for ASIL, we created a $comE^{D58A}$ mutant, in which the phosphorylable aspartate residue at position 58 was replaced by alanine. The $comE^{D58A}$ mutant autolysed like the wt strain (R801) at acidic pH, demonstrating that phosphorylation in this residue was not necessary for ASIL activation.
We had described that the serine threonine kinase StkP controls competence, virulence, and autolysis. We also found that StkP was participating in the ComE-controlled ASIL pathway, being required for induction of *comE* transcripts. In vitro phosphorylation assays revealed that ComE is a target of StkP, being phosphorylated exclusively at threonine residues. These findings support the idea that upon acidic stress StkP activates ComE by phosphorylation and triggers the autolytic response.

MI-P29.
ACCase 5 IS AN ESSENTIAL ACYL-COA CARBOXYLASE IN *Mycobacteria*
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Mycolic acids are essential for the survival, virulence and antibiotic resistance of the human pathogen *Mycobacterium tuberculosis*. Two acyl-CoA carboxylases, ACCase 5 and ACCase 6, carboxylate both acetyl- and propionyl-CoA to produce malonyl- and methylmalonyl-CoA, the building blocks for fatty acid, mycolic and mycocerosic acid biosynthesis. ACCase 6 is a dedicated acetyl-CoA carboxylase *in vivo*, and *in vitro* experiments showed that ACCase 5 carboxylates propionyl-CoA more efficiently than acetyl-CoA. To find out the physiological role of ACCase 5, we constructed a conditional mutant in the *Mycobacterium smegmatis* carboxyltransferase gene *accD5*, in which the addition of anhydrotetracycline (ATc) to the media turns off *accD5* expression. This mutant could only be obtained in the presence of wild type copies of AccD5, and stopped growing after ATc addition, showing that AccD5 is an essential carboxyltransferase component of the ACCase 5 enzyme complex. Furthermore, we observed that, in *accD5* conditional mutant cell-free extracts, propionyl-CoA carboxylase activity decreases after ATc addition, suggesting that ACCase 5 is a dedicated propionyl-CoA carboxilase *in vivo*, and that none of the other ACCases present in this bacterium can compensate for the loss of this activity. A more exhaustive characterization of the mutant is in progress.

MI-P30.
CHARACTERIZATION OF REGULATORY MECHANISMS OF SHLA HEMOLYSIN EXPRESSION IN *Serratia marcescens*
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The ShlA hemolysin of *Serratia marcescens* is one of the major virulence factors of this opportunistic pathogen. ShlA together with ShlB, an outer membrane protein, required for secretion and activation of ShlA, form a two-partner system encoded by the *shlBA* operon. It has been previously reported that ShlA presents cytotoxic activity in several cellular lines, and we have recently demonstrated that it is also responsible for the autophagic phenotype induced by *Serratia* in epithelial cells. We have also previously determined that the Rcs regulatory system modulates flagellar biogenesis and the expression of the PhlA phospholipase in response to structural alterations in the bacterial envelope. In this work, we analyze the influence of the Rcs phosphorelay system on ShlA synthesis regulation. We found that the *rcsB* mutant strain presents higher hemolytic activity than the wild type strain, in different growth conditions. This observation is also supported by immunodetection assays, which show higher levels of ShlA in the *rcsB* mutant. In agreement with this result, we have found a putative RcsB binding site in the promoter region of *shlBA* operon, suggesting that Rcs system controls *shlA* expression at the transcriptional level. Together, our results reveal that, in *S. marcescens*, the Rcs phosphorelay system is key for the regulation of bacterial virulence determinants.

MI-P31.
DELETION OF RHOMBOID PROTEASE GENE AFFECTS PROTEIN GLYCOSILATION AND CELL MORPHOLOGY IN *Haloferax volcanii*
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Rhomboids (Rho) are a recently discovered family of widely distributed intramembrane serine proteases. In eukaryotes and bacteria these enzymes have diverse biological functions including regulation of growth factor signalling, mitochondrial fusion, parasite invasion and *quorum sensing*. However, nothing is known on the biology of Rho proteases in Archaea.
The aim of our research is to characterize archaeal Rho using *Haloferax volcanii* as a model organism. To identify possible Rho substrates, we compared SDS-PAGE profiles of wt and a Rho protease (*rhoII*) deletion mutant (MIG1). Protein staining evidenced two polypeptides (200 and 98 kDa, respectively) that were enriched in the membrane fraction of the mutant strain MIG1. By LC-MS/MS analysis, these species were identified as the S-layer glycoprotein (200 kDa) and a putative periplasmic substrate-binding protein (98 kDa) of a dipeptides/oligopeptides ABC transporter. PAS staining confirmed that both polypeptides were glycosilated and revealed a different electrophoretic profile of glycosilated species in the wt compared to MIG1. In accordance with defective glycosilation of the S-layer protein, cell shape was affected in the mutant strain, as observed by SEM. Altogether our results suggest that RhoII may be involved in regulation of the protein glycosilation pathway in haloarchaea.
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MI-P32.
HIGH MASS MOLECULAR PROTEINS SECRETED BY *Clostridium septicum* INDUCE MACROPHAGE APOPTOSIS
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Clostridium septicum is an anaerobic bacillus, causal agent of myonecrosis diseases in man and animals that evolve with a high mortality rate. The only lethal factor informed is the alpha toxin (molecular mass of 46.5 kDa) that induces programmed necrosis. In this work we evaluated the capability of high molecular mass (>100 kDa) extracellular proteins of *C. septicum* to induce apoptosis or necrosis in murine peritoneal macrophages. Culture supernatants obtained at mid log phase from *C. septicum* ATCC 10092 were used to prepare concentrated and partial purified proteins by ultrafiltration: (i) >100, (ii)100 to 30, (iii)30 to 10 and (iv)< 10 kDa that were added to macrophages at 1, 2.5 and 5% v/v. Apoptotic and necrotic indices were established. Apoptosis was detected by morphological changes observed by optic microscopy, Annexin V CY3 and DNA ladder. Apoptosis was observed in macrophages treated with fractions (i) and (ii) at concentrations of 1 and 2,5%. Higher concentrations exerted a marked necrotic effect, particularly in fraction (ii). We have recently identified a *C. septicum* piruvato flavodoxin/ferrodoxin oxidorreductase of 130 kDa. It has been reported that the over- expression of this enzyme in mammalian cells induce apoptosis. These results show that *C. septicum* expresses high molecular mass virulence factors different from others than the alpha toxin.

MI-P33.

ANTI-APOPTOTIC EFFECT OF *Clostridium septicum* ALPHA TOXIN

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Clostridium septicum, the causative agent of atraumatic gas gangrene, produces a single lethal factor, alpha-toxin. The infection with this pathogen has been associated with colonic cancer. The objective of this work was to investigate the mechanism of action of the *C. septicum* alpha toxin on epithelial HT29 cell line and murine macrophages. The gene for *C. septicum* alpha-toxin was cloned and expressed in BL21 *Escherichia coli* strain. The purification of 6xHis-tagged recombinant proteins expressed in *E. coli* was performed by the Ni-NTA Purification System. The expression of the nuclear factor kappa beta (NF- B) was determined by Western blot. Macrophage and HT 29 cells were transferred to 96 well tissue culture plates, treated with different concentration of *C. septicum* alpha toxin and incubated for 6, 12, 24 and 48h in presence and absence of the pro-apoptotic drug staurosporine. The viability of the cells was assessed by 3-(4,5-dimethylthiazol-2-yl) (MTT) assay. Increased concentration of recombinant alpha toxin decreased the viability of cells but increased viability of staurosporine treated cells. Also, alpha toxin increased I ß expression and decreased NF- ß expression. In vitro experiments, showed an anti-apoptotic protective role on epithelial cells of intestinal mucosa and a possible contribution to the onset of colonic cancer.

MI-P34.

THE LENGTH OF THE EXOPOLYSACCHARIDE XANTHAN IS MODULATED BY IN TANDEM GUMB AND GumC OVEREXPRESSION

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Xanthan is a polymer of pentasaccharide repeat units, secreted by *Xanthomonas campestris* (Xc). Its biosynthesis involves an operon composed of 12 genes (*gumB* to *gumM*). Our aim was to study the roles GumB and GumC play in the polymerization and secretion of xanthan. We have already shown that *gumB* and *gumC* mutant strains did not produce xanthan, but synthesized the lipid-linked repeat units. Here, our strategy was to express increasing amounts of GumB and GumC, individually or in tandem, and to analyze the effect on xanthan polymerization. The corresponding genes were cloned in an arabinose-inducible vector and introduced in the wild-type Xc. Overexpression of GumB, GumC, or GumB-GumC neither affected the total amount nor the chemical composition of xanthan. We measured xanthan viscosity as indicator of polymer length. While GumB overexpression did not affect viscosity, a moderate increase in viscosity was achieved by raising GumC levels 5-times. In this condition, GumC was partially degraded. Expression of GumB in tandem with GumC diminished GumC degradation and resulted in higher xanthan viscosity than normal xanthan produced by the wild-type Xc. Moreover, longer polymer chains were observed by atomic force microscopy. These results suggest that modulation of xanthan chain-length involved both GumB and GumC proteins.

MI-P35.

NITRIC OXIDE AND TRUNCATED HEMOGLOBIN IN *Azospirillum brasilense*

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Azospirillum is a plant growth-promoting rhizobacteria that produces nitric oxide (NO) by nitrate dissimilation pathway under anaerobic respiration. Under aerobic conditions and growing with NO_3^-, we previously determined that *A. brasilense* Sp245 (wt) produces ca. 120 nmol $NO.g^{-1}$ bacteria, whereas an isogenic mutant in periplasmic nitrate reductase (Faj164) only produces ca. 6 nmol $NO.g^{-1}$ bacteria. As high concentrations of NO cause damage to proteins and membranes, we hypothesized on mechanisms of bacterial protection based on the occurrence of hemoglobins. We identified a putative truncated hemoglobin (trHb) of 147 amino acids in *A. brasilense* Sp245 genome (AztrHb). Bioinformatics analysis showed that AzTrHb have 71% similarity to trHb of *Campilobacter jejuni* (Ctb) belonging to phylogenetic group III. Additionally, semi quantitative RT-PCR analysis indicated that AztrHb expression was higher at late exponential growth in both strains when O_2 was deficient, but in O_2 sufficiency *wt* strain showed higher levels of AztrHb than Faj164 strain, according to its higher NO production. This is the first report on hemoglobin expression in the rhizobacteria *A. brasilense*

MI-P36.

DIVERSITY AND ABUNDANCE OF MARINE BACTERIAL AND ARCHAEAL COMMUNITIES FROM POTTER COVE, ANTARCTICA

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Temporal and spatial variability of Bacterial and Archaeal communities were studied during a one-year period at Potter Cove, Antarctica. Photosynthetic pigments, suspended particulate matter, salinity and temperature were also measured. Water samples from 3 sites were taken: E1 (inner cove), E2 (outer cove) and E3 (near the opening of a creek). Genomic DNA from filtered samples (0.22 µm) was analyzed using DGGE. Sequencing revealed that most bands were related to members of Class -*Proteobacteria* and some to -*Proteobacteria*. Principal Component Analysis using environmental data and diversity index, grouped the samples according to the different seasons, with component 1 (86.5%) grouping together the summer and spring samples and differentiating it from winter and autumn ones because of its high chlorophyll-a content, level of organic and inorganic suspended matter and lower bacterial and archaeal diversity. Discriminant Analysis confirmed the overlapping between spring and summer data and its significant differences compared with autumn and winter. Bacterial and archaeal DGGE patterns from E3 differentiate from E1 and E2, suggesting a spatial variability, probably due to glacial melting in summer. Results evidenced that environmental factors prevailing in summer and spring decrease microbial diversity and suggested annual changes in richness and abundance of *Bacteria* and *Archea*.

MI-P37.

ISOLATION OF A FUNGUS FROM A HYDROCARBON CONTAMINATED ANTARCTIC SOIL AND CHARACTERIZATION OF IT EXUD

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A psychrotolerant fungus was isolated from hydrocarbon-contaminated soils from Jubany station. After 30 days of growth at 15°C, the isolate produce an intense yellowish-colored exudate which completely dyed the culture medium (potato-dextrose–chloranphenicol agar). The exudate can be collected as small drops from the surface of colonies and the Petri dish. Based on previous reports, it was supposed that the molecule responsible of the yellow color could be an anthraquinone-related compound. In order to find out the composition of the exudate, several analytical procedures were applied (thin layer chromatography TLC, isoelectrofocusing, UV spectra analysis and reverse-phase high performance liquid chromatography). The results showed that the exudate is composed by at least four components, one of them exhibiting high polarity. The UV analysis suggested that two different molecules are responsible of the yellow color. Reverse-phase TLC evidenced the presence of two compounds with high absorption at near UV (366 nm) and isolectrofocusing showed that at least one of these compounds is a protein with isoelectric point near 3. Colonies of the fungus showed to have antifungal properties, inducing an evident inhibition halo when it was cultivated with other fungus in the same plate. The antifungal compound could represent a novel molecule with clinical or biotechnological potential uses.

MI-P38.

CITRATE METABOLISM IN *Lactobacillus casei* ATCC 334

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Citrate metabolism has been extensively studied in the lactic acid bacterium (LAB) by our labs. The first step of the citrate fermentative pathway following the uptake from the medium by the precursor/product exchanger, is the conversion to oxaloacetate and acetate by the citrate lyase. Then, oxaloacetate is decarboxylated to pyruvate by a cytoplasmic oxaloacetate decarboxylase termed CitM. The pathway generates proton motive force by an indirect proton pumping mechanism.
Lactobacillus casei ATCC334 is a probiotic microorganism used in the manufacture of different dairy fermented products. The genetic organization in the cit cluster in Lb casei differs from the organization found in others LABs. The steps in the pathway are the same but they are catalyzed by different enzymes. Uptake of citrate is catalyzed by a member of the Me2+-citrate transporter family and oxaloacetate is decarboxylated by a membrane bound complex (OAD) that in Gram-negative bacteria is a Na+ pump. The main objective of this work is to characterized citrate uptake and utilization in Lb casei.
Resting cells of Lb casei require the presence of Ca2+-ions to metabolise citrate indicating that the citrate is taken up in complex with Ca2+. Similarly, Ca2+ was required for the generation of a pH gradient by the pathway. Expression of the pathway was studied under different growth conditions.

MI-P39.

AN APPROACH TO ELUCIDATE THE MECHANISM OF ACTION OF THIACETAZONE ON *Mycobacterium smegmatis*

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Thiacetazone (TAC) is an inexpensive antitubercular drug that acts on mycobacteria although the essential target affected by it is still a matter of debate. To exert its effect TAC needs to be activated through the action of the mycobacterial mono-oxigenase EthA. It was reported that TAC inhibits the cyclopropanating mycolic acids synthases on *Mycobacterium bovis* BCG Pasteur. These enzymes are methyltransferases which add chemical modifications leading to mature mycolic acids although they are non essential enzymes. *Mycobacterium smegmatis* is a rapid growth mycobacteria reported to be resistant to TAC. In a first attempt to elucidate the cause of this resistance the seven methyltransferases of *M. smegmatis* were cloned and over-expressed. The minimum inhibitory concentration (MIC) of TAC did not change with respect to the parental strain although changes in the mycolic acid profile were observed. This result indicates that these enzymes are not involved in the resistance to TAC in this mycobacteria. Other possibility was that *M. smegmatis* lacked the activation step, for this reason the effect of the over-expression of ethA was studied. This made *M. smegmatis* susceptible to TAC with a MIC of 50 µg/ml. Analysis of the effect of TAC on this strain showed inhibition of mycolic acid synthesis. This results points to a still unidentified target of TAC on mycobacteria.

MI-P40.

VIRULENCE PHENOTYPIC MARKERS IN *Acinetobacter* SHOW INDEPENDENCE OF LUX SYSTEM

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Acinetobacter baumannii, an opportunistic Gram negative bacterium, represents a growing nosocomial threat due to its ability to acquire antibiotic resistance to most current therapeutic agents. We investigated the role of the AHL-mediated quorum sensing system in the expression of several pathogenic traits. For this purpose, the LuxI/LuxR system was studied in nine species-level characterized isolates from environmental and nosocomial sources. Lipase and siderophore secretion, hemolysis, biofilm formation, antibiotic resistance and cell motility showed independence of the expression of quorum sensing signals. Interestingly, only isolates belonging to the *A. calcoaceticus-A. baumannii* group, which contains the most clinically relevant strains, displayed quorum sensing signals and carried *luxI/R* genes. In addition, a *A. junii* isolate, showed extremely high level of biofilm formation (ca. 20 fold higher than the basal level) with no detectable quorum signals nor LuxI/R system, contradicting published work linking both markers. It was also determined that *Acinetobacter* isolates obtained from patients produced higher amounts of siderophores than those from non-clinical sources ($p<0.03$). Phylogenetic sequence analysis of translated *luxI/R* genes suggests that LuxI/R components in *Acinetobacter* were originated early in the genus speciation.

MI-P41.
BLUE LIGHT AND IRON AVAILABILITY REGULATE MOTILITY IN *Acinetobacter baylyi* STRAIN ADP1
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Light is a ubiquitous signal that many organisms use to assess the status of their environment and respond to adjust their physiology accordingly. Four putative Blue Light-sensing Using Flavin (BLUF) domains were found in the sequenced genome of *A. baylyi* (ADP1). This strain produces a polar thick pili implicated in motility, which is suppressed by light (Bitrian SAIB2008). Moreover, recent results in *Acinetobacter baumannii* ATCC 17978 have demonstrated that the blue-light sensing protein A (BlsA) is responsible for cell motility inhibition (Mussi *et al.*, 2010).
The aim of this study was to determine the wavelength which was involved in motility inhibition and which were the genes whose expression was regulated by light.
Cell motility was assayed as previously described (Mussi *et al.*, 2010). Briefly, Petri plates prepared with swimming medium were inoculated with 3 µl of freshly grown cultures (OD_{600} 0.3) and incubated at 24°C for 15 h in the dark or under blue or red light-emitting diode (LED), intensity ~ 6 µmol m^{-2} s^{-1} (LI-COR LI-1800).
We have demonstrated that motility in *Acinetobacter baylyi* (ADP1) is inhibited by blue light, whereas red light does not affect this phenotype. A microarray analysis revealed that a group of genes related with iron homeostasis are regulated by blue light. In addition, we showed that motility is also inhibited under iron-limited condition.

MI-P42.
PHOTOPROTECTION BY L-HISTIDINE AND MANNITOL IN *Escherichia coli* EXPOSED TO NATURAL SUNLIGHT
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The lethal effect of solar radiation on bacteria depends on the photodynamic action of its UVA component. Oxidative damage induced by UVA has been ascribed to the generation of different reactive oxygen species and the aim of the present work was to study the eventual generation of singlet oxygen and hydroxyl radical during exposure of *E. coli K*12 to natural sunlight. Survival curves were obtained in the presence or absence of L-histidine or mannitol, and without scavengers but under a nitrogen atmosphere. The addition of L-histidine or mannitol reproduced in a considerable extent the effect of oxygen depletion, and at the concentrations used both scavengers produce similar changes in the sunlight response of *E. coli*. If the rate constants for the reactions occurring in the cell are proportional to their *in vitro* values, and intracellular concentrations of the scavengers are proportional to their concentrations in the irradiation medium, the results strongly suggest that hydroxyl radical is responsible for most of the photodynamic action of solar radiation, since L-histidine and mannitol are efficient scavengers for this radical, but the reactivity of mannitol toward singlet oxygen is poor. The production of superoxide and hydrogen peroxide, precursors of the hydroxyl radical, could be a relevant characteristic determining the sunlight susceptibility of a bacterial species.

MI-P43.
STEROL BIOSYNTHESIS COMPARTMENTALIZATION IN *Trypanosoma cruzi*
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Sterol-enriched membrane microdomains are involved in many vital cellular processes as endocytosis, replication, compartmentalization and signaling. CYP51, a Cytochrome P450 (CYP) enzyme is a key enzyme in sterol synthesis present in Trypanosomatids. Moreover, ergosterol biosynthesis pathway is considered to be a selective drug target in Fungi, Leishmania and Trypanosoma, since their mammalian host use cholesterol instead. *Tc*CPR-A, *Tc*CPR-B and *Tc*CPR-C are members of putative Cytochrome P450 reductases (CPR) family in *T. cruzi*. The recombinant proteins *Tc*CPR-B and *Tc*CPR-C expressed in *E. coli* were able to complement CYP activities in an *in vitro* system. Moreover, ergosterol content was significantly increased in *Tc*CPR-B and C overexpressing parasites. We present here the results of complementation experiments showing that the introduction of *T. cruzi* cpr-B gene into a cpr knock-out *Saccharomyces cerevisiae* strain (cpr-) WRDelta can restore the yeast normal growth. Interestingly, immunofluorescence studies in *T. cruzi* localize these enzymes to different subcellular compartments. On the contrary, *Tc*CPR-A overexpression was lethal, displaying aberrant cells, with abnormal morphology and ultrastructural alterations.

MI-P44.
MONOAROMATIC HYDROCARBON BIODEGRADATION BY *Pseudomonas* STRAINS
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Monoaromatics hydrocarbons like benzene, toluene and xylene (BTX) are toxic compounds present in oil contaminated sites. *Pseudomonas* spp. KA-08 (KA) and KB-08 (KB) were isolated from activated sludge in our lab using kerosene as sole carbon source. As alkane dehydrogenase was not found in these strains, we proposed that BTX fraction in kerosene could support their growth. The aim of this work was to analyze degradation of BTX compounds by *Pseudomonas* spp. KA and KB. Both strains were able to grow with benzene, toluene and xylene as sole carbon source when the concentrations were 0,1%, 0.1% and 5% v/v respectively. KA was also able to grow with xylene at high concentrations (up to 90%). Xylene degradation was assayed qualitatively using an adaptation of the Microtox method by testing luminescence of *Vibrio fischerii*. Detection of genes involved in BTX degradation was analyzed by PCR amplification. Fragments corresponding to catechol dioxygenase (*xylE*) and xylene monooxygenase (xylA) genes were detected in both strains, while an aromatic dioxygenase (todC1) gene was present only in KA. Capability to produce biosurfactants in cultures with xylene 1% v/v was assayed by analyzing emulsifying activity (EA). EA was 17% and 22% in KB and KA, respectively. Our results suggest that these strains showed appropriated characteristics to be considered as monoaromatics bioremediation agents.

MI-P45.
GENOTYPING OF *Staphylococcus aureus* ISOLATED FROM DAIRY HERDS IN ARGENTINA

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Staphylococcus aureus is an important pathogen implicated in bovine mastitis. Virulence factors associated with pathogenesis are not well understood. The aim of this work was to examine 12 virulence-associated genes by PCR in 32 *S. aureus* strains isolated from infected animals. Milk samples were obtained from 25 dairy herds located in the central region of Argentina. Identification of strains was carried out by PCR-RFLP *groEL* gene. The genes studied were: *nuc, clfA, coa, spaA, hla - hlb, fnbB- fnbA, cap8 - cap5, icaA - icaD*. PCR analysis detected that *coa* and *protA* genes were found in 100% of the strains while *clfA* and *nuc* were found in 78% and 90%, respectively. The *hla* and *hlb* genes were present in 50% of the isolates while *fnbA* and B were found in 44% and 84%, respectively. The *cap8* gene was absent in all strains and *cap5* gene was present only in 28% of the strains. The *icaA* and *icaD* genes were found in 53% and 87.5%, respectively. A large number of virulence profiles could be identified, showing that isolates with different profiles of virulence are able to cause mastitis. No predominant virulence profile was determined. As yet, nothing has been reported about the occurrence of virulence-associated genes among *S. aureus* strains from dairy herds in Argentina. The results of this study contributes to a better understanding of the pathogenicity of this bacteria.

MI-P46.
BIOFILM PRODUCTION BY *Streptococcus uberis* STRAINS ISOLATED FROM BOVINE MASTITIS

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Streptococcus uberis is a major cause of mastitis in dairy cattle. Persistent and recurrent mastitis infections could be related to the formation of biofilms. The objectives of this study were to determine in vitro biofilm formation and the presence of several genes associated with biofilm formation such as competence (*comEA, comEC, comX*) and quorum sensing *luxS*, in *S. uberis* strains collected from bovine mastitis in Argentina. Thirty four strains of *S. uberis* were tested by Congo Red agar, microtiter plate, air-liquid interface with and without sucrose. The strains were classified as negative (9,1%), weak (30,31%) and strong (60,60%) biofilm formers. In this study, sucrose has an effect on biofilm production. All the strains were additionally investigated for the presence of competence and *luxS* genes. More than half of the strains were positive for the genes involved in biofilm production. The present study reveals that *S. uberis* is capable of forming biofilms in vitro on an abiotic surface. The results contribute to a better understanding of the pathogenicity of this bacterium and will allow for development of strategies to better manage in order to prevent mastitis caused by this pathogen.

MI-P47.
VIRULENCE GENES OF *Staphylococcus aureus* ISOLATED FROM BOVINE MASTITIS IN ARGENTINA AND BRAZIL

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Reports of the importance of Staphylococcus species as pathogens in animal infections have been described worldwide. Among coagulase-positive species, *Staphylococcus aureus* is considered the most pathogenic one, especially due to its ability to produce a large range of virulence factors that enables it to colonize different tissues of a large range of animal species. The present work evaluated the presence of the genes *fbnA, fbnB, cap5* and *cap8* that codifies for the protein fibronectin and the polissacharidic capsule in 66 coagulase-positive Staphylococci isolates from bovine mastitis in Argentina and Brazil. Polymerase chain reaction analysis was carried out using the primers and respective amplification programs available in literature. Brazilian isolates provided nineteen strains (38%) confirmed by rDNA amplification as *S. aureus*. PCR for fbnA gene were positive for 32% of the isolates. None isolates were positive for *fbnB*. Amplification of *cap* genes yielded 4% positive strains for *cap5* and only 2% positive strain for *cap8* gene. On the other hand, Argentinean isolates yielded *fbnA* gene for 26% of the isolates. *cap5* gene was only present in 60% of the isolates. The results showed that the genes analysed had a different occurrence according to the countries.

MI-P48.
DRAFT GENOME SEQUENCE OF *Enterococcus mundtii* CRL1656

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Enterococcus mundtii is a coccoid, Gram-positive, pigmented bacterium that belongs to the suborder Enterococcaceae. *E. mundtii* strain CRL1656 was isolated from raw cow milk from the region of Trancas (Tucumán, Argentina). This bacteriocinogenic lactic acid bacterium has been proposed as a probiotic microorganism to prevent mastitis in cows. We present the draft genomic sequence of this strain which was assembled in 450 contigs. The sequence was obtained using a whole-genome shotgun strategy (231,679 reads totaling ~89 Mb; ~29-fold coverage) with a 454 GS Titanium pyrosequencer. The genome was 3,102,251 bases in length, with a mean GC content of 38.31%. A total of 2,764 coding sequences (CDS) and 65 structural RNAs (49 tRNAs) were predicted. Annotation covered 297 RAST subsystems (43%) with 1,176 CDS of which 64 CDS were labeled as hypothetical proteins. As this strain is adapted to the dairy and plant environment a large set of genes related to sugar metabolism was detected (406 genes). We also found 22 genes that might be related to resistance to antibiotics and toxic compounds. Noteworthy, 3 of them encoded putative ß-lactamases, 4 resistance to fluoroquinolones and 2 multidrug efflux pumps. A cluster of 3 genes involved in bacteriocin synthesis was also found. Further comparative and experimental work will be needed to determine the probiotic characteristics of this microorganism.

MI-P49.
COMPARATIVE ANALYSIS OF GLYCOGEN SYNTHESIS ENZYMES IN CYANOBACTERIA
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Plants, green algae, and cyanobacteria (CN) synthesize storage polysaccharides by a similar ADPG-based pathway. CN, as other bacteria, accumulates glycogen, while plants and algae synthesized starch. Glycogen and starch synthesis are very complex processes. Each group present special features, but the two first steps, carried out by ADP-glucose pyrophosphorylase (AGPase) and glycogen synthase (GS)/starch synthase, are present in all organisms. CN represent a very interesting group because storage metabolism pathways of photosynthetic eukaryotes have evolved from a cyanobacterial ancestor via endosymbiosis. Thus, we perform a global comparative bioinformatic analysis of all AGPase and GS proteins of cyanobacterial available genomes and representative sequences of Viridiplantae and Bacteria with the aim of detecting key diversity patterns that could reflect functional differences. The results reveal that all CN have a single AGPase, which is extremely conserved within this group. However, cyanobacterial GS presents major variability and one or two isoforms (GSA1 and GSA2), with the exception of UCYN-A, which lacks GS homologs. GSA1 is found in almost all CN analyzed, however GSA2 is only found in 25/58 genomes. The most important differences in domains, 3D structure and phylogeny related with protein function will be discussed.
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MI-P50.
***IN VITRO* AND *IN VIVO* CHARACTERIZATION OF *Bordetella bronchiseptica* LIPOPOLYSACCHARIDE DEFECTIVE MUTANTS**
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Bordetella bronchiseptica is a respiratory pathogen of different mammals, including humans. The lipopolysaccharide (LPS) of this pathogen plays an important role in bacterial host interaction not only as adhesin but also in protection.
Here we describe two genes, BB3398 and BB3394,that potentially codifiy to known glycoyltransferases involved in the biosynthesis of the *B. bronchiseptica* core oligosaccharide. By insertionally inactivating these genes and studying the resulting LPS structures, we show that BB3398 presented a deep rough phenotype in SDS-PAGE while BB3394 mutant profile was similar to parental LPS. In addition, we demonstrate that mutations in both genes affect the sensivity to bactericidal activity of mice naïve sera and to detergent. Furthermore, we found that inactivation of the responsible glycosyltransferases reduces bacterial colonization in mice. These results represent another evidence showing the relevance of the complete structure of LPS in bacterial host interaction.

MI-P51.
IMPACT OF MISMATCH REPAIR SYSTEM (MRS) DEFICIENCY ON THE EVOLUTION OF *Pseudomonas aeruginosa*
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Cystic fibrosis (CF) patients acquire *P. aeruginosa* (PA) during early childhood later on developing chronic airway infections (CAI) established by a same bacterial lineage, which persists through their entire life by using various adaptive mechanisms. Among them, PA suffers phenotypic diversification based on mutagenic events, leading to the emergence of adaptive variants such as mucoids, sessile and avirulents. Mutators, mainly due to MRS deficiency, are also highly prevalent in CAI and have been associated with increased antibiotic resistance. However, their role remains to be completely elucidated. This work aims to study the impact of hypermutability on PA phenotypic and genetic diversification through many generations of persistence in CF patients. We set up longitudinal (20 years) and cross-sectional (90 isolates) collections of isolates from CF patients harboring mutator strains, which are being analyzed by using a combination of whole genome sequencing, BIOLOG metabolic profiling and phenotypic characterization. Results to date on metabolic and phenotypic diversity indicate that mutator and nonmutator clones follow convergent evolution into the same adaptive traits. Notably, mutators with different MRS mutations can be observed into a single patient, even coexisting at the same time, confirming strong selective pressures for mutators in the harsh CF lung environment.

MI-P52.
MECHANOSENSITIVE CHANNELS ARE REQUIRED FOR BACTERIAL SURVIVAL FROM SEVERE THERMAL STRESS
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Mesophilic bacteria like *Escherichia coli* can sustain growth in a temperature range between 15 and 49 ºC. Higher temperatures (> 50 ºC) promote cell death and are therefore defined as severe or extreme. The cellular targets responsible for cell death are still obscure, but ribosome melting, protein aggregation, and denaturation of membrane proteins are thought to represent main targets of extreme temperature exposure. The cellular mechanisms that contribute to bacterial survival and/or recovery from severe heat stress (SHS) are still ill-defined.
Here, we present evidence that mechanosensitive channels (MSC) are required for bacterial survival from SHS. Cell death curves indicated that *E. coli* mutants lacking major MSCs were much more sensitive to SHS at 50ºC than isogenic wild-type (wt) cells. Expression in the mutants of the corresponding *msc* genes from plasmids restored the wt SHS resistant phenotype, discarding pleiotropic effects due to the mutations. Protein aggregation and cell buoyant density analyses using wt cells indicated that SHS provoked a hyposmotic-like stress, most probably resulting from the massive cytoplasmatic protein denaturation and exposure of buried protein sectors to the solvent. We propose that MSC are necessary to relieve the increases in cell turgor resulting from the SHS, thus allowing cell survival and recovery when extreme temperatures recede.

MI-P53.
THE *SBMA* LOCUS IS INVOLVED IN VIRULENCE-ASSOSIATED PHENOTYPES

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SbmA protein is involved in the transport of MccB17, MccJ25, bleomycin and proline rich peptides into the *Escherichia coli* cytoplasm. In *Brucella abortus*, *Sinorhizobium meliloti* and *Mycobacterium tuberculosis* the SbmA homologue, BacA, is essential for the chronic infection of these pathogens. We evaluated SbmA role in the *Salmonella* Typhimurium ability to infect and replicate within macrophages. The *sbmA* mutant strain showed increased intracellular replication compared to parental strain. Further assays demonstrated that this phenomenon was caused by increased resistance to the acidic pH present in the macrophage environment. Biofilm formation, aggregation and swarming are important bacterial traits that affect pathogen chances of survival in competitive environments and the ability to infect hosts. Assays demonstrated that *sbmA* absence significantly enhanced biofilm, aggregation and swarming phenotypes. Additionally, mutation in *yaiW*, gene transcriptionally related to *sbmA*, affected biofilm and swarming in the same way as *sbmA* mutation did. However, the *yaiW* mutant showed a reduction in the aggregation phenotype. A gene profile expression assay of *sbmA* and *yaiW* mutans showed that the transcription levels of genes implicated in swarming and motility were enhanced in those mutants. These results suggest that *sbmA* is involved in important physiological roles related to virulence.

NS-P01.

ANGIOTENSIN II AT_2 RECEPTORS MODULATE NEURONAL DIFFERENTIATION

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Neuronal differentiation is a complex process characterized by neurite outgrowth. Binding of nerve growth factor (NGF) to the receptor TrKA induces differentiation by activating ERK pathway. In the past years it has become clear that, in addition to its cardiovascular roles, the octapeptide Angiotensin II (Ang II) has many functions in the brain by acting through two receptor subtypes, AT_1 and AT_2. However its role in neuronal differentiation is not well defined. Thus, we decided to examine the participation of Ang II and NGF in differentiation of Neuro2A cells with special focus in ERK activation. By RT-PCR and western blot we determined that Neuro2A cells express AT_1, AT_2 and TrKA receptors. Cells were stimulated with Ang II, CGP42112 (AT_2 specific receptor agonist) or NGF and active ERK detected in cell lysates by western blot with specific antibodies. As expected, NGF induces ERK phosphorylation in a time dependent manner. Likewise, Ang II and CGP42112 stimulate ERK, suggesting an AT_2 receptor-dependent effect. To study differentiation, cells were treated with agonists for 24 h and observed under optic and fluorescent microscopy. Both Ang II and CGP42112 induce differentiation and expression of ßIII tubulin, a neuritogenesis marker. These results suggest that AT_2 receptor activation induces ERK phosphorylation, expression of ßIII tubulin and differentiation in Neuro2A cells.

NS-P02.

GANGLIONIC EFFECT OF PROLACTIN ON THE LUTEAL REGRESSION ON DAY 4 POSTPARTUM IN NON-LACTATING RATS

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We have shown that prolactin (PRL) promotes the regression of the corpus luteum (CL) at the end of pregnancy through neural pathway. However, this effect was not manifested because of its high endogenous levels in the postpartum period in lactating rats. The aim of this study was to investigate the effect of PRL in the coeliac ganglion (CG), through superior ovarian nerve (SON) on the luteal regression on day 4 pospartum in non-lactating rats. The *ex vivo* system CG-SON-Ovary was incubated in metabolic bath keeping the CG and the ovary, connected by the SON, in separate compartments. PRL was added (10^{-7}M) into the ganglion compartment [(PRL)g]. Controls were not stimulated. Periodic extractions of the ovary incubation liquid were taken at different times during 240 min to measure progesterone (P) by RIA and nitrites by the Griess methods. At 240 min, the luteal mRNA expression of 3ß-HSD, 20 -HSD (P synthesis and degradation enzymes, respectively) and iNOS, were analysed by RT-PCR. ANOVA I followed by Tukey test with a statistical significance of $p<0.05$ was used. [(PRL)g] increased the release of P and the expression of 3ß-HSD and decreased 20 -HSD; it also increased the release of nitrites without modify the expression of iNOS respect to the control. In conclusion, PRL, whose serum levels are low in non-lactating rats, modulates the luteal regression through the neural pathway.

NS-P03.

GSH REDUCTASE EXPRESSION IS CIRCADIAN IN THE HIPPOCAMPUS AND DAMPENED IN THE VITAMIN A DEFICIENCY

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Reduced glutathione (GSH) is the major endogenous antioxidant produced by cells and is critical for the maintenance of the redox potential in the brain. Our objectives were to investigate whether GSH reductase (GR) displays a circadian expression pattern in the rat hippocampus and to evaluate to which extent vitamin A deficiency (VAD) could modify temporal patterns of clock, RARs and GR expression. Holtzman rats weaned at 21 d of age were immediately assigned to either a vitamin A-free diet (DE group) or the same diet containing 4000 IU of vitamin A/Kg diet (CO group) during 3 months. Both groups were maintained under 12h-Light:12h-Dark or 12h-Dark:12h-Dark conditions during 10 days before the experiment. Hippocampi were taken every 4 h. GR transcript levels were determined by RT-PCR. RAR , RARß, BMAL1 and PER1 protein levels were determined by immunoblotting. We found mRNA levels of GR display a circadian pattern in the rat hippocampus peaking at the middle of the night ($P<0.01$). RAR , RARß, BMAL1 and PER1 also exhibited circadian rhythmicity ($P< 0.05$, $P<0.01$) in this brain area. VAD dampened the oscillating GR expression and phase shifted rhythms of clock and RARs proteins.Thus, VAD might affect the circadian expression of GR and consequently, the availability of GSH at a given time during the day, probably, by altering the circadian patterns of key clock and RARs proteins.

NS-P04.

NEUROPROTECTIVE EFFECT OF THE ACTIVATION OF CREB AGAINST OXIDATIVE STRESS IN NEURONAL CELLS

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CREB (cAMP response element binding) protein is a cellular transcription factor that mediates responses to different physiological and pathological signals. Previously, we have demostrated that this transcription factor is phosphorylated in response to DNA damage and cellular stress caused by H_2O_2 treatment and that this modification seems to be necessary but not sufficient to trigger its activation. Here, we studied the role of CREB activation during the DNA damage response against neuronal stress. We observed an upregulation of CREB mRNA and protein levels (RealTimePCR and Western blot) following H_2O_2 treatment, but no significant ser133 phosporylation.
Downregulation of CREB resulted in enhanced apoptosis (20,7% vs. 6,1%) and increased number and size of DNA damage foci as well as H2Ax protein levels in neuronal cells treated with H_2O_2.
The neuroprotective effect mediated by CREB appears to require phosphorylation at another residue, different from ser133, and to be regulated by different pathways.
In summary, regulation and activation of CREB and its transcriptional modulation of target genes promote the neuroprotective effect of CREB.

NS-P05.
ARC PROTEIN IS UPREGULATED AT A mRNA LEVEL AFTER DNA DAMAGE
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Early Growth Response 1 (EGR1) is a transcription factor that belongs to the immediate early gene (IEG) family, a group of genes whose expression is rapidly increased following synaptic firing. Under these conditions EGR1 activates the transcription of Arc, another IEG also involved in synaptic plasticity and memory formation.
On the other hand EGR1 is also involved in cell proliferation and apoptosis and becomes upregulated following DNA damage. The aim of this work was to study the expression of both IEG following DNA damage in order to find out if Arc transcription is increased after a genotoxic insult. The expression of both genes was upregulated in cell lines of neural origin after the treatment with three different genotoxic agents (UV light, H_2O_2 and neocarzinostatin), whether they were differentiated or not under the appropiate conditions. EGR1 mRNA stability after the addition of actinomycin D was unmodified in the presence or absence of a genotoxic agent, but was more stable in differentiated cells. Finally Arc and EGR1 expression was also induced by the addition of the calcium ionophore A23187, and required the release of intracellular calcium for its upregulation after DNA damage in non-differentiated cells.
Overall our results show for the first time that Arc is upregulated after DNA damage, and suggest a possible role of this protein in the DNA damage response.

NS-P06.
ANGIOTENSIN II AT2 RECEPTORS IN THE AUDITORY PATHWAY DURING DEVELOPMENT
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Angiotensin II (Ang II) is associated with fluid homeostasis and blood pressure control and can also modulate neuronal activity in regions involved in cognition, motor control and sensory integration. Ang II recognizes two receptor subtypes, named as AT1 and AT2. Previously, we studied Ang II receptor localization during cerebellar development, which peaked at P15. The inferior colliculus (IC) is a relay station in the ascending auditory pathway at the midbrain, serving as a major integrative center within the central auditory system. We performed a study of Ang II receptor localization by autoradiography and immunohistochemistry and mRNA expression by RT-PCR during postnatal development in rat midbrain and hindbrain. Specific binding at the IC increased with age, maximal in P15 rats ($P < 0.001$) with prevalence of AT2 receptors, in coincidence with maximal mRNA AT2 receptor expression at this stage. Interestingly, the ventral and dorsal coclear nuclei, involved in the auditory pathway also exhibited binding to AT2 receptors in the P8 and P15 rat brainstem. The pattern of Ang II AT2 receptors expression in the IC encompasses the pattern of brainstem maturation and connection with the cerebral system. Since the onset of hearing in the rat, happens around P12, we can assume that AT2 receptors might play a potential role in organogenesis and the establishment of neuronal circuits

NS-P07.
PROTEIN KINASE D1 (PKD1)-DEPENDENT NEUROTROPHIN RECEPTOR TRKA TRAFFICKING AND SORTING
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After determining the role of several proteins, such as PKD1, in the regulation of intracellular trafficking, we have decided to study the role of these regulatory proteins in the establishment of neuronal polarity, specifically through the analysis of TrkA Neurotrophin Receptor transport and sorting.
This receptor is actively localized in the axonal terminal, reaching that specific neuronal region by ligand (NGF)-dependent transcytosis at the dendrites.
Without NGF, EGFP-TrkA normal distribution is mostly somatodendritic. In the absence of an active PKD1, using shPKD1 or PKD-KD, we have observed a remarkable decrease in the TrkA distribution at the neuronal processes, together with its accumulation in large vesicles at the neuronal soma. In addition, there was a significant reduction in the length and number of dendrite branches.
On the other side, we have analyzed the effect of the lack of PKD1 activity when NGF was present. Even though the length of dendrites was reduced as it was observed without NGF, there was a change in the arborization pattern and in the morfometric variables of dendritic branches.
These results would confirm that PKD1 play a key role in the fission regulation of vesicles carrying TrkA, and that the presence of this receptor at the neuronal surface would be essential for dendrite lenght development but not for branching of these neuronal processes.

NS-P08.
NEW PERSPECTIVES IN GLAUCOMA: UNRECOGNIZED SYMPTOMS AND LOOKING FOR NEW THERAPEUTIC STRATEGIES
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Glaucoma is a leading cause of blindness, due to retinal ganglion cells (RGC) death and optic nerve damage. Retinal ischemia participates in glaucomatous damage. Recent evidences indicate that a population of RGC is intrinsically photosensitive (through the expression of a fotopigment, melanopsin), and transmits light information to the suprachiasmatic nuclei (SCN), the principal pacemaker for circadian rhythms. We analyzed: 1) the effect of ischemic conditioning on retinal damage induced by experimental glaucoma, and 2) the non-image forming visual system in experimental and human glaucoma.Weekly injections of vehicle or chondroitin sulfate were performed in the rat eye anterior chamber for 10 weeks. Ischemic conditioning was induced by weekly increasing intraocular pressure to 120 mmHg for 5 min. Brief ischemia pulses reversed the effect of glaucoma on retinal function and histology. Experimental glaucoma induced alterations in melanopsin levels, light suppression of nocturnal pineal melatonin, and light-induced c-Fos expression in the SCN. Glaucomatous animals exhibited an increase in the diurnal activity, and glaucomatous patients showed a significant decrease in the sleep quality. Conclusion: The induction of ischemic tolerance could constitute a new therapeutic strategy for glaucoma treatment. Glaucoma induced significant alterations in the non-image forming visual system.

NS-P09.
LOCALIZATION OF RETINALDEHYDE ISOMERASE IN THE CHICKEN INNER RETINA
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Retinal cone and rod photoreceptor cells (PRC) are responsible for day and night vision respectively while the inner retina has been mainly involved in the transmission of the nerve impulse from PRC to the brain. However, a third group of PRC has been shown recently to be present in the inner retina, specially in intrinsically photosensitive retinal ganglion cells (reviewed in Guido *et al.*, 2010). Moreover, different non-visual opsins such as Opn4 , Opn5 and RGR were shown to be expressed in the inner retina. However, it is still unknown the mechanism used to regenerate the photopigment chromophore. RPE65 is the main isomerohydrolase in the vertebrate eye expressed in the retinal pigment epithelium but not in the neural retina.The zebra fish has three different isoforms for the RPE65 enzyme of which, RPE65c is expressed in the retina. In the chicken, we observed the expression of the RPE65c homolog in the inner retina only, especially in the inner nuclear and inner plexiform layers. Using specific cell markers we found that expression was mainly restricted to amacrine cells. In addition, we found isomerohydrolase activity in this retinal area. In conclusion, chicken amacrine cells display the expression and activity of this non-typical isomerase. Results provide first evidences on the mechanism by which the inner retina may regenerate the chromophore linked to non-visual opsins.

NS-P10.
NOVEL ISOLATION OF CHICKEN RETINAL HORIZONTAL CELLS
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We described that the non-visual photopigment melanopsin X is expressed mainly in horizontal cells of the chicken retina (Verra *et al*., 2011). In this work our aim was to purify and culture horizontal cells (HC´s) from the chicken embryonic retina for further characterization.
Disaggregated retinas of chicken embryos at day 14 were subjected to a bovine serum albumin (BSA) discontinuous gradient of concentrations ranging from 1 to 5%. After centrifugation, cells collected from the different phases were cultured for 4 days and characterized by immunochemistry and cell morphology. Phases were examined with specifics antibodies against HC markers: PROX-1 and Islet-1. Results showed that only the fraction corresponding to 2.5% of BSA was highly enriched in PROX-1 positive cells ($\geq$ 95%) displaying a typical HC morphology. In fact, some cells in this fraction resembled axon-less candelabrum-shaped HC's. Preliminary results showed that around 50% of the PROX-1 cells in this phase were positive for Islet-1. Moreover, Western blot assays demonstrated that mainly the phase at 2.5% BSA, exhibited positive PROX-1 immunoreactivity (MW: 83kDa).
In conclusion the BSA gradient proved to be the most effective method to separate different retinal cell populations, and particularly HC´s which will allow us to characterize them as potential photoreceptors by biochemical and pharmacological studies.

NS-P11.
RESPONSE OF FLY MUTANTS FOR THE METABOLISM OF N-ß-ALANYLDERIVATIVES TO CROWDING STRESS
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In *Drosophila melanogaster*, Ebony and Tan proteins are responsible for the synthesis and hydrolysis, respectively, of N- -alanylderivatives like N- -alanyldopamine (NBAD). Together, they establish a system that regulates dopamine (DA) and other neurotransmitter levels in insects, maintaining central nervous system (CNS) homeostasis; which is altered in null-function mutants. *ebony* presents reduced levels of NBAD and excess of DA; the opposite is true for *tan*. In *Drosophila* DA plays central regulatory roles controlling sleep and wakefulness. In a crowded environment, a high release of neurotransmitters occurs, so flies need to recycle DA in order to avoid potential oxidative stress in the CNS. Our aim was to compare wild type and mutants response to crowding stress. Main parameters studied were sleep patterns (recorded in activity monitors), lipid peroxidation (indicative of oxidative stress) and NBAD metabolism (indicator of DA recycling). Both *ebony* and *tan* mutant strains exhibited differences in sleep patterns. *ebony* flies showed a decrease in total sleeping time at night. *tan* showed difficulties maintaining sleep, with a decreased in the duration of sleep bouts, but an increase in their frequencies. Differences in lipid peroxidation in response to stress were observed between the strains. These results suggest a role of the Ebony-Tan system in the maintenance of CNS homeostasis.

NS-P12.
NOT JUST ANY FREE FATTY ACID INHIBITS THE NICOTINIC ACETYLCHOLINE RECEPTOR
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To elucidate the mechanism involved in the non-competitive inhibition of the nicotinic acetylcholine receptor (AChR) caused by free fatty acids (FFAs), we studied the effect of FFAs with a single double-bond at different positions (6, 9, 11 and 13, *cis*-18:1) on different AChR properties. Two FFAs (6 and 9) reduced the duration of the channel open-state. The briefest component of the closed-time distribution remained unaltered, suggesting that 6 and 9 do not behave as typical open-channel blockers but rather as allosteric blockers. Fluorescence resonance energy transfer studies showed that all FFAs locate at the lipid-AChR interface, 6 being restricted to annular sites and all others occupying non-annular sites. Fluorescence quenching studies of pyrene-labeled AChR indicate that all *cis*-FFAs produce AChR conformational changes at the transmembrane level. Using the AChR conformational-sensitive probe crystal violet, we observed that all unsaturated FFAs increase its K_D in the AChR desensitized state, but only 9, 11 and 13 *cis*-18:1 decrease its K_D in the resting state. In conclusion, some FFAs appear to directly inhibit AChR function probably by localizing at superficial sites inside the membrane, whereas other FFAs modulate the receptor´s conformational states by a different mechanism.

NS-P13.

SEXUALLY DIMORPHIC INTERACTION BETWEEN ESTRADIOL AND NOTCH/Ngn3 SIGNALING IN HYPOTHALAMIC NEURONS

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Previous results have demonstrated that sexually segregated hypothalamic neurons respond differentially to the hormonal environment before the masculinizing actions of gonadal steroids. At the embryonic stage 16, 17ß-estradiol (E2) induces axonal growth only in male hypothalamic neurons. Recent studies have shown that E2 and Notch signaling converge to control neuritogenesis in hippocampal neurons. Activation of Notch is associated with an enhancement of the expression of the transcription factors Hes1 and Hes5, which negatively regulate the proneural gene Ngn3 that is involved in neuritic growth. Using real time PCR we have detected that E14 hypothalamic neurons express Ngn3 in a sexually dimorphic pattern: female neuronal cultures had significantly higher Ngn3 mRNA levels than males. Moreover, E2 (10 nM) significantly increased Ngn3 mRNA in males, but not in females. This effect was completely blocked by the ER /ß-mediated transcription antagonist ICI 182,780 (10 µM). In addition, male neuronal cultures had significantly higher Hes1 mRNA levels than females and E2 significantly reduced Hes1 mRNA levels in males, but not in females. The effect of the hormone on Hes1 was also blocked by ICI 182,780. These results suggest that E2 and Notch signaling pathways interact in hypothalamic neurons with a sexually dimorphic pattern even before the organizational effect of gonadal steroids.

ST-P01.

KLF6 TUMOR SUPPRESSOR IS INDUCED UPON EXPRESSION OF ACTIVATED RAS: ANTI-ONCOGENESIS OR ONCOGENESIS ?

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KLF6 is a member of the Krüppel-like factor family of transcriptional regulators and a tumor suppressor gene whose loss of expression or activity, due to loss-of-heterozygocity LOH, mutation, or promoter methylation, occur in many types of cancer. We demonstrated that this protein interacts with the c-Jun oncoprotein and induces its degradation leading to inhibition of cell proliferation. c-Jun is a major component of the AP-1 transcription factor, an important cooperating partner of oncogenic Ras in cell transformation, which also requires the function of Jun N-terminal Kinases (JNKs). This work focuses on biochemical and molecular mechanisms involving KLF6 as a factor that could regulate the oncogenic activation triggered by the Ras pathway.

To investigate a possible Ras-KLF6 crosstalk, we took advantage of a Ras-inducible model. We stable transduced NIH3T3 fibroblasts to express a constitutively active H-Ras (G12V) under the control of a tetracycline inducible promoter. Here we demonstrate that H-RasG12V induction leads to a progressive increase of KLF6 protein in a time-dependent manner. This KLF6 increase can be repressed by a JNKs specific inhibitor. Further, in experiments where KLF6 is over expressed after Ras induction we observed a decrease in cell proliferation rate.

Thus, results are consistent with the ability of KLF6 to impair enhanced cell proliferation following activation of Ras oncogene.

ST-P02.

ANTIAPOPTOTIC EFFECTS OF TESTOSTERONE AND 17ß-ESTRADIOL IN SKELETAL MUSCLE

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We previously described that testosterone (T) and 17ß-estradiol (E2) protect C2C12 muscle cells from apoptosis induced by H_2O_2. Thus, H_2O_2 promoted typical changes of apoptosis such as nuclear fragmentation, cytoskeleton disorganization, mitochondrial reorganization/dysfunction and cytochrome c release that were abolished upon treatment of cells with the hormones. In the present work we studied further in depth the mechanism of action of these steroids in apoptosis. Competitive binding assays, immunocytochemistry and immunoblottings revealed that the androgen receptor with a non-classical localization (mainly mitochondria) is involved in the protective role of T. Thus, T inhibits PARP cleavage and Bax expression induced by H_2O_2 but in presence of flutamide this antiapoptotic effect is not observed. By flow cytometry using the fluorescent dye JC-1, it was also demonstrated that T prevents mitochondrial membrane potential depolarization in response to H_2O_2, as described before for E2. This loss of membrane potential may be due to the opening of the mitochondrial permeability transition pore (MPTP), as flow cytometry and fluorescence microscopy with calcein-AM demonstrated that E2 can inhibit the continuous activation of MPTP by H_2O_2. Altogether, our studies suggest a role of T and E2 in the regulation of apoptosis with a clear action at the mitochondrial level in muscle cells.

ST-P03.

EXTRACELLULAR ATP INDUCES THE INACTIVATION OF FOXO TRANSCRIPTION FACTORS THROUGH PI3K/AKT

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Forkhead O transcription factors (FoxOs) play a pivotal role in the regulation of cell proliferation and cell death. Thus, perturbation of their function can result in diseases such as cancer. In this work, we studied the effect of extracellular ATP on the phosphorylation and expression of FoxO transcription factors in human breast cancer MCF-7 cells. Western blot analysis showed that 5 µM ATP rapidly (15 min) phosphorylated FoxO1/3a at threonine 24/32. However, ATP did not induce changes in the phosphorylation of FoxO3a at serine 318 or FoxO1 at serine 256, and in the expression of FoxO1 and FoxO3a. In addition, the use of several purinergic agonists ATP S, ADP, ADPßS, UTP, UDP and adenosine (5 µM) showed that the phosphorylation of FoxO1/3a was due to ATP action and not to its degradation products, and suggested the involvement of ATP/UTP-sensitive P2Y receptors in that effect. Moreover, the PI3K inhibitor Ly294002 suppressed the phosphorylation of FoxO1/3a at Thr 24/32 in response to ATP. Immunocytochemistry and subcellular fractionation studies showed that ATP induces the translocation of FoxO3a from the nucleus to the cytoplasm. The results obtained demonstrate that ATP induces the inactivation of FoxO1/3a in a PI3K dependent manner in MCF-7 cells, suggesting a role for these transcription factors in the regulation of breast cancer cells proliferation induced by ATP.

ST-P04.

ING1B TUMOR SUPPRESSOR REQUIRES FUNCTIONAL CHECKPOINT KINASE 1 TO EXERT ITS DNA REPAIR ACTIVITY

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Inhibitor of Growth 1b (ING1b), a member of the ING protein family, is involved in senescence, cell cycle arrest, apoptosis and DNA repair. Here, we studied the role of endogenous ING1b in DNA repair, in wild type and Ing1 deficient g/g MEFs, treated with H_2O_2 or neocarzinostatin (NCS) as genotoxic agents. In absence of ING1 DNA repair is impaired in unscheduled DNA synthesis (UDS) assays for both treatments. Moreover, transfection of Ing1b antisense oligonucleotide in wt MEFs reduced DNA repair to levels in g/g MEFs. g/g MEFs showed higher levels of H2AX, a marker of DSBs, relative to wt MEFs. These displayed a slower kinetics of H2AX appearance than g/g MEFs indicating that ING1b deficiency results in an exacerbated response to DNA damage. We showed that ING1b transcript and protein are up-regulated by H_2O_2, NCS and UV in MEFs, HCT116 and H1299 cells and this induction is independent of p53. We also detected ING1b phosphorylation in HCT116 cells by metabolic labelling after DNA damage, independently of p53. Moreover, ING1b DNA repair activity in wt MEFs is completely abolished when Chk1 is inhibited by SB218078. In summary, ING1b improves DNA repair in response to a variety of genotoxic agents. We propose that physiological levels of ING1b are required to trigger a proper DNA damage response. Finally, phosphorylation of ING1b, probably by Chk1 is necessary to exert its DNA repair activity.

ST-P05.
PARTICIPATION OF FAK IN ANG II AT2 RECEPTOR SIGNALING PATHWAY IN RAT HINDRAIN
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The functional role of AT2 receptors is unclear and it activates unconventional signaling pathways. In the present study, we aimed to investigate the transduction mechanism of Ang II AT2 receptors in PND15 rat hindbrain, a physiological developmental condition. Previously, we demonstrated the participation of the PTPase SHP-1 and c-Src family kinase (SFK) members in the signaling pathway of Ang II AT2 receptors. Membrane preparations of PND15 rat hindbrain were stimulated with Ang II, solubilizated with Triton X-100 and immunoprecipitated with anti AT2 or anti-SHP-1 antibody. Since AT2 receptors are involved in neuron migration, we tested the presence of FAK and paxillin in immunocomplexes. Surprisingly, AT2-immunocomplexes contained p85FAK, while paxillin and p125FAK were absent. Thus, a complex containing AT2/SHP-1/c-Src/p85FAK was obtained, suggesting a potential role of Ang II AT2 receptors in cerebellar development and neuronal differentiation. p85FAK/AT2 association was time-dependent. The presence of c-Src inhibitor PP2 and PTPase inhibitor Na_3VO_4 induced the uncoupling of p85FAK from AT2 receptors. Besides, we showed the association of p125FAK and p85FAK to SHP-1 in SHP-1-immunocomplexes. In summary, we demonstrated the presence of an active signal transduction mechanism in PND15 rat hindbrain, a developmental stage critical for cerebellar development.

ST-P06.
ANALYSIS OF SCAFFOLD-PROTEIN MEMBRANE RECRUITMENT DYNAMICS DURING PHEROMONE RESPONSE IN *Saccharomycfes cerevisiae*
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The yeast mating pheromone response pathway is a prototypical example of a signaling pathway that uses a scaffold protein. The scaffold, Ste5, has several interaction domains, some of which mediate essential steps necessary for the activation of its associated three-tiered MAPK cascade, while others function in higher-order regulatory behaviors, such as feedback phosphorylation by the MAPK Fus3[1] and inhibitory phosphorylation by the cell-cycle dependent kinase[2]. This work is aimed at determining the role of Ste5 interaction domains in the dynamics of the pheromone response.
To do this, we followed in real-time recruitment of Ste5 to the membrane in single cells upon pheromone treatment, analyzing the redistribution of a Ste5 YFP fusion with a confocal microscope. We obtained quantitative measurements from single-cells using Cell-ID coupled data analysis with R, following a method previously developed in our lab[3].
We measured several Ste5 mutants to assess the role of Ste5 modular domains in pathway dynamics. Mutations at Ste5 regulatory sites, mimicking constitutive (de)phosphorylation, produced changes in the dynamic of membrane binding, suggesting the importance of such modifications for the fine-tuning of the response.
[1]Yu *et al*. Nature 456:755(2008)
[2]Strickfaden *et al*. Cell 128:519(2007)
[3]Chernomoretz *et al*. Curr Protoc Mol Biol 84:14.18.1(2008)

ST-P07.
STUDYING THE CELL CYCLE AND PHEROMONE RESPONSE PATHWAY INTERACTION IN YEAST *Saccharomycfes cerevisiae*
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In haploid yeast, mating pheromone triggers a fate decision: arrest of the cell cycle in G1 and initiation of mating events. To do that, the MAP kinase of the pheromone response pathway (PRP) Fus3 activates the cyclin dependent kinase inhibitor Far1, which binds to and inhibits all three G1 cyclins complexes, Cdc28/Clns. Cdc28/Cln2 activity is essential to pass the START G1 checkpoint, while Cdc28/Cln3 is required earlier in G1 to drive the expression of Cln2. This has two important consequences: a) inhibition of Cdc28/Clns causes cells to arrest in G1, and b) since Cdc28/Cln2 can block pheromone response, its inhibition by Far1 acts as a positive feedback loop. Because the signaling pathway is physically supported by a network of interacting proteins, evolving in space and time according to fundamental laws of reaction, diffusion and transport, here we propose a basic mathematical model, with a small number of parameters and dynamical variables, that reproduces the observed biological phenomena: the interaction between the cell cycle and the PRP. As a first approximation, we used a set of nonlinear ordinary differential equations (ODE) that describe the dynamics of the simultaneously occurring reactions. We tested our model using fluorescent microscopy, measuring fluorescent proteins used as reporters of the PRP and morphology for the cell cycle.

ST-P08.
LIGHT-DEPENDENT DAGK ACTIVITY IN A PURIFIED PHOTORECEPTOR NUCLEAR FRACTION FROM BOVINE RETINAS
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Diacylglycerol kinase (DAGK) activity, which synthesizes phosphatidic acid (PA) from 3H-diacylglycerol (3H-DAG) and ATP, was observed in isolated nuclei from bovine retinas (AIVO, 2010). The purpose of this study was to analyze the effect of light on DAGK activity in isolated nuclei from photoreceptor cells. To this end, bovine eye cups were exposed to light (dark/light model). Nuclei isolation procedure was slightly modified to purify nuclei from photoreceptor cells. Isolated retinas were homogenized in 0.25 M sucrose, filtrated and combined with a sucrose solution to obtain 1.6 M sucrose as a final concentration (H1), layered on the top of a discontinous gradient of 2.3 M and 2.5 M sucrose and centrifuged during 70 min at 25000 rpm. N1 and N2 (interphases) and N3 (pellet) were thus obtained. Small nuclei from photoreceptor cells were found in N3. Results derived from immunofluorescence (IF) with CRX, a transcription factor from photoreceptor cell nuclei. A detergent-lipid mixed micelar assay was used to evaluate DAGK activity. An increased activity (two times) was observed in the N3 nuclei fraction from light-exposed retinas with respect to that found in dark-adapted retinas. IF studies revealed an increased light-dependent signal for anti-DAGK dzeta and épsilon. These data indicate that light induces an increased presence and activity of DAGK in nuclei from photoreceptor cells.

ST-P09.

EXTRACELLULAR ATP-INDUCED CYTOSKELETAL REORGANIZATION IN OSTEOBLASTIC CELLS

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The aim of this study was to investigate the role of extracellular ATP in the regulation of cytoskeletal reorganization in osteoblastic cells and to evaluate the participation of the PI3K/Akt signaling pathway in this effect. Immunocytochemical experiments showed that ATP induced the appearance of actin stress fibers and filopodia in a time-dependent manner. The reorganization of the actin cytoskeleton was also observed after UTP treatment, a $P2Y_2$ receptor agonist but not with ADP or ADPßS, known agonists at the $P2Y_1$ receptors. None of the purinergic agonists employed were able to induce a rearrangement of the intermediate filament vimentin. The use of Ly294002, a PI3K inhibitor, suggested that the PI3K/Akt pathway does not participate in the ATP-induced cytoskeletal reorganization. Wound healing assays combined with actin staining revealed that osteoblastic cells did not migrate after a 6 h-ATP treatment. However, changes observed in cell morphology along with actin reorganization suggest that cells may migrate later. In conclusion, ATP affects actin reorganization in osteoblastic cells in a PI3K/Akt independent pathway.

ST-P10.

IS AtPLD A TARGET FOR NO-MEDIATED S-NITROSYLATION?

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Previously, we demonstrated that phospholipase Dd (AtPLD), one of the 12 Arabidopsis thaliana PLDs, is downstream of nitric oxide (NO) signalling during stomatal closure. How NO activates PLD is still unknown. Since NO is a lipophilic compound, membrane proteins are theoretically more exposed to its chemistry. NO could directly act on proteins by nitrosylation of cysteins (S-nitrosylation). So far, there are no reports of S-nitrosylation of PLDs in either animals or plants. The aim of this study is to analyze whether AtPLD could be a target for S-nitrosylation. In order to determine whether any PLD from plants have the S-nitrosylation motif we did a PHI-Blast analysis. Many PLDs, including PLD , have the motif. In order to analyze whether AtPLD is S-nitrosylated, we transiently overexpress AtPLD with c-Myc tag in tobacco leaves. We observed the presence of AtPLD transcript by RT-PCR and measured *in vitro* increase PLD activity. However we could not detect the PLD -c-Myc protein. Interestingly, under well-water conditions these plants showed a reduced stomatal conductance respect to control ones. Under water deprivation they showed a wilting phenotype. These results support the role of AtPLD in water status balance. Unfortunately data about S-nitrosylation is still lacking. We are currently expressing AtPLD in bacteria.

ST-P11.

AGED-RELATED CHANGES IN ENDOCANNABINOID HYDROLYSIS IN RAT CEREBRAL CORTEX SYNAPTOSOMES

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The enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) are both involved in the hydrolysis of endocannabinoids 2-arachidonoilglycerol (2-AG) and anandamide (AEA), respectively. The aim of this study was to evaluate how the breakdown of 2-AG and AEA was modified by agonists and antagonists of CB1 and CB2 receptors during physiological aging. Cerebral cortex synaptosomes from adult (3 mo) and aged (28 mo) rats were isolated by differential centrifugation and purified in ficoll gradients. MAGL and FAAH activities were assayed using 2-arachidonoyl-[^{3}H]glycerol or arachidonyl-[^{3}H]etanolamine, respectively; and their products, [^{3}H]glycerol and [^{3}H]etanolamine, were quantified from aqueous phase by liquid scintillation. The presence and activity of both enzymes was observed in synaptosomes. MAGL activity was increased and FAAH activity was decreased by aging. MAGL and FAAH activities in aged synaptosomes were abolished by the action of their respective inhibitors. CB1 and CB2 agonist (WIN) did not modify MAGL activity although CB1 antagonist (SR1) increased MAGL activity in adults and decreased it in aged synaptosomes. Our results show important differences in endocannabinoid metabolism during aging, which are modulated through cannabinoid receptors.

ST-P12.

RELEVANCE OF DIFFERENT ERK1/2 PHOSPHORYLATION CONSENSUS SITES IN MKP-1 STABILITY IN LEYDIG CELLS

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In MA-10 Leydig cells, hCG/cAMP up-regulate MAP kinase phosphatase -1 (MKP-1), which in turn attenuates the hormonal action on MAP kinase activity and steroidogenesis. hCG/cAMP lead to MKP-1 accumulation through gene transcription activation and posttranslational modifications including ERK-dependent phosphorylation. Since ERK1/2 phosphorylation in S359 and S364 results in MKP-1 stabilization while in S296 and S323 is associated with protein degradation, our aim was to determine the role of these sites in MKP-1 stabilization by cAMP. We also analyzed a possible link between MKP-1 acetylation and stabilization. Western blot analysis performed with an anti-flag antibody showed that, in MA-10 Leydig cells overexpressing flag-MKP-1 wild type (WT), this protein reaches a null or weak expression level in unstimulated cells and a strong and transient expression in 8Br-cAMP-stimulated cells. Pulse chase experiments showed that, in stimulated cells, the WT protein half life is 120 min, while the double mutants S359A-S364A and S296A-S323A exhibit a shorter (45 min) and longer half life (>150 min), respectively. Thus, the hormonal stabilization of MKP-1 could be the net effect of ERK1/2 phosphorylation in these four sites. Also, acetylation could contribute to MKP-1 stabilization, as cell exposure to a histone deacetylase inhibitor increased flag-MKP-1.

ST-P13.

PKA-CHROMATIN ASSOCIATION IN STRESS RESPONSIVE TARGET GENES FROM *Saccharomyces cerevisiae*

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Regulation of gene expression by intracellular stimulus-activated protein kinases is essential for cell adaptation to environmental changes. There are three PKA catalytic subunits in *S.cerevisiae*:Tpk1,Tpk2 and Tpk3 and one regulatory subunit:Bcy1. Previously, we had demonstrated that both catalytic and regulatory subunits of PKA were associated to transcribed regions and promoters of target genes in a stress dependent manner. Now, using ChIP-real time assay we analyze the requirement of kinase activity and the role of Bcy1 protein on Tpk chromatin association. Inactive versions of Tpk1 and Tpk2 do not associate to chromatin. Deletion of BCY1 promotes higher Tpk1 association, whereas it abolished Tpk2 association. We then analyse the possible role of Tpk on chromatin remodelling in response to stress conditions. Deletion of Tpk1 or Tpk2 affects the density of H3 on defined gene regions. Additionally, we analyse the kinetics of binding chromatin remodellers in the regions bound by Tpk1 and Tpk2 in response to stress. During stress stimulus there is a transient binding to chromatin of Arp8, Rsc1 and Snf2-chromatin remodellers followed temporarily by Tpk association. We also observe co-occupancy of Tpk2 and DNA-binding transcription factor Rap1p. These results suggest that PKA could participate in gene expression by *in situ* phosphorylation of transcriptional or chromatin regulators.

ST-P14.

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

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Transcriptional regulation is a key mechanism that controls the fate and response of cells to diverse signals. Therefore, the identification of the DNA-binding proteins, which mediate these signals, is a crucial step in elucidating how cell fate is regulated. We applied a functional genomic approach to scrutinize the promoters of the yeast PKA subunits: TPKs and BCY1 to contribute to the understanding of the specificity of cAMP-PKA pathway. We have used a two-color GFP-RFP reporter system: Reporter-Synthetic Genetic Array to systematically assess the effects of gene deletions on the TPK1, TPK2 TPK3 and BCY1 promoter activities. The screen allows measurement of a promoter–GFP reporter gene and a control promoter–RFP reporter gene in an array of yeast deletion mutants and provides quantitative measures of promoter gene activity in each mutant background. We identified potential transcription factors, many of which have no perfect consensus site within the promoters and also that transcription of these genes are repressed by the PKA activity itself, and also in presence of glucose, but not in presence of a nonfermentable carbon source. Our analysis also supports the view that although comparative analysis can provide a useful guide, functional assays are required for accurate identification of TF-binding site interactions in complex promoters

ST-P15.

DIFFERENTIAL ROLES OF PROTEIN KINASE A REGULATORY SUBUNIT ISOFORMS IN *Mucor circinelloides*

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PKA is a tetramer composed of two Regulatory (R) and two Catalytic subunits (C). We have demonstrated the existence of 4 genes for R and 10 genes for C in *M. circinelloides*. In a previous work we had disrupted the gene *pkaR1* (R1) and described its role in growth and morphology. R1 showed a reduction in radial growth, alterations in germination rate, cell volume, germ tube length, and asexual sporulation. In the present work, we have disrupted the genes *pkaR2* (R2), *pkaR3 (R3)*, or *pkaR4* (R4) with the aim of analyzing if each PKAR isoform has a different role in the differentiation process. The germ tube emission in R2 is earlier than in the wild-type strain. PKAR4 appears to be essential for the fungus survival. The homokaryotic R4 strain was not stable; with the successive cultures it became heterokaryotic. R4 strain had an impediment to emit the germ tube under aerobic conditions and after the transition from anaerobic to aerobic conditions; it also has an increased cell volume. R3 shows slight differences from wt. The expression of the pkaR genes was assessed in r1, R2 and R3 by semiquantitative RT-PCR. Different mRNA expression was detected in the knockout strains for each pkaR gene. The results indicate that each PKAR isoform has a different role in *M. circinelloides* physiology, thus contributing to the specificity of cAMP-PKA pathway.

ST-P16.

PI3K-DEPENDENT INACTIVATION OF FOXO TRANSCRIPTION FACTORS IN A HIPPOCAMPAL MODEL OF OXIDATIVE STRESS

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We have previously demonstrated the alteration of cell viability, morphology and lipid peroxidation as well as the activation of PI3K/Akt/GSK3ß pathway in a model of iron-induced neurodegeneration. The exposure of a mouse hippocampal neuronal cell line (HT22) to different concentrations of Fe^{2+} (25-200 µM) for 24 h led us to define a mild oxidative injury status (25-50 µM Fe^{2+}). The aim of this work was to investigate the involvement of FoxO transcription factors, known downstream effectors of PI3K, during iron-triggered mild oxidative stress. Under this experimental condition, reactive oxygen species measured by fluorescence microscopy increased with small changes in cell viability, and SOD1 levels decreased whereas catalase levels showed no changes with respect to controls. The localization of phosphorylated and non-phosphorylated FoxO3a was studied in nuclear and cytosolic fractions. Levels of phospho-FoxO3a (the inactive form) increased in the cytosolic fraction of cells treated with iron in a PI3K-dependent manner. Consistent with this, total FoxO3a content decreased in the nuclear fraction. FoxO1 phosphorylation also increased in the cytosolic fraction. Our results show that iron-induced neurotoxicity activates PI3K/Akt/GSK3ß promoting FoxO3a/FoxO1 phosphorylation, their inactivation and their cytosolic localization under mild oxidative injury in hippocampal neurons.

ST-P17.

CHARACTERIZATION OF VPS15: A PUTATIVE REGULATORY KINASE OF VPS34 IN *Trypanosoma cruzi*

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Class III phosphoinositide 3-kinases (PI3Ks), known as Vps34, are important regulators of vesicular trafficking, autophagy and nutrient sensing. Previously, we have functionally characterized the first class III PI3K reported in *Trypanosoma cruzi*, named TcVps34, which has a role in vital processes such as osmoregulation, acidification, and receptor-mediated endocytosis. In yeast and other organisms, Vps34 exerts its lipid kinase activity by forming a protein complex with a Ser/Thr protein kinase known as Vps15. In this work, we initiated the characterization of TcVps15, its homologue in *T. cruzi*. The recombinant TcVps15 was able to phosphorylate both the comertial histone mix (H2As) and the myelin basic protein, showing a cation preference for Mn^{+2}. Moreover, transgenic parasites overexpressing TcVps15 showed an increase in their kinase activity comparing to wild type cells. In addition, western blot analysis of *T. cruzi* parasites overexpressing TcVps34 or TcVps15 showed that both enzymes are localized mainly at the membrane fraction. However, both proteins were partially released with high salt concentration. Interestingly, the localization of these proteins was not affected by the treatment with wortmannin, a PI3K inhibitor. Further experiments will be needed to address the mechanism by which TcVps15 regulates TcVps34, as well as the function of the kinase activity of TcVps15.

ST-P18.

BIOCHEMICAL CHARACTERIZATION OF RAB3 AND RAB27 DURING HUMAN SPERM EXOCYTOSIS

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The two secretory Rabs, Rab3 and Rab27, regulate distinct steps of vesicle exocytosis in neuroendocrine cells. Sperm contain a single, large dense-core granule that is released by regulated exocytosis (the acrosome reaction, AR) during fertilization. The AR utilizes the same fusion machinery as neurons and neuroendocrine cells. Here, we characterize the behaviour of Rab3 in human sperm and show for the first time the requirement of Rab27 during the AR. We detected Rab27 in human sperm by Western blot and indirect immunofluorescence. Calcium was unable to elicit the AR when permeabilized human sperm was loaded with anti-Rab27 antibodies or with Slac2-b, a Rab27 effector, indicating that this small GTPase is essential for exocytosis. Anti-Rab27 and anti-Rab3 antibodies had different effects when added to sperm after cis SNARE complex disassembly and when exocytosis was triggered by high calcium concentrations. Rab27 was predominantly membrane-bound at all times, whereas the population of membrane-bound Rab3 augmented in response to exocytosis inducers. By means of pull down assays with specific protein cassettes we found that a substantial proportion of these Rabs was active (GTP-bound) in resting cells; AR inducers increased the levels even further but to different degrees for each Rab. These results indicate that there are distinct points of action for these two small GTPases

ST-P19.

Akt ALLOWS CYTOSOLIC REGULATION OF ENDOPLASMIC RETICULUM STRESS PATHWAYS

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In mammalian cells, Akt protein kinases are recruited to the plasma membrane where they are phosphorylated in response to a wide variety of extracellular signals. Traditionally, Akt regulates a multitude of targets that promote cell survival. On the other hand, the Unfolded Protein Response (UPR) pathways, which typically respond to Endoplasmic Reticulum (ER) signals, have been shown to mantain homeostasis by regulating cell survival and cell death. We studied UPR and Akt activity levels by western blot and RT-PCR and we monitored simultaneously these levels in individual cells by working with specifically designed fluorescent protein-based reporters of IRE1, ATF6, Akt1 and Akt2. Here we propose, by genetic analysis and fluorescence microscopy, that transient modulation of AKT activity allows ER membrane residing Akt molecules to activate UPR. Our results unravel Akt as a novel cytosolic regulator of UPR pathways. This crosstalk emerges as a master control mechanism of cell decision making in terms of survival or death showing the remarkable flexibility of signaling pathways, which can direct cells to opposing fates depending on the dynamics of their activation.

EN-P01.

PURIFICATION AND FUNCTIONAL CHARACTERIZATION OF A CYS-LESS VERSION OF *Bacillus subtilis* THERMOSENSOR

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Histidine kinases (HKs) play a major role in signal transduction in prokaryotes for the cellular adaptation to environmental conditions and stresses. The HK DesK of *Bacillus subtilis* is a member of the two component system DesK/DesR responsible for *Bacillus* adaptation to cold shock. Its sensor region is confined to the five transmembrane segments (TMS) whose unknown conformational rearrangements are essential for sensing and transducing the cold signal to the cytoplasmic catalytic domain.
Site-directed spin labeling (SDSL) and Electronic Paramagnetic Resonance (EPR) have proven to be useful for elucidating structures of a great variety of integral membrane proteins. Since these techniques require having only one cysteine (Cys) in the protein at the site of study and DesK catalytic domain has two Cys residues, which seemed to be essential for the kinase activity, we first generated a Cys-less variant of DesK by overlap PCR. Then we found conditions in which DesKCys-less can be expressed and purified in a soluble form at high yields from *E. coli* cells. Finally, the biochemical characterization showed that DesKCys-less enzyme has less autokinase and phosphotransferase activities than DesK wild type, but it still kept cold shock regulation. These results will allow us to initiate the determination of DesK TMS folds and conformational dynamics using spin-labeling EPR spectroscopy.

EN-P02.

CHARACTERIZATION OF THE ENZYMATIC ACTIVITY OF *Pseudomonas aeruginosa* MUTL PROTEIN

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It was described that in the mismatch repair system of some bacterial and eukaryotic organism devoid of MutH and Dam methylation, MutL has a divalent cation-dependent endonucleolytic activity, which could have an *in vivo* MutH homolog function. In addition, our group recently described that *P. aeruginosa* MutL (PaMutL) has endonucleolytic activity. Using supercoiled plasmid, this activity was evidenced by the presence of relax plasmid caused by single strand break and lineal DNA fragment due to a second hydrolysis event. In this work we studied the activity of this enzyme but using as a substrate lineal dsDNA fluorescent labeled at the 5' ends. The products of the reaction were detected by automated capillary electrophoresis and denaturing polyacrylamide gels, where DNA is visualized as single strand allowing nicks identification. Double strand breaks detection was performed by native agarose gel electrophoresis. Our results indicated that besides the endonucleolytic activity, PaMutL would also have an exonucleolytic activity, removing one or few nucleotides from the 5' end. Whereas the hydrolysis of supercoiled DNA plasmid by PaMutL generated nicked plasmid and lineal DNA products, the treatment of lineal dsDNA with PaMutL did not produce double strand breaks or internal nicks. This could indicate that PaMutL enzymatic activity is regulated by DNA topology or sequence context.

EN-P03.

INSIGHT INTO THE BASIS OF GLYCOGENIN INACTIVATION CAUSING GLYCOGENOSIS BY MUTATIONAL APPROACH

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Glycogenin-1 is one of the two human glycogenin isoforms, mainly expressed in muscle. It displays 93% sequence identity with the rabbit enzyme, the best studied member of this protein family and the only one whose three dimensional structure has been solved. A missense mutation, Thr82Met, in one allele of the glycogenin-1 gene *GYG1*, producing human glycogenin inactivation and deficient priming of glycogen synthesis, was recently described. To analyze the structural basis of the loss of enzyme activity responsible for the human glycogenosis phenotype, we introduced the Thr82Met mutation into rabbit muscle glycogenin and solved its crystal structure. Thr82Met substitution resulted in only a few altered intramolecular residues interactions, while those with UDP-glucose were conserved. The results are consistent with the enzyme inactivation due to the prevented essential involvement of Asp162 in UDP-glucose activation, produced by loss of Thr82 to Asp162 hydrogen bonding, favored hydrophobic interactions between the replacing methionine and the neighboring amino acids, or both. Here we report the characterization of glycogenin mutants obtained by substitution of Thr82 with residues of different size and hydrophobicity, in an attempt to distinguish between the above proposed causes of inactivation.

EN-P04.

A 2[4FE-4S] FERREDOXIN IS SUBSTRATE OF FERREDOXIN-NADP$^+$ REDUCTASE FROM *Leptospira interrogans*

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Ferredoxins (Fds) are small iron-sulfur proteins with low redox potential which act as electron donors in various metabolic pathways. They can be grouped in three distinct families, the plant and mammalian type [2Fe–2S], the thioredoxin-like [2Fe-2S] and the 2[4Fe-4S] ferredoxins, first isolated from anaerobic bacteria. The latter may differ in cluster type (3Fe or 4Fe), number (one or two), and length of polypeptide chain. Ferredoxins can be substrates of ferredoxin-NADP$^+$ reductases (FNR) in redox metabolisms in mitochondria, plastids and bacteria. We have identified and cloned a 2[4Fe-4S] ferredoxin (LFd2) from *Leptospira interrogans*, a parasitic bacterium of animals and humans. We succeeded in expressing and purifying the recombinant protein with its Fe-S cluster properly bound, by co-expressing the biogenesis iron-sulfur system (ISC) from *Escherichia coli*. An O_2-free atmosphere was required during the purification protocol. LFd2 displayed spectral similarities with typical 2[4Fe-4S] ferredoxins. We were able to measure cytochrome *c* reductase activity with *L. interrogans* FNR and LFd2. Our results suggest that electron transfer between the reductase and LFd2 is optimal at pH 6,5 and low salt concentration. Our studies show that in *L. interrogans* a plastidic type FNR exchanges electrons with a bacterial type ferredoxin, process which has not been previously observed in nature.

EN-P05.
EFFECT OF ORGANOPHOSPHATE FENITROTHION ON BIOCHEMICAL PARAMETERS OF THE CRUSTACEAN *Macrobachium borellii*
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Several agrochemicals like organophosphates are used in agricultural practices in Argentina. Although most of them have low persistence in aquatic ecosystems, they adversely affect non-target fauna.
We evaluated metabolic alterations that could be used as pollution biomarkers in the area of Río de La Plata, using the prawn *Macrobachium borellii* exposed to the pesticide fenitrothion (FS) as a model. The digestive gland (DG) from adults exposed to sublethal concentrations (0.1 to 0.7 ppb) for 1 to 7 days was studied. The activity of catalase (CAT), glutathione-S-transferase (GST) superoxide dismutase (SOD) and peroxidation levels were determined. Inhibition of hemolymph acetylcholinesterase (ACT) and hemocyte DNA damage was also determined. SOD activity increased at 0.7 ppb FS on day 2 and 4. Exposure to 0.4 ppb FS increased CAT activity on day 1 and 4, while GST increased on day 2 and 7 in the DG of exposed prawn. LPO levels increased at 0.4 ppb except on day 7. Hemolymph analysis showed that while ACT activity decreased inversely with FS concentration after day 2, hemocytes displayed most DNA damage after 7 day exposure to FS. As a whole, these results indicate that though there is no correlation between these parameters and FS concentration and/or days of exposure, these biochemical parameters were highly sensitive and could be employed in the evaluation of FS aquatic pollution.

EN-P06.
CHARACTERIZATION OF ENZYMES INVOLVED IN UDP-GLUCOSE METABOLISM IN *Escherichia coli*
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UDP-Glc is a key glycosyl donor for carbohydrate metabolism in bacteria. It is produced from UTP and Glc-1P by UDP-Glc pyrophosphorylase (GalU). Another protein (GalF), with high sequence identity to GalU, was reported in *Escherichia coli*. We compared the kinetic and structural properties of both recombinant proteins. Values of $S_{0.5}$ for GalU were determined to be 0.16 mM (UTP) and 0.035 mM (Glc-1P), whereas those for GalF were 0.91 mM and 0.32 mM, respectively. GalU displayed a four order of magnitude higher V_{max} than GalF. *In silico* analysis showed that GalF lacks two key catalytic residues; thus, we constructed the mutant GalF M15T/H16R, which exhibited similar affinity for both of the substrates, but had a 10-fold higher V_{max} than GalF. Although the latter V_{max} is substantially lower than that of GalU, mutations effectively enhanced the catalytic ability of GalF. Size exclusion chromatography revealed that GalU is tetrameric, whereas GalF and its mutant are monomers; which could account for the only partial "resurrection" observed for mutated GalF. We hypothesize that, after gene duplication, GalU retained the catalytic function and GalF acquired new roles, including the modulation of GalU activity.
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EN-P07.
A MUTATION MIMICKING ACETYL RESIDUE ON LECTIN DOMAIN OF ppGalNAc-T2 AFFECTS ITS BIOLOGICAL ACTIVITY
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ppGalNAc-T2 is a type II membrane protein with an intraluminal glycosyltransferase domain followed by R-type lectin domain. We previously described the effect of acetylation on catalytic and glycan-binding abilities of ppGalNAc-T2. Acetylation on K103, S109, K111, K363, S373, K521 and S529 of ppGalNAc-T2 reduced the enzymatic activity and modified the carbohydrate recognition of lectin domain. (QXW)3 motive is a main characteristic of R-type lectin family and K521 is part of QKW motif of ppGalNAc-T2 lectin domains. This work studies the relevance of K521 on catalytic activity of glycosyltransferase ppGalNAc-T2 as well as its contribution on the carbohydrate-binding ability of lectin domain when K521 is mutated mimicking an acetyl residue. Mutant ppGalNAc-T2K521Q was cloned in Baculovirus expression vector pAcGP67, expressed in Sf9 insect cells, purified by affinity chromatography to homogeneity and acetylated *in vitro*. Competitive assays revealed a carbohydrate-binding specificity of lectin domain from mutant ppGalNAc-T2K521Q similar to acetylated ppGalNAc-T2. In addition, catalytic activity of enzyme was reduced by mutation, as observed by acetylation on ppGalNAc-T2. In conclusion, the mutation K521Q of ppGalNAc-T2 modifies the protein in similar form to acetylation. Acetylation on K521 could have a regulatory role on biological function of ppGalNAc-T2.

EN-P08.
INHIBITORY EFFECT OF ACETYLATED LECTIN DOMAINS ON GLYCOSYLTRANSFERASE ACTIVITY OF ppGalNAc-T2
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Polypeptide GalNAc transferases (ppGalNAc-Ts) are a family of enzymes that catalyze initiation of mucin-type O-glycosylation. All ppGalNAc-Ts in mammals are type II transmembrane proteins having a Golgi lumenal region that contains a catalytic domain with glycosyltransferase activity, and a C-terminal "ricin-like" lectin domain. We investigated the effect of acetylated lectin domains of ppGalNAc-T3 and ppGalNAc-T4 on GalNAc-transferase activity of ppGalNAc-T2. Lectin domains and ppGalNAc-T2 were expressed as soluble recombinant proteins in insect cells. Constructs contain 6xHis and T7 tags. Recombinant proteins were purified to homogeneity using Ni^{2+} affinity chromatography. Lectin domains were acetylated *in vitro*, and this chemical modification was detected by western-blot using anti-acetylated lysine antibody. Enzymatic activity of ppGalNAc-T2 was analyzed by mass spectrometry and colorimetric assay. The presence of acetylated lectin domains from ppGalNAc-T3 and ppGalNAc-T4 produced a clear inhibitory effect on catalytic activity of ppGalNAc-T2. Direct binding of lectin domains to (glycosylated) acceptor peptides was not affected by acetylation. In addition, competitive lectin assays showed that acetylation do not change the carbohydrate recognition of these lectin domains. Taken together, these findings suggest a control of ppGalNAc-T2 activity by acetylated lectin domains

EN-P09.

DIGESTIVE RESPONSES OF ANTICARSIA GEMMATALIS LARVAE TO SOYBEAN (*Glycine max*) PROTEASE INHIBITORS

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Anticarsia gemmatalis is a major pest of soybean (*Glycine max*). However, soybean has digestive protease inhibitors (PI) as a defense against insects attack. Although these PI are an effective defense many herbivores, the mortality of *A. gemmatalis* is very low. Thioredoxins proteins (Trxs) are able to reduce disulfide bonds in some proteins such as those found in PI. The Trx in the gut of *A. gemmatalis* could be a component of the resistance to the soybean PI. The aim of this study was to sequence Trx from *A. gemmatalis* to analyze Trx expression from guts of larvae that fed on diets with different levels of PI. We designed primers from Trxs of related species and specific cDNA were obtained and sequenced. Larvae were fed on diets containing different percentages of soybean seeds. We obtained total coding sequence with 98% of similitude to related species, and its conserved active site. Trx expression increase 120% in larvae that fed on diet containing 80% of seeds, and 70% expression in larvae that fed diet with 20% of seeds compared to PI-free diet (control). Survival and weight gain of larvae was also measure. Larvae that fed on 80% raw soybean had 60% survival compared to the control, and a weight gain 90% less, in the case of larvae that fed with 20% of raw soybeans showed 8% lower weight gain. This suggests a direct involvement of the *A. gemmatalis'* Trx against soybeans PI.

EN-P10.

PROTEASES INHIBITORS OF *Glycine max* SEEDS DECREASE DIGESTIVE ENZYME ACTIVITY OF *Nezara viridula*

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Although currently pests are controlled by transgenic crops, no transgenic soybeans (*G. max*) can resist stinkbugs (*N. viridula*) herbivory. These bugs damage young pods and decrease crop yield. However, soybean naturally responds to insect herbivory by up-regulating anti-herbivore defenses, such as protease inhibitors (PI), which affect digestion and amino acid assimilation in the gut of insects. Our aim was to determine the effects of soybean's PI on proteolytic activity in the gut of stinkbugs. Field collected insects were kept on control diet for 15d, and then placed for 72h on two soybean genotypes, Williams and a resistant cultivar (PI227687) to insect herbivory. Total and cysteine protease activity in the gut of stinkbugs were decreased after 72h of feeding on soybean pods.This activities decresed 43% and 33% when stinkbugs fed on cv Williams, and 72% and 66% on cv PI227687, compared to the control. Cystein proteases showed the highest activity in the insect gut extracts (30-45%), followed by the aspartyl and serine proteases (10-30%). Experiments with soybean PI showed that stinkbugs that fed on control diet had higher levels of sensitive digestive proteases to PI than those that fed on the PI227687.Our results suggest that although digestive enzymes of stinkbug can be inhibited by soybean PI, these insect compensate the lost activity by inducing enzymes resistance against PI.

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